GERMPLASM PROGRAM LEGUMES

Annual Report for 1994

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International Center for Agricultural Research in the Dry Areas
P.O. Box 5466, Aleppo, Syria

This report was written and compiled by program scientists and represents a working document of ICARDA. Its primary objective is to communicate the season's research results quickly to fellow scientists, particularly those within West Asia and North Africa, with whom ICARDA has close collaboration. Owing to the tight production deadlines, editing of the report was kept to a minimum.

GERMPLASM PROGRAM-LEGUMES
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1. INTRODUCTION

Sustainable increases in the productivity of rainfed farming systems in the West Asia and North Africa (WANA) region require judicious incorporation of cool-season legumes in the cereal dominated cropping common in the region. This necessitates development of legume cultivars that are not only high yielding but are also able to withstand stresses imposed by environment (drought, cold and heat) and biological factors (diseases, insect-pests, parasitic weeds, etc). The diverse cropping systems in which they have to be incorporated in different agroecological zones in WANA, and other parts of the developing world, impose specific demands on the phenology and the adaptation to environments in the cultivars to be developed.

The objective of the legume improvement work of the Germplasm Program at ICARDA is to develop a range of cultivar of different cool-season annual legume species, adapted to specific niches in the cropping systems, through cooperative research with national programs. Research and training activities during the 1994/95 season were continued with this objective and covered such legume species as vetches and chickling (grasspea) amongst annual forage legumes, and kabuli chickpea, lentil, faba bean and dry pea among the food legumes.

Research on kabuli chickpea was partly supported by and jointly done with International Crops Research Institute for the Semi-Arid Tropics (ICRISAT). Research on faba bean was done mainly through the national programs participating in the faba bean research networks operating in China, the Nile Valley and North Africa. *Orobanche* research was done in collaboration with the University of Hohenheim, Germany. Work on nematodes was done with the

Institute of Agricultural Nematology, Bari, Italy. Molecular-marker work was done in collaboration with the University of Frankfurt, Germany and Washington State University, Pullman, USA. Linkages were also forged with the Cooperative Research Centre for Legumes in Mediterranean Agriculture (CLIMA), Western Australia.

Our research work was mainly centered at Tel Hadya site of ICARDA, but good use was also made of other subsites in Syria (Breda and Jindiress) and Lebanon (Kfardan and Terbol). Generation advancement of lentil and kabuli chickpea was done in the summer season at Terbol. Research stations of several national programs were used, jointly with national scientists, for research on developing breeding material with specific resistance to some key biotic and abiotic stresses because of the presence of ideal screening conditions there. The process of decentralization of crop improvement work was continued with the help of appropriate national programs and the International Legume Testing Program was further targetted to specific conditions to better meet the changing needs of NARSs.

The weather conditions during the season at Breda, Tel Hadya, Jindiress, Terbol and Kfardan are depicted in Section 11. The long-term average precipitation at these sites is 280, 328, 470, 600 and 380 mm. During 1993/94 the total seasonal precipitation at the first four locations was 290.4, 373.3, 5337 and 475 mm, respectively. Thus the rainfall at the sites in Syria was above long-term average while at Terbol, it was below long-term average. The winter, in general, was mild, which prevented good screening for cold tolerance. The temperatures at all the sites were abnormally high in mid-April, with large difference in day and night temperatures. This adversely affected the reproductive phase of lentil and annual forage legumes. Because of

extended wet period with temperatures conducive for disease development, chickpea crop in several parts of Syria was severely affected by Ascochyta blight. These weather conditions permitted good screening of chickpea material for resistance to Ascochyta blight.

During the season some staff changes occurred. The Chickpea Pathologist, the Visiting Scientist in Crop Physiology from ICRISAT and the Visiting Scientist in Chickpea Breeding left the Program at the end of their tenure by August/Sept 1994. The time input of Legume Entomologist was reduced to half for personal reasons.

The Cereals Training Scientist started doing the training coordination work on legumes as well. The Virologist moved from GRU to the Germplasm Program along with his support staff. Dr. Wafa Choumane of Tishreen University joined the biotechnology group as a Visiting Scientist. No major changes occurred in the support staff of the group.

Results of the work done during the 1993/94 season are presented in this volume. Also, the training and networking activities and the publications of the Program have been listed. Much of the work reported here has been done in collaboration with the national programs in WANA, to whom we are indebted. - **M.C. Saxena.**

2. KABULI CHICKPEA IMPROVEMENT

The kabuli chickpea improvement is a joint program with ICRISAT, India. The main objective of the program is to increase and stabilize kabuli chickpea production in the developing world. Of the five main regions where chickpea is grown, the Mediterranean region and Latin America produce mostly kabuli-type chickpea. Five to ten percent of the area in the other three main production regions (Indian subcontinent, East Africa and Australia) is also devoted to the production of the kabuli type. Kabuli chickpea is also grown in high elevation areas (>1000 m elevation) in West Asia, especially in Turkey, Iraq, Iran, and Afghanistan; and in the Atlas mountains of the North Africa.

Ascochyta blight and wilt are the two major diseases of chickpea. Leaf miner in the Mediterranean region and pod borer in other regions are major insect pests. Drought is the major abiotic stress throughout the chickpea growing areas and cold assumes importance in Mediterranean environments and the temperate region especially when introducing winter planting. Kabuli chickpea is mainly grown as a rainfed crop in the wheat-based farming system in areas receiving between 350 mm and 600 mm annual rainfall in the West Asia and North Africa (WANA) region. In Egypt and Sudan and parts of South Asia, West Asia and Central America, the crop is grown with supplemental irrigation.

In West Asia and North Africa, where the crop is currently spring-sown, yield can be increased substantially by advancing sowing date from spring to early winter. With the introduction of winter sowing the chickpea cultivation can be extended to lower rainfall regions, to as low as 300 mm. Increasing plant density and reducing row width can also increase yield

significantly, especially during winter sowing. Winter sowing allows the crop to be harvested by machine. Major efforts are underway to stabilize chickpea productivity by breeding cultivars resistant to various stresses, such as diseases (ascochyta blight and fusarium wilt), insect pests (leaf miner), parasites (cyst nematode), and physical stresses (mainly cold and drought). Exploitation of wild *Cicer* species for transfer of genes for resistancey to different stresses is another area receiving high research priority at the Center. DNA fingerprinting in *Ascochyta rabiei* is being pursued with great promise.

During 1994, several collaborative projects continued to operate. In the project "Development of chickpea germplasm with combined resistance to ascochyta blight and fusarium wilt using wild and cultivated species", four Italian institutions collaborated with ICARDA. The screening for cyst nematode was carried out in association with the Istituto di Nematologia Agraria, C.N.R., Bari, Italy. Fusarium wilt resistance screening was done in association with the Institut de la Recherche Agronomique de Tunisie (IRAT), Tunisia and the Departamento de Patologia Vegetal, Cordoba, Spain. Screening for tolerance to cold was done in cooperation with agricultural research institutes in Turkey. The University of Saskatchewan, Canada is collaborating in studies of genetic diversity in kabuli chickpea. Studies on mechanism of drought and cold tolerance and aspects of biological nitrogen fixation are being conducted in collaboration with INRA, Montpellier, France. Studies on application of restriction fragment length polymorphism (RFLP) in characterizing chickpea genotypes and *Ascochyta rabiei* isolates are carried out in collaboration with the University of Frankfurt, Germany.

2.1. Chickpea Breeding

Major objectives of the breeding are (1) to produce cultivars and genetic stocks with high and stable yield and to develop segregating populations and materials to support National Agricultural Research Systems (NARSs) and (2) to conduct strategic research on methodologies to improve germplasm. Specific objectives in the development of improved germplasm for different regions are:

1. Mediterranean region: (a) winter sowing (30% of resources): resistance to ascochyta blight, tolerance to cold, suitability for machine harvesting, medium to large seed size; (b) spring sowing (30% of resources): cold tolerance at seedling stage, resistance to ascochyta blight and fusarium wilt, tolerance of drought and early maturity, medium to large seed size;

2. Indian subcontinent and East Africa (20% of resources): resistance to ascochyta blight and/or fusarium wilt, drought tolerance, early maturity, small to medium seed size, response to supplemental irrigation;

3. Latin America (5% of resources): resistance to fusarium wilt, root rot and virus diseases, large seed size;

4. High elevation areas (15% of resources): spring sowing, cold tolerance at seedling stage, resistance to ascochyta blight, terminal drought tolerance, early maturity, and medium to large seed size. **K.B. Singh.**

2.1.1. Use of Improved Germplasm by NARSs

2.1.1.1. International nurseries/trials and other breeding lines

During 1994, 13,874 chickpea entries were shipped to NARS in 338 sets through international nurseries and special requests for breeding lines to 42 countries. Eighty-eight percent of the international trials and nurseries were furnished to developing countries and the remaining 12% to industrialized countries (Table 2.1.1). The nurseries were in demand from all the six continents from Chile to China and from Canada to Australia-New Zealand. Despite our encouragement to national programs to accept more segregating and crossing block materials, the demand for the finished material is increasing, suggesting that breeders have found ICARDA-ICRISAT chickpea lines useful for their direct exploitation. The kabuli-chickpea network is well established among chickpea scientists. **K.B. Singh, R.S. Malhotra and M.C. Saxena.**

2.1.1.2. On-farm trials in Syria

Two chickpea lines (FLIP 86-5C and FLIP 86-6C) were selected by the Directorate of Agriculture and Scientific Research (DASR), Ministry of Agriculture and Agrarian Reform, from the ICARDA/ICRISAT international trials and were evaluated in researcher-managed on-farm trials throughout Syria from 1991/1992 to 1993/94 (Table 2.1.2). The two new entries were resistant/ tolerant to cold and ascochyta blight except in north-eastern Syria. Both produced marginally lower yields than the two checks, Ghab 1 and Ghab 3. However, the two new entries excelled the checks in two key characters. Both FLIP 86-5C and FLIP 86-6C have 50% larger seed size, an attribute in great demand in the WANA region. Both the new

Table 2.1.1. Number of entries furnished in the form of
international yield trials and specific
nurseries in 1994.

Country	Trial or nursery		Breeding	Total
	No. of sets of trial/nursery	No. of entries	lines (no.)	entries (no.)
Afghanistan	1	64	–	64
Algeria	10	299	9	308
Australia	10	423	21	444
Bangladesh	2	62	–	62
Bhutan	1	49	–	49
Bulgaria	2	65	–	65
Canada	4	192	473	665
Chile	3	97	–	97
China	3	106	–	106
Colombia	3	103	–	103
Cyprus	1	24	–	24
Egypt	10	303	239	542
Ethiopia	16	551	–	551
France	4	163	6	169
India	27	979	246	1225
Iran	13	406	–	406
Iraq	9	250	6	256
Italy	17	539	2	541
Jordan	4	120	47	167
Kuwait	–	–	5	5
Lebanon	7	275	1	276
Libya	2	55	–	55
Mexico	3	104	–	104
Morocco	17	633	–	633
Myanmar	17	579	–	579
New Zealand	8	291	–	291
Oman	1	31	–	31
Pakistan	13	574	45	619
Peru	2	73	–	73
Poland	–	–	20	20
Portugal	7	276	–	276
Saudi Arabia	3	86	–	86
Slovakia	1	64	–	64
Spain	2	73	–	73
Sudan	5	134	–	134
Syria	44	1922	–	1922
Thailand	3	97	–	97
Tunisia	18	874	–	874
Turkey	35	1362	16	1378
U.K.	1	51	–	51
U.S.A.	1	51	9	60
Total	338	12,719	1145	13,874

Table 2.1.2. Seed yield (kg/ha) of chickpea entries in the on-farm trials conducted jointly with the Directorate of Agriculture Scientific Research, Syria and ICARDA during the years 1991/92, 1992/93, 1993/94.

Entry	Seed yield 1991/92[a]	1992/93[b]	1993/94[c]	Mean	100-seed[d] weight (g)	Plant[d] height (cm)	Days to[d] flower	Days to[d] maturity	Protein[e] content (%)	Ascochyta[d] blight score	Cold[d] score
FLIP 86-5C	2084	2013	1287	1793	41	55	115	158	18.3	6	3
FLIP 86-6C	1977	2012	1323	1767	38	54	112	156	17.9	6	3
FLIP 84-15C	-	2146	1129	1636	42	49	111	157	17.8	7	3
Ghab 1	1925	2201	1318	1809	28	42	106	151	17.5	7	4
Ghab 3	1931	2054	1525	1832	27	48	111	153	17.8	5	3
Location mean	1980	2085	1316		35	49	111	155	17.86		
S.E. of mean	45.6	40.9	78.7		0.5	0.3	0.2	0.2	0.1		
LSD at 0.05	131.0	160.6	309.1		2.0	1.4	1.0	0.8	0.4		
C.V. (%)	8.3	2.7	8.4		2.0	1.0	0.3	0.2	0.9		

a/ Mean of 13 locations
b/ Mean of 13 locations
c/ Mean of 14 locations
d/ Data were collected during 1993/94 on 1-9 scale, where 1= no damage, 9= complete kill.
e/ Data collected in Tel Hadya during 1992/93 on 1-9 scale, where 1= no damage, 9= complete kill.

cultivars have tall growth habit and thus are suitable for combine harvest, important for large scale introduction of winter chickpea. Based on large seed size and tall stature, DASR has identified FLIP 86-5C for release as a commercial cultivar in Syria.

The ICARDA-ICRISAT Kabuli Chickpea Project was involved in the conduct of on-farm trials in many other countries including Algeria, Iraq, Jordan, Lebanon, Morocco, Tunisia, and Turkey. The degree of our involvement varied from complete association (e.g. Lebanon) to only providing advice (e.g. Turkey). Results have been encouraging as demonstrated by a large number of releases of cultivars and their adoption by farmers. **NARSs scientists and K.B. Singh.**

2.1.1.3. Pre-release multiplication of cultivars by national programs

Forty lines have been chosen by 14 NARSs during 1994 from the ICARDA/ICRISAT international trials for the pre-release multiplication and on-farm testing (Table 2.1.3). However, we do not have yet the full information. The new lines have resistance to ascochyta blight and tolerance of cold. They have large seed size, thus they meet consumers' demands. If grown in winter, they attain a minimum height of 40 cm and can be harvested by machine. Seeds of some of the promising lines are being multiplied at ICARDA to meet potential initial demand of NARSs. **K.B. Singh, R.S. Malhotra and M.C. Saxena.**

Table 2.1.3. Chickpea lines identified for pre-release multiplication and on-farm testing by NARSs in recent years.

Country	Lines
Afghanistan	FLIP 82-4C, FLIP 82-9C, FLIP 82-16C, FLIP 82-20C
Algeria	FLIP 83-49C, FLIP 83-71C, FLIP 84-109C, FLIP 84-145C, FLIP 85-17C
Cyprus	FLIP 85-10C
Egypt	FLIP 80-30C
France	FLIP 84-188C
Iraq	FLIP 86-6C, FLIP 88-85.
Jordan	FLIP 84-15C, FLIP 85-5C
Lebanon	FLIP 82-150C, FLIP 88-85C
Libya	FLIP 84-79C, FLIP 84-93C, FLIP 84-144C
Mexico	FLIP 81-293C
Morocco	FLIP 83-48C, FLIP 84-79C, FLIP 84-145C, FLIP 84-182C
Syria	FLIP 86-5C
Tunisia	FLIP 83-47C
Turkey	FLIP 81-70C, FLIP 82-74C, FLIP 82-161C, FLIP 82-269C, FLIP 83-31C, FLIP 83-41C, FLIP 83-47C, FLIP 83-77C, FLIP 84-79C, FLIP 85-13C, FLIP 85-15C, 87AK 71112.

2.1.1.4. Release of cultivars by NARSs

During 1994, four cultivars were released by two countries. Turkey released 'Damla' (FLIP 85-7C) and 'Azizyie' (FLIP 84-15C) and USA released 'Dwelley' (Surutato x FLIP 85-58C) and 'Sanford' (Surutato x FLIP 85-58C) (Table 2.1.4).

NARSs in 20 countries have released over the duration of this project 56 lines as cultivars from material furnished from ICARDA (Table 2.1.4). Forty-three of them have been released for winter sowing in the Mediterranean region, nine for spring sowing including four in China, two for winter sowing in more southerly latitudes for sowing with irrigation, and one each in Oman and Pakistan. With these releases chickpea cultivars bred at the Center have been released in all the four major regions of chickpea production. **NARS Scientist, K.B. Singh, R.S. Malhotra and M.C. Saxena.**

Table 2.1.4. Kabuli chickpea cultivars released by national
programs.

Country	Cultivars released	Year of released	Specific features
Algeria	ILC 482	1988	High yield, blight res.
	ILC 3279	1988	Tall, blight res.
	FLIP 84-79C	1991	Cold, blight res.
	FLIP 84-92C	1991	Blight res.
China	ILC 202	1988	Tall, for Ginghai pr.
	ILC 411	1988	High yield, for Ginghai pr.
	FLIP 81-71C	1993	High yield
	FLIP 81-40C	1993	High yield
Cyprus	Yialousa	1984	Tall, blight res.
	Kyrenia	1987	Large seeds
Egypt	ILC 195	1993	Blight, wilt res.
France	TS1009	1988	Blight res.
	TS1502	1988	Blight res.
	Roye Rene	1992	Cold, blight res.
Iraq	ILC 482	1991	Blight res., high yield
	ILC 3279	1991	Tall, blight res.
Italy	Califfo	1987	Tall, blight res.
	Sultano	1987	Tall, blight res.
Jordan	Jubeiha 2	1990	High yield, blight res.
	Jubeiha 3	1990	High yield, blight res.
Lebanon	Janta 2	1989	High yield, wide adaptation
	FLIP 85-5C	1993	Green seed consumption
Libya	ILC 484	1993	High yield, blight res.
Morocco	ILC 195	1987	Tall, blight res.
	ILC 482	1987	High yield, blight res.
	Douyet	1992	Large seed, blight res.
	Rizki	1992	Large seed, blight res.
Oman	ILC 237	1988	High yield, irrigated cond.
Pakistan	Noor 91	1992	High yield, blight res.
Portugal	Elmo	1989	Blight res.
	Elvar	1989	Blight res.
Spain	Fardan	1985	Tall, blight res.
	Zegri	1985	Mid-tall, blight res.
	Almena	1985	Tall, blight res.
	Alcazaba	1985	Tall, blight res.
	Atalaya	1985	Mid-tall, blight res.
Sudan	Shendi	1987	High yield, irrigated cond.
	Jeb el Mara	1993	Resistant to heat and wilt
Syria	Ghab 1	1986	High yield, blight res.
	Ghab 2	1986	Tall, blight res.
	Ghab 3	1991	High yield, cold & blight res.

Tunisia	Chetoui	1986	Tall, blight res.
	Kassab	1986	Large seeds, blight res.
	Amdoun 1	1986	Large seeds, wilt res.
	FLIP 84-79C	1991	Blight, cold res.
	FLIP 84-92C	1991	Large seed, blight res.
Turkey	ILC 195	1986	Tall, blight res.
	Guney Sarisi 482	1986	High yield, blight res.
	Damla 89	1994	Blight res.
	Aziziye	1994	Blight res.
	Akcin	1991	Tall, blight res.
	Aydin 92	1992	Large seed, blight res.
	Menemen 92	1992	Large seed, blight res.
	Izmir 92	1992	Large seed, blight res.
USA	Sanford	1994	Large seed, blight res.
	Dwelley	1994	Large seed, blight res.

2.1.2. Screening for Stress Tolerance

2.1.2.1. Wilt resistance in chickpea

Diseases are the major constraints to chickpea (*Cicer arietinum* L.) production. Among the 50 diseases reported, fusarium wilt induced by *Fusarium oxysporum* Schlecht. emend. Snyd. & Hans. f.sp. *ciceri* (Padwick) Snyd. & Hans. is the second most important disease worldwide. In WANA, it is prevalent in parts of North Africa and in the Nile Valley. *F. oxysporum* f. sp. *ciceri* is both soil-borne and seed transmitted. It can survive in the soil for long periods. Inoculum in infected seed can be eradicated by seed dressing with fungicide. The most practical and economical method for the control of fusarium wilt is the use of resistant cultivars. Accordingly, resistance breeding has been one of the main objectives in chickpea improvement. In this effort, the major complication arises from the presence of different races of the pathogen. Seven races of the pathogen have been reported. Therefore, more efforts are needed to increase the effectiveness and stability breeding material for disease

resistance.

Screening in the field must be conducted in a wilt-sick plot. Development of wilt-sick plot involves growing a highly susceptible cultivar after inoculating the seed with the pathogen for 3-4 seasons and incorporating parts of wilted plants into the soil. Following this method, a wilt-sick plot has been developed at ICARDA. Likewise, wilt sick plots have been developed, either artificially or selecting a wilt-infested field and improving it further, in many WANA countries (Tunisia, Egypt, Sudan, Ethiopia) and elsewhere including Spain.

In absence of a wilt-sick plot at ICARDA until 1994, screening and breeding work was conducted in collaboration with the Universidad de Córdoba, Spain, Plant Pathology Research Institute, Rome, Italy and INRAT, Tunisia. In Spain, 3019 germplasm accessions were screened in two wilt-sick plots at Montilla and Santaella (Córdoba) since 1987 (Figure 2.1.1). Some of kabuli lines with high level of resistance were ILC 267, ILC 1278, ILC 1300, FLIP 86-93C, FLIP 87-33C, and FLIP 87-38C. Three desi lines, FLIP 85-20C, FLIP 85-29C, and FLIP 85-30C also gave highly resistant reaction. Above FLIP lines are also tolerant to cold.

In an attempt to find new sources of resistance to wilt, 102 accessions of six wild annual *Cicer* species were evaluated in Italy in the greenhouse using a liquid conidial suspension of the fungus. The seeds of each accession, after treatment with sodium hypochlorite, were germinated on moistened perlite in plastic trays. After two weeks, the seedlings were transferred to 200-ml plastic containers having 150 ml of inoculum. The accessions were evaluated after 40 days of inoculation.

No. of lines
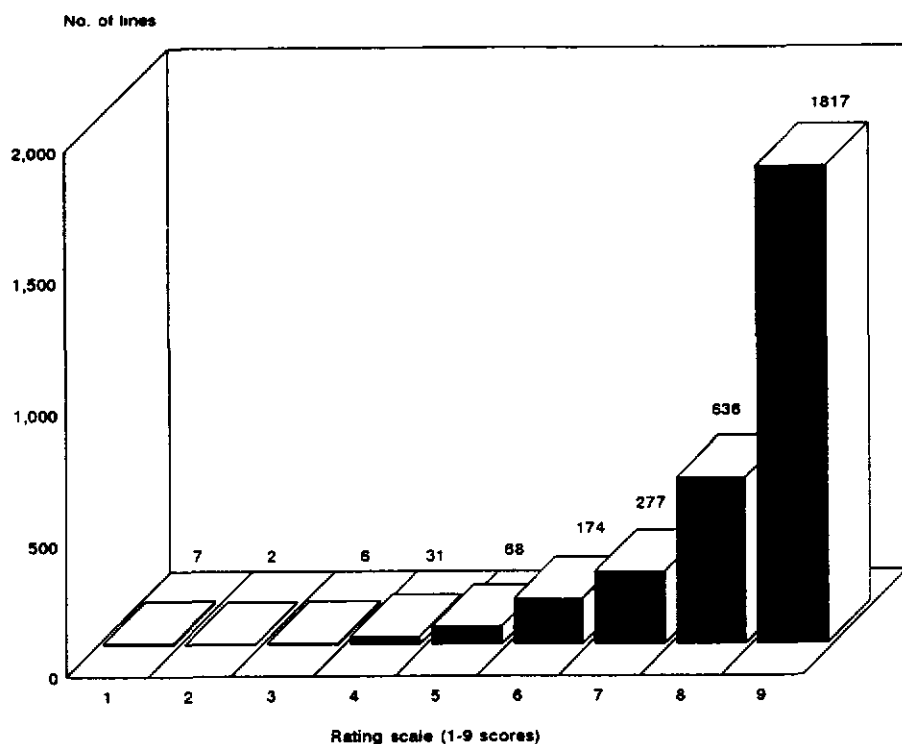
Figure 2.1.1. Screening of chickpeas for fusarium wilt, 1987-1993, at Córdoba, Spain by Universidad de Córdoba and ICARDA.

Highly resistant reaction to wilt was shown by all accessions of *C. bijugum* and some of *C. echinospermum, C. judaicum, C. pinnatifidum* and *C. reticulatum* (Table 2.1.5). This evaluation has helped in identifying new and diverse sources of resistance to wilt for potential use in chickpea breeding. These were ILWC- 64, -71, -73, -76, -80, -83 of *C. bijugum*, ILWC-126, -130 of *C. reticulatum* and ILWC -186 of *C. judaicum*.

Table 2.1.5. Reaction of accessions of wild *Cicer* species to fusarium wilt (seed artificially inoculated in water culture) at the Institute of Plant Pathology, Rome, Italy.

Cicer Spp.	No. of accessions tested	Accessions with score (1-9 scale)*		
		0 %	0-1 %	>1-4 %
C. judaicum	25	4	32	64
C. bijugum	23	26	70	4
C. reticulatum	23	8	22	70
C. pinnatifidum	25	0	20	80
C. echinospermum	4	0	25	75
C. yamashitae	2	0	0	100

* 1= no disease; 9=most severe disease reaction.

The chickpea improvement program in the Legumes Laboratory of INRAT, Tunisia, has as main objective breeding for resistance to ascochyta blight [*Ascochyta rabiei* (Pass.) Lab.] and wilt. Over 4000 germplasm accessions have been evaluated. Many resistant sources were identified and evaluated for yield and other agronomic traits. A high yielding and wilt-resistant line, Amdoun 1, has been released for commercial cultivation in Tunisia. Furthermore, the program has developed a breeding scheme for screening in alternate generations for blight and wilt. High-yielding lines with resistance to blight and wilt are selected. Following this scheme, lines with combined resistance have been developed such as B91-L1/268, B91-L13/268, B91-L1/274 and B91-L1/247. These lines had a 4.5 rating for ascochyta blight on a 1 to 9-point scale (where 1 = free from damage and 9 = all plants killed) and 0% of wilt incidence. They weigh 35-37 g per 100-seed.

Sources of resistance to fusarium wilt which have been developed through these collaborative efforts have

been disseminated through the Chickpea International
Fusarium Wilt Nursery over the past five years to national
programs. Many countries, including Egypt, Ethiopia and
Sudan have confirmed resistance under their conditions.
Some of the resistant lines also have high yield and they
may be released as cultivars.

Whereas no research on fusarium wilt was conducted 15
years ago in WANA, several institutions are now engaged in
research on this important disease. Consequently, many
resistant sources have been identified. Lines with
combined resistance to fusarium wilt and ascochyta blight
in Tunisia, and fusarium wilt and cold at ICARDA have been
developed and a resistant cultivar released in Tunisia.
Speedy progress in solving fusarium wilt problem in WANA
is expected. With the establishment of a wilt-sick plot
in Tel Hadya, major field work will be initiated in the
1994/95 season. **R. Jimenez-Diaz (Spain), H. Halila
(Tunisia), A. Porta-Puglia (Italy) and K.B. Singh
(ICARDA).**

2.1.2.2. Identification of sources of resistance to different stresses in wild species

The most important cause for variation in chickpea yield
is that farmers pay little attention to crop management
and hardly use production inputs - improved cultivars,
fertilizer, and pesticide including weed control. If
chickpea yields are to increase and stabilize, major
attention should be given to resistance breeding. The
number of lines evaluated between 1978 and 1994 for
different resistance/tolerance to major stresses are shown
in Figure 2.1.2. Resistant sources have been identified
for all stresses except to seed beetle (*Callosobruchus
chinensis* L.) and cyst nematode.

No. of lines
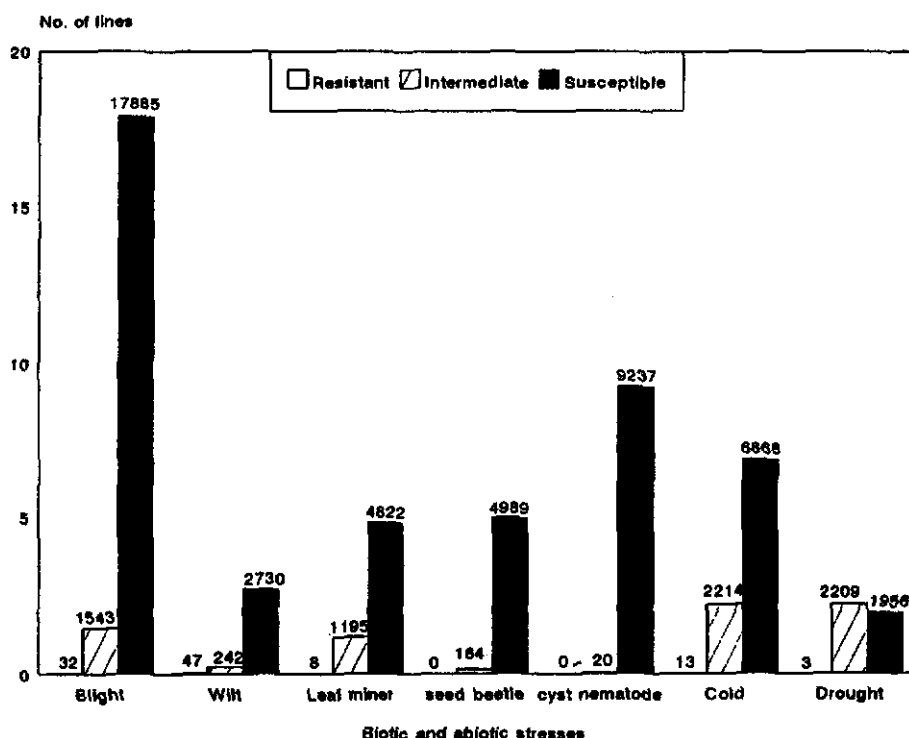
Figure 2.1.2. Reaction of chickpea germplasm accessions to biotic and abiotic stresses at Tel Hadya between 1978 and 1992.

Evaluation of eight annual wild *Cicer* species has been conducted to identify sources of resistance to multiple stresses. The results are summarized in Figure 2.1.3. Sources of resistance were found for all seven stress factors. Wild species were the only source of resistance so far found to seed beetle and cyst nematode and had higher level of resistance than the cultivated species for fusarium wilt, leaf miner, and cold. The most important source for resistance to different stress factors was *C. bijugum*, while *C. yamashitae* was the least important.

No. of lines
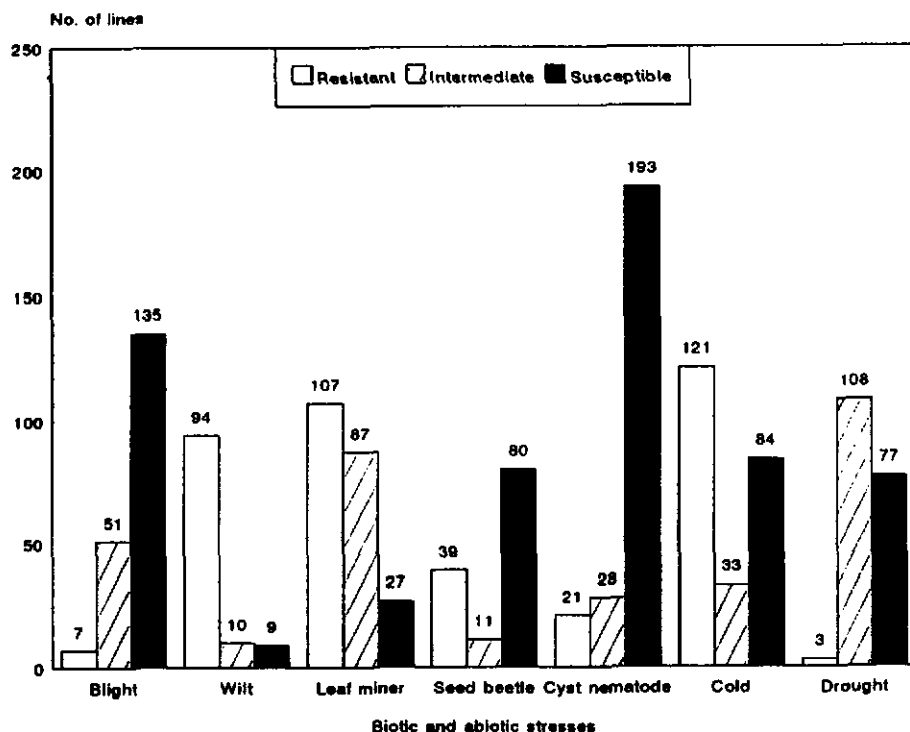
Figure 2.1.3. Reaction of wild *Cicer* accessions to seven stresses at Tel Hadya, 1987-1994.

Sources of resistance to biotic and abiotic stresses in chickpea and *Cicer* species developed at ICARDA and released to researchers are shown in Table 2.1.6. Sources of resistance are available individually to all seven stresses for which research is being conducted. Sources with multiple stress-resistance have been identified in

Cicer species. These sources have been shared with NARS through the distribution in the Legumes International Testing Program. They have been used in crossing programs at ICARDA and many national programs. High yielding lines with combined resistance to ascochyta blight and cold have been bred at ICARDA and shared with the national programs. Research at ICARDA has acted as a catalyst and many national programs are engaged in resistance breeding. **K.B. Singh, S. Weigand, M.C. Saxena, R.S. Malhotra, M. Omar (ICARDA), M.V. Reddy, C. Johansen (ICRISAT, India), A. Porta-Puglia, N. Greco and M. Di Vito (Italy), H. Halila (Tunisia), R. Jimenez-Diaz (Spain).**

Table 2.1.6. Sources of resistance to biotic and abiotic stresses developed at ICARDA, and released for use in breeding programs.

Stresses	Sources of resistance
<u>Single</u>	
Ascochyta blight (AB)	ILC 200, 6482, ICC 4475, 6328, 12004, FLIP 90-98C, 91-2C, 91-18C, 91-22C, 91-24C, 91-46C, 91-50C, 91-54C
Fusarium wilt (FW)	ILC 267, 1278, 1300, FLIP 86-93C, 87-33C, 87-38C
Leaf miner (LM)	ILC 3800, 5901, 7738
Seed beetle (SB)	ILWC 39, 104, 179, 181
Cyst nematode (CN)	ILWC 119
Cold (CO)	ILC 8262, 8617
Drought (DR)	FLIP 87-59C, 88-42C
<u>Multiple stresses</u>	
AB, LM, CO	ILWC 37 (*C. cuneatum*)
FW, LM, SB,CO	ILWC 39 (*C. echinospermum*)
AB, FW, SB, CN, CO	ILWC 62, ILWC 70, ILWC 73 (*C. bijugum*)
FW, LM, SB	ILWC 98, ILWC 102 (*C. judaicum*)
FW, CO	ILWC 141 (*C. reticulatum*)
FW, SB, CO	ILWC 112 (*C. reticulatum*), ILWC 179, ILWC 181 (*C. echinospermum*)
AB, LM, CN	ILWC 250 (*C. pinnatifidum*)

2.1.2.3. Screening for resistance to blight, wilt, and drought during 1993/94

The 1993/94 evaluations included 514 new lines for ascochyta blight resistance, 119 new lines for fusarium wilt, and 1184 lines for drought (Table 2.1.7). In addition 293 promising lines were tested in the leaf miner nursery and 225 lines in the drought nursery for confirmation. Screening against these stresses resulted in identification of three resistant lines each of to leaf miner and drought and one to wilt. **K.B. Singh, S. Weigand, M.C. Saxena, M. Omar, M.T. Mmbaga (ICARDA), R. Jimenez-Diaz (Spain) and C. Johansen (ICRISAT, India).**

Table 2.1.7. Reaction of chickpea germplasm to ascochyta blight, fusarium wilt and drought at Tel Hadya, during 1994.

Rating scale*	Ascochyta blight	Fusarium wilt**	Drought
1	0	0	0
2	0	0	0
3	0	1	0
4	2	0	16
5	35	1	112
6	48	4	269
7	105	8	318
8	97	13	347
9	227	92	122
Total	514	119	1184

*   Rating scale = 1 = no effect of stress; 9 = high damage from stress.
** Screening done at Cordoba, Spain during 1993.

2.1.3. Germplasm Enhancement

2.1.3.1. Seed yield improvement through increased biomass yield

Seed yield is directly and positively correlated with biomass production in chickpea. Low biomass yield is a major limiting factor in farming systems in West Asia and North Africa. Results from yield trials from Tel Hadya (a dry site, 330 mm annual rainfall) and Terbol (a wet site, 575 mm annual rainfall) over the past eleven years have shown that the maximum biomass was only 7 and 9 t ha^{-1}, respectively, at these two locations. It was also observed that the tall genotypes gave higher biomass than the shorter ones. Besides low biomass, harvest index is also low in chickpea. Therefore, to substantially increase seed yield there is a need to increase both biomass and harvest index. To achieve this we initiated a project during 1989 to increase production of chickpea through increased biomass production. The approach used was to cross tall x tall genotypes of diverse origin. Materials were advanced through bulk breeding method. During 1991/92, single plant selections were made. The F_4 and F_5 progenies were grown during 1992/93 and progenies with tall and high biomass yield were selected visually. During 1993/94 a yield trial was conducted with 11 progenies and ILC 3279 as tall and high biomass check entry. The results on yield and other agronomic characters are presented in Table 2.1.8.

In seven out of 11 test entries the biomass yields were significantly higher than the check cultivar. The best line was S 92260 which produced 38% higher biomass and 44% higher seed yield than ILC 3279. It was also 24% taller than the check cultivar and had 120% more secondary branches. These results confirm increased height

Table 2.1.8. Biomass and seed yield (kg/ha) and other agronomic traits of lines developed to increase biomass in chickpea, Tel Hadya, 1993/94.

Entry	PlHt	PrBr	SecBr	Pod/Pl	Sd/Pl	BYLD	SYLD	HI	100SW
S 92217	79	3.8	6.5	24	23	5428	1951	36.0	38.7
S 92218	83	4.4	5.6	22	21	6244	2122	34.1	39.0
S 92249	84	3.3	6.3	24	28	6297	2620	41.6	35.9
S 92260	86	4.3	11.0	61	56	7317	2848	39.1	37.5
S 92307	76	3.6	4.4	27	27	5889	2346	39.7	32.2
S 92310	80	4.0	5.8	32	36	5511	2101	38.3	29.5
S 92312	79	3.1	6.1	27	30	5000	1896	38.0	34.1
S 92440	86	3.9	6.1	33	28	6311	2081	33.4	34.6
F_6 Sel 93TH 34856	85	4.4	11.6	52	54	6361	2102	33.1	30.8
F_6 Sel 93TH 34858	86	3.6	6.8	56	48	6417	2333	36.5	29.0
F_6 Sel 93TH 34840	88	4.1	9.5	54	51	6522	2584	39.6	35.0
ILC 3279 (Check)	70	3.0	5.0	34	31	5328	1969	37.0	25.0
Location mean	81.8	3.9	7.2	37.1	36.1	6052	2246	37.2	33.4
SE of mean	1.57	0.37	1.15	3.7	4.1	293.0	88.0	1.2	0.50
LSD at 0.05	4.51	1.071	3.32	10.8	12.0	843.3	253.3	3.6	1.45
C.V. %	3.8	19.6	32.0	20.2	23.2	9.6	7.8	6.7	3.0

PlHt = plant height (cm); PrBr = number of primary branches per plant; SecBr = number of secondary branches per plant; Pod/Pl = number of pods per plant; Sd/Pl = number of seeds per plant; BYLD = biological yield per hectare; SYLD = seed yield per hectare; HI = harvest index in percentage; 100Sw = 100-seed weight (g).

and secondary branches can contribute to increased biomass and seed yield. **M. Omar and K.B. Singh.**

2.1.3.2. Ascochyta blight

Ascochyta blight is the major disease of chickpea and it has assumed a greater significance with the introduction of winter chickpea in the Mediterranean basin. Without resistance to this disease chickpea cannot be grown during winter. Therefore, the major emphasis in breeding at ICARDA is to develop ascochyta blight-resistant cultivars. When resistant lines were tested across locations and years none of the cultivars were found resistant at all locations indicating the presence of physiologic races. Research at ICARDA identified six races in Syria and 13 races in the Mediterranean basin. Therefore, efforts began to combine genes for resistance to ascochyta blight. Several approaches are being followed.

The principal mechanism is to cross resistance sources of diverse origin (gene pyramiding) assuming that they have different genes for resistance and combine them into a durable resistant lines. This program was initiated in 1989/90 and materials developed by the end of 1993/94 season are shown in Table 2.1.9. During the 1993/94 season five crosses were made, and 436 thirty-six F_2, F_4, F_5, F_6, and F_7 plants were selected. All plants had a rating of 3 on 1-9 scale, where 1 = free from damage and 9 = all plants killed. It is likely that some of them may be escapes, and they will be retested. Materials selected in the project have been passed on to the biotechnology unit for evaluation using molecular-marker technique. **K.B. Singh and M.T. Mbaga.**

Table 2.1.9. Number of progenies/plants selected for ascochyta blight resistance in the gene-pyramiding project at Tel Hadya, 1993/94.

Generation	No. of progenies/bulks in each rating class							No. of plants selected
	1-2[a]	3	4	5	6	7-9	Total	
F_7 progenies	0	8	23	14	9	6	60	22
F_6 progenies	0	82	81	7	0	0	170	96
F_5 progenies (R x R)	0	110	126	51	11	0	298	116
F_4 bulks (R x R)	0	0	2	2	1	0	5	100
F_2 bulks (R x R)	0	1	1	3	0	0	5	102

[a]/ Rating scale: 1 = no visible damage, 9 = all plants killed.

2.1.3.3. Cold tolerance

The materials sown in the cold tolerance nursery at Tel Hadya during 1993/94 included 5 F_2 bulks, 136 F_3 progenies, 4 F_4 bulks, 2 F_5 bulks, 6 F_6 progenies, 2 F_7 progenies, and 25 F_8 progenies. Unfortunately, the season turned out to be warm, hence no screening for cold tolerance was possible.

In some interspecific crosses, we observed plants flowering early. We selected 12 plants which flowered around 2nd of March and harvested them individually. These plants flowered about 3 weeks earlier than normal flowering during spring and if they maintain their early flowering trait, they will be useful in breeding program for lengthening the reproductive phase in chickpea. Another interesting observation was partial sterility in some plants from interspecific crosses. Seventeen plants with 2-3 seeds had been selected. We will study these plants next season for their sterlity behaviour. **K.B. Singh and R.S. Malhotra**.

2.1.4. Development of Improved Germplasm for Wheat-based Farming Systems

2.1.4.1. Segregating generations

During the 1993/94 season, 212 crosses were made and F_1 advanced in the off-season during 1994. F_2 and F_4 bulks were grown in the main season and F_3 bulks again in the off-season (Table 2.1.10). A total of 13,976 progeny rows were grown for winter and spring seasons. A total of 329 promising and uniform F_5 and F_6 progenies were bulked. These bulked lines were purified and multiplied in the off-season for multi-location evaluation. Due to infestation by wilt-root-rot complex, late maturity, and poor growth habit, 80 lines had to be rejected, leaving only 249 for evaluation in the yield trials next season. Ascochyta blight developed in epiphytotic form and effective selection was made. **K.B. Singh**.

Table 2.1.10. Chickpea breeding material grown at Tel Hadya during winter and spring and at Terbol during off-season, 1993/94.

Generation	No. of/bulk progenies	No. of plants selected	No. of bulked progenies
F_0	212 crosses	–	–
F_1	212 crosses	4022	–
F_2 Bulk	160 crosses	225	–
		79*	–
F_3 Bulk	85 crosses	84*	–
F_4 Bulk	188 crosses	9674	–
F_5 Progeny (large)	178 progenies	–	15
F_5 Progeny (early)	2565 progenies	–	110
F_5 Progeny (others)	7401 progenies	55	204
Total:			
$F_2/F_3/F_4$ Bulks	433	13,976	–
F_5 Progenies	10,144	–	329

2.1.4.2. Yield performance of newly bred lines

Three hundred and fifty newly-bred lines were evaluated in 6 preliminary yield trials (PYTs) at three locations (Tel Hadya, Jindiress and Terbol) and in two seasons (winter and spring). Several lines were superior in yield over the checks Ghab 1 in winter and ILC 1929 in spring, although only a few were significantly better yielding (Table 2.1.11). The 1993/94 was a normal season, but the rainfall distribution was poor. However, the yield in winter plantings was higher than previous years, and was lower in spring plantings as compared to previous years. Over the three locations, winter chickpea produced 2093 kg ha^{-1}, giving an increase of 124% over spring planting. **K.B. Singh**.

Table 2.1.11. Performance of newly developed lines during winter and spring plantings at Tel Hadya (6 trials each season), Jindiress (6 trials each season) and Terbol (5 trials each season), 1993/94.

Location and season	No. of entries*			Yield (kg/ha)**		Range for	
	T	> check	Sig. > check	LM	MHY	C.V. (%)	LSD (P≤0.05) (kg/ha)
Tel Hadya							
-Winter	350	137	3	2228	2858	11-19	486-818
-Spring	350	22	12	864	1358	14-33	242-438
Jindiress							
-Winter	350	326	12	1719	2722	19-25	630-846
-Spring	350	143	23	989	1450	20-27	364-464
Terbol							
-Winter	320	39	14	2333	3207	9-13	438-634
-Spring	320	46	4	947	1267	12-18	236-340
Overall							
-Winter	-	-	-	2093	2929	-	-
-Spring	-	-	-	933	1358	-	-

* T = Total; >check = Exceeding check; Sig> check = Significantly excuding check.
** LM = Location mean; MHY = Mean of highest yield.

2.1.4.3. Winter sowing

2.1.4.3.1. Performance of newly bred lines at ICARDA sites

A comparison of spring versus winter sowing has been made over 11 years (1983/84 to 1993/94) at three sites (Tel Hadya, Jindiress and Terbol), using breeding lines (ranging in no. between 72 and 486). The winters of 1984/85, 1988/89, 1989/90, 1991/92 were colder than normal and the springs of 1983/84, 1988/89, 1989/90, 1990/91, and 1992/93 (especially at Tel Hadya) were drier than normal.

The seed yield data in Figure 2.1.4 showed that winter-sown trials on average produced 1712 kg/ha against 1023 kg of spring-sown trials, giving 67% or 689 kg/ha more yield. The yield differences between winter and spring were larger during dry seasons than in normal or wet seasons. During an abnormally cold year (1984/85), yields of winter-sown trials were lower than spring-sown trials. This was, however, no more true starting with the 1988/89 season which was also very cold. This is because of the deliberate selection for cold tolerance since 1984/85.

Breeders usually select the top 10% lines for further evaluation; this 10% top yielders in winter sowing produced 126% or 1295 kg/ha more grain than the mean yield produced in spring over eleven years. Many lines produced more than 4 t/ha seed yield during winter, especially in the favorable environment of Terbol. Obviously, there is a big advantage of winter sowing over spring. **K.B. Singh**.
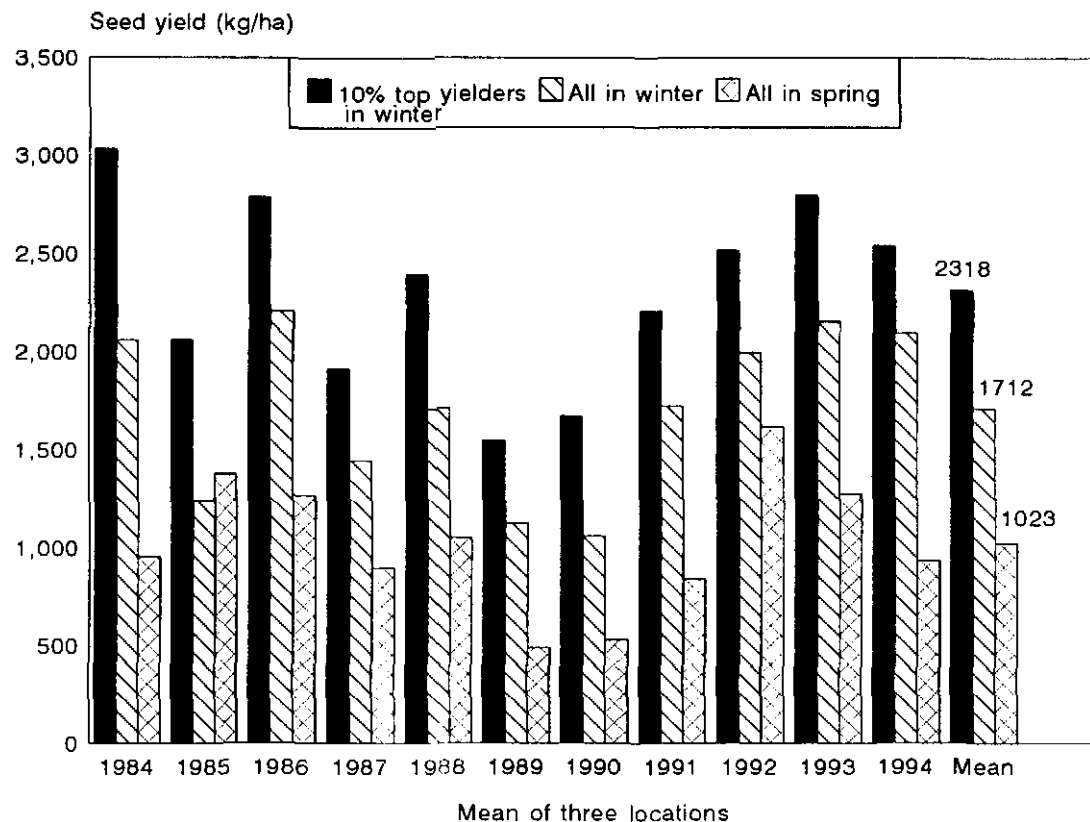
Figure 2.1.4. Mean seed yield (kg ha^{-1}) of chickpea grown in winter and spring at three locations and eleven years.

2.1.5. Strategic Research

2.1.5.1. Development of early mutants with resistance to ascochyta blight or leaf miner

The joint ICARDA/ICRISAT Kabuli Chickpea Project in Syria has bred lines with resistance to six races of ascochyta blight [caused by *Ascochyta rabiei* (Pass.) Lab.], but none of them is early. Thus most of them are unadapted to spring sowing in West Asia and North Africa (WANA). The project has also identified a line, ILC 5901, resistant to leaf miner (*Liriomyza cicerina* Rond.) from an evaluation of over 5500 germplasm accessions. This line is very late maturing and poses problems in utilization. Mutation technique has been used to develop new genetic variability for these traits. Mutagenic studies were initiated during the 1991-92 season to develop early lines with resistance to ascochyta blight and leaf miner.

M_1 **Generation:** Two ascochyta blight-resistant lines (FLIP 84-92C and FLIP 90-73C) and one leaf miner-resistant line (ILC 5901) were chosen for this study. One thousand seeds of each of these lines were exposed to three dosages, 40, 50 and 60 kr of gamma rays. The M_1 generation was sown at Tel Hadya, Syria, during the 1991/92 winter. Germination (after 30 days), survival and sterile plants at maturity were recorded (Table 2.1.12). Germination was reduced with the increasing dosage in all lines, especially in ILC 5901. A drastic reduction in the survival at maturity occurred in all genotypes, especially at 60 kr for FLIP 90-73C and ILC 5901. The percentage of sterile plants was greater with 40 kr gamma rays, as compared to 50 and 60 kr gamma rays. A total of 3292 plants survived and were harvested individually.

Table 2.1.12. Percentage germination, survival at maturity and sterile plants in M_1 populations of three chickpea genotypes irradiated with gamma rays.

Genotype	Gamma rays (Kr)	Ger. (%)	Survival at maturity (%)	% of sterile plants
FLIP 84-92C	0	91.0	90.0	0.0
	40	85.1	49.7	35.4
	50	62.7	49.7	13.0
	60	53.6	43.3	10.3
FLIP 90-73C	0	99.0	96.0	0.0
	40	93.6	49.5	44.1
	50	63.0	45.2	17.8
	60	34.7	16.9	17.8
ILC 5901	0	79.0	77.0	0.0
	40	63.4	46.2	17.2
	50	27.8	21.8	6.0
	60	9.2	6.8	2.4

M_2 Generation: Plant rows of the 3292 surviving plants of the M_1 generation were sown on 20 March 1993. The material was evaluated for earliness to flower in 5 categories: very early (< 36 days), early (36-40 days), medium (41-45 days), late (46-50 days) and very late (>50 days) (Table 2.1.13). This scale is applicable to late spring-sown chickpea. Out of 3292 progenies and 75 parental lines, one progeny was rated very early and two progenies as early. Another 295 progenies were medium and the remaining 2994 progenies were late to very late. Only six early flowering plants were selected from the three very early and early progenies and harvested individually.

Table 2.1.13. Evaluation of M_2 progenies of three chickpea genotypes for earliness to flower at Tel Hadya during spring 1993.

Genotype/ dose	Very	Early early[a]	Medium	Late to very late	Total
FLIP 84-92C					
control	0	0	0	25	25
40 kr	1(1)[b]	0	55	440	497
50 kr	0	0	53	445	498
60 kr	0	0	104	329	433
FLIP 90-73C					
control	0	0	0	25	25
40 kr	0	1(3)	35	460	495
50 kr	0	0	15	437	452
60 kr	0	0	1	168	169
ILC 5901					
control	0	0	0	25	25
40 kr	0	1(2)	14	447	462
50 kr	0	0	15	203	218
60 kr	0	0	3	65	68
Total	1(1)	2(5)	295	3069	3367

[a] Very early = <36 days to flower; early = 36-40 days, medium = 41-45 days; late = 46-50 days; very late = >50 days.
[b]/ Number of individual plants selected is given in paranthesis.

M_3 Progenies: The M_3 generation was sown on 27 March 1994. The evaluation of six progenis is presented in Table 2.1.14. The early maturity of five out of the six was confirmed (Table 2.1.14). None of these segregated for maturity or for other observable characters. The agronomic characteristics of these five progenies (mutants) are given in Table 2.1.15. The mutant from FLIP 84-92C was 12 days earlier in flowering and 15 days earlier in maturity than its parent. The two mutants from FLIP 90-73C were 25 days earlier in flowering and 30 days earlier in maturity.

Likewise, the performance of the two mutants from ILC
5901 was equally impressive, as they flowered and
matured 25 days earlier than their parent ILC 5901.
Despite their earliness, these mutants produced a
higher seed yield than their parents.

Table 2.1.14. Evaluation of M_3 progenies of three
chickpea genotypes for flowering at Tel
Hadya during spring 1994.

Genotype/dose	Very early	Early	Medium
FLIP 84-92C			
40 kr	0	1	0
50 kr	0	0	0
60 kr	0	0	0
FLIP 90-73C			
40 kr	0	2	1
50 kr	0	0	0
60 kr	0	0	0
ILC 5901			
40 kr	0	2	0
50 kr	0	0	0
60 kr	0	0	0
Total	0	5	1

Table 2.1.15. Agronomic traits of the early chickpea
mutants during the spring of 1994.

Entry	DFL[*]	DMAT	Seed yield (g/plant)	100 SW (g)
FLIP 84-92C (control)	65	105	6.3	35.0
FLIP 84-92C-1 (mutant)	53	90	9.3	34.1
FLIP 90-73C (control)	70	105	1.2	33.8
FLIP 90-73C-1 (mutant)	45	75	4.5	32.2
FLIP 90-73C-2 (mutant)	44	75	5.1	29.7
ILC 5901 (control)	68	100	2.1	21.4
ILC 5901-1 (mutant)	42	75	5.0	22.3
ILC 5901-2 (mutant)	40	75	4.6	22.0

[*] DFL = days to flower; DMAT = days to mature; 100 SW
= 100-seed weight.

Conclusions: Mutation work proved effective in generating early mutants in genotypes resistant to ascochyta blight or to leaf miner. Three mutants with resistance to ascochyta blight and early maturity, developed from FLIP 84-92C and FLIP 90-73C, will be useful genetic stocks. So will be the case for the other two mutants with resistance to leaf miner and early maturity, developed from ILC 5901. Such genotypes are not available in the germplasm accessions. A small quantity of seed is available from the Germplasm Program.

2.1.5.2. Mutation study for long pod

In 1991/92 mutation work was initiated to induce genetic variability in chickpea. One thousand seeds of cv ILC 5901 (multipinnate leaf) were irradiated with 40, 50 and 60 kr gamma rays. The M_2 generation was planted at Tel Hadya in 1992/93. Out of 22,500 plants only one plant with a long pod was observed from the 40 kr treated progeny. The character was true breeding in M_3 generation as shown in Table 2.1.16. **M. Omar and K.B. Singh.**

Table 2.1.16. Characteristics of a long pod mutant compared with its parent.

Characteristics	Parent ILC 5901	Mutant ILC 5901-long pod
Plant height (cm)	35	37
Pods per plant (no.)	11.7	16.3
Pod length (cm)	1.7	2.8
100 seed weight (g)	21.4	22.7
Days to flowering	68	62
Days to maturity	100	95

2.1.5.3. Effect of nitrogen on cold tolerance in chickpea

Screening for cold tolerance is essential to develop chickpea cultivars adapted for winter sowing. Therefore, a field screening technique was developed for use at Tel Hadya. A high level of soil nitrogen is known to decrease cold tolerance in peas and winter cereals. The present study was designed to examine whether through increased nitrogen application the discrimination in the chickpea genotypes for their cold susceptibility could be enhanced and thus selection pressure for cold tolerance could be increased.

Twenty genotypes with differential reaction to cold and three levels of nitrogen treatments, 0, 100 and 200 kg N ha^{-1}, were chosen for this experiment which was conducted in pots. The material was sown on October 1, 1990. Three plants were grown in each pot and six pots comprised one treatment. The pots were arranged in a randomized block design with six replications. These pots were placed in the field in the cold tolerance nursery at Tel Hadya. Because of the placement of pots in the furrow, the soil level in the pot was same as in the field. The nitrogen was applied in two split doses, half on 15 November, 1990 and the other half on 15 December 1990. The 1990/91 season had 35 days of below zero temperatures with absolute minimum temperature dropping down to only -6.4°C. Thus, the season was not conducive for field screening for cold tolerance. The rating for cold tolerance was done at the beginning of March 1991 on a 1 to 9 scale, where 1 = free (no damage) and 9 = all plants killed.

The analysis of variance for reaction to cold

revealed that differences between cold reaction due to nitrogen, and genotypes were highly significant. Mean cold reaction of genotypes at different levels of nitrogen is given in Table 2.1.17. A large variation for reaction to cold tolerance in genotypes was observed. It varied from 1.67 to 4.67 with 0 N, 3.33 to 9.00 with 100 N, and 3.67 to 9.00 with 200 N treatments. Although the mean cold susceptibility reaction *increased as level of nitrogen application* increased, the difference between 100 and 200 N was not significant ($P \leq 0.05$). The genotype x nitrogen interaction was significant as well. The difference in the cold susceptibility of tested genotypes was not as large without nitrogen application as with high level of nitrogen. The N application was thus effective in enhancing discrimination amongst genotypes for their reaction to cold. Some genotypes, for example ILWC 151, ILWC 165 and FLIP 86-87C, were least effected by N and remained tolerant to cold at all levels of N application, while others such as ILC 794, ILC 3468 and ILWC 57, showed tolerant reaction to cold with no application of N and highly susceptible reaction with application of N. Perhaps the increased nitrogen supply encourages rate of growth, which accentuates susceptibility to sudden cold.

It is concluded from this study that nitrogen application was effective in enhancing the discrimination amongst genotypes for their reaction to cold and nitrogen application could be used in preliminary field screening of cold susceptibility even in the seasons when the cold is not severe. **R.S. Malhotra, K.B. Singh and M.C. Saxena.**

Table 2.1.17. Cold tolerance reaction (1-9 scale) of chickpea genotypes at different levels of nitrogen application at Tel Hadya, Syria during 1990/91.

Entry Name	Kg N ha^{-1}		
	0	100	200
ILC 668	4.00	5.50	7.50
ILC 794	3.83	7.50	8.50
ILC 1929	4.17	8.33	8.97
ILC 3465	3.00	4.33	4.50
ILC 3468	3.50	8.17	8.67
ILC 3470	3.17	5.67	6.00
ILC 3476	3.33	5.50	8.33
ILC 6001	3.50	7.67	8.83
ILC 8617	2.67	4.00	4.33
ILWC 57	3.67	9.00	9.00
ILWC 76	2.00	3.83	4.00
ILWC 104	2.00	4.00	4.33
ILWC 151	2.67	4.00	4.00
ILWC 165	1.67	3.33	3.67
ILWC 183	2.17	4.00	5.17
FLIP 81-16C	4.00	8.67	8.83
FLIP 81-21C	4.67	8.83	9.00
FLIP 82-114C	4.00	4.50	8.00
FLIP 86-86C	3.67	3.83	4.50
FLIP 86-87C	3.50	4.00	4.00
Mean	3.26	5.73	6.51
Susceptible check	4.67	8.83	9.00
LSD at P≤0.05 for two N means		0.154	
LSD at P≤0.05 for two E means		0.375	
LSD at P≤0.05 for comparisons among N x E interaction means		0.673	

2.1.5.4. Development of early flowering and podding lines under low temperature

When the four cool-season food legumes are sown in late November, faba bean starts flowering in late February while temperatures are still low and occasionally freezing. Field pea and lentil begin

flowering in early March while temperatures are low but no frost occurs. However, chickpeas begin flowering in early April, almost 4-6 weeks later than the other food legumes. Under favourable conditions, both faba bean and pea produce substantially higher seed yield than chickpea perhaps because of the long reproductive phase in faba bean and pea. In an attempt to increase the reproductive period in chickpea, three studies were initiated during 1993/94.

(a) <u>Mutation</u>: Five thousand M_3 plants in each of three treatments (40, 50 and 60 kr of gamma rays) and three lines (ILC 5901, FLIP 84-92C and FLIP 90-73C) totalling 45,000 plants were sown in early December 1993. Observations were recorded on flowering. Forty-six plants flowered in the first week of March 94, about 15-20 days earlier than normal flowering time of winter-sown chickpeas (Table 2.1.18). These plants were selected and plant rows were grown in the off-season at Terbol where 76 early flowering plants were selected for further testing.

(b) <u>Early maturity germplasm</u>: Eight hundred twenty-six early maturity germplasm lines were selected and sown in early December 1993. Thirty-two lines flowered two to three weeks earlier than normal winter chickpea (Table 2.1.18). These were harvested individually in June 1994 and sown in off-season at Terbol. At Terbol, 38 plants flowered very early. These were harvested individually and will be tested again next season.

(c) <u>Intraspecific crosses for early flowering</u>: Sixteen crosses were made between early maturity and cold tolerant lines during 1992/93. The F_2 bulks of these 16 crosses were sown in December 1993. During early March 125 plants were selected that flowered

early and were harvested individually for further testing (Table 2.1.18).

(d) Selection of early flowering plants from interspecific crosses: Twelve plants that flowered on 2nd March were selected and harvested individually (Table 2.1.18).

Table 2.1.18. Materials sown for the development of early flowering lines under low temperature at Tel Hadya, 1993/94.

Material	No. of plants	
	Sown	Harvested
M_3 mutants of 3 chickpea lines, 3 trteatments	45,000	46
Early flowering germplasm	826 lines	119 (from 32 lines)
Intraspecific crosses (16)	16,000	125
Interspecific crosses (5)	5000	12

2.1.6. Interspecific Hybridization

2.1.6.1. Yield improvement

Interspecific hybridization is generally made to understand the phylogenetic relationship among species, to transfer useful genes from the wild to cultivated form and to increase the genetic variability in cultivated species. In recent years, wild species have contributed to the yield improvement in cultigen of various crops. In chickpea yield could be improved by incorporation of genes from wild species.

A 9 x 9 diallel cross was carried out during the 1987/88 season in the field at Tel Hadya. The F_2s of four successful crosses involving the cultigen as one of the parents, i.e. *C. arietinum* (ILC 482) × *C. echinospermum* (ILWC 35), *C. arietinum* (ILC 482) × *C. reticulatum* (ILWC 36) and their reciprocals, were grown in the field at Tel Hadya during 1989/90. From each cross-combination about 10% of F_2 plants phenotypically similar to cultigen were selected. The chosen plants underwent pedigree selection in the subsequent generations. The off-season advancement was carried out at Terbol, Lebanon. Finally, 135 F_5/F_6 uniform and promising progenies were selected during 1992/93 and evaluated for seed yield and other traits during 1993/94. These entries were sown on 5 December 1993 along with parents in preliminary yield trial. The plot size was 2 rows 4 m long. Spacings between and within rows were 45 cm and 10 cm, respectively. The experimental design was incomplete blocks with two replications. Observations were made on 12 characters, namely days to flowering, days to maturity, synchrony in maturity, pod dehiscence, biological yield (kg ha^{-1}), seed yield kg ha^{-1}), harvest index, 100-seed weight (g), plant height (cm), seed color (beige, orange and brown) and seed shape (owl shaped, round and angular). Genstat 5 release 3 program was used for the analysis of the data.

Superior lines to the check in seed yield, plant height and 100-seed weight, are shown in Table 2.1.19. The F_6 material flowered between 102 to 123 days, while the parent cultigen (ILC 482) flowered in 105 days. The differences among the earliest lines and the cultigen were not significant. Lines matured between 155 and 171 days. Few progeny lines matured earlier than the cultigen parent, which matured in 156 days. The plant height ranged from 20 to 59 cm.

Thirty-eight progenies were taller than ILC 482 and nine of them significantly. These were all derived from *C. arietinum* (ILC 482) × *C. echinospermum* (ILWC 35) cross. These taller progenies showed erect and semi-erect growth habit, were uniform in maturity and free of pod dehiscence. Seeds were round mostly of beige color.

The biological yield of the F_6/F_7 progenies ranged from 1262 to 6705 kg ha^{-1}. Twenty-one progenies produced more biological yield than the cultigen parent but only one differed significantly from the cultigen. The F_6/F_7 lines produced 151 to 2802 kg ha^{-1} seed yield. The highest yielding line was derived from *C. reticulatum* (ILWC 36) × *C. arietinum* (ILC 482), while the second and third highest were from *C. arietinum* (ILC 482) × *C. echinospermum* (ILWC 35). They were all uniform in maturity, with no pod dehiscence and showed kabuli type seeds. The harvest index of F_6/F_7 progenies studied varied from 10 to 52%. Although 16 progenies showed harvest index higher than that of the cultivated parent (harvest index 42%), none of them were statistically better.

The 100-seed weight of progenies varied from 13 to 37 grams. Although 35 progenies produced heavier seeds as compared to the cultivated parent (100-seed weight was of 27 grams), only 10 were significantly different. They showed a growth habit ranging from erect to semi-spreading, were mostly uniform in maturity and free from pod dehiscence. Furthermore, their seeds were light colored and round or owl-shaped. The two lines with the heaviest seeds were derived from the cross involving *C. reticulatum* (ILWC 36) as female parent, while the remaining were derived from the cross involving *C. echinospermum* (ILWC 35) as male parent. These three progenies were all free from

Table 2.1.19. Lines from interspecific crosses superior to the check in seed yield, and significantly superior to check in plant height and 100-seed weight.

Line no.	Cross/Prog.	DFL no.	DVA no.	PH cm	BYD kg ha⁻¹	SYD kg ha⁻¹	HI	SW g	Characters evaluated GH	UM	PD	SC	SS
Lines superior to check in seed yield												
53	RA/F7	104	155	43.4	6705	2802	0.42	31.5	erect	1	1	beige	owl
95	AE/F6	105	159	44.1	5645	2720	0.48	19.4	erect	1	1	beige	round
5	AE/F7	102	159	44.6	4961	2494	0.48	26.9	semi-spreading	1	1	orange	round
81	AE/F7	115	159	55.4	5531	2392	0.42	33.2	semi-erect	1	1	beige	round
55	AE/F7	117	160	43.4	5039	2339	0.48	30.9	semi-spreading	1	2	beige	owl
49	AE/F7	113	158	41.5	5361	2306	0.44	24.7	erect	1	1	beige	owl
73	AE/F7	108	158	41.8	4892	2300	0.46	30.5	erect	1	3	brown	angular
82	RA/F7	121	161	37.6	4460	2284	0.50	36.5	semi-erect	1	1	beige	owl
96	AE/F6	117	158	43.3	5395	2265	0.43	n.a.	erect	1	2	beige	round
80	AE/F7	119	165	55.9	5528	2224	0.40	31.3	semi-erect	1	1	beige	round
86	RA/F6	105	156	42.3	4752	2131	0.43	30.2	semi-erect	1	1	beige	owl
18	AE/F7	111	158	39.2	4539	2102	0.48	31.7	semi-spreading	1	1	brown	round
89	AE/F7	112	160	41.3	4705	2061	0.43	30.4	semi-erect	1	1	brown	angular
116	AE/F7	113	163	49.7	4924	2041	0.43	28.9	semi-erect	1	2	orange	round
2	AE/F7	108	158	42.8	5388	2033	0.36	31.8	semi-erect	1	1	orange	round
126	AE/F6	102	161	48.0	4275	1949	0.46	31.8	semi-spreading	1	1	orange	round
41	AE/F7	119	171	46.9	5431	1931	0.41	35.8	semi-spreading	2	1	beige	owl
7	AE/F7	114	161	38.1	3922	1908	0.48	22.4	semi-erect	1	1	brown	angular
50	AE/F7	109	164	44.5	4415	1906	0.44	29.5	semi-erect	2	1	beige	round
63	AE/F7	110	162	46.2	5093	1894	0.36	26.9	erect	1	1	beige	round
Lines significantly superior to check in plant height												
111	AE/F6	118	163	59.1	3659	1595	0.40	32.0	semi-erect	2	1	orange	round
17	AE/F7	118	161	58.7	4644	1637	0.36	24.9	erect	1	1	beige	round
79	AE/F7	109	163	56.2	4199	1516	0.35	31.2	erect	1	1	beige	round
80	AE/F7	119	165	55.9	5528	2224	0.40	31.3	semi-erect	1	1	beige	round
81	AE/F7	115	159	55.4	5531	2392	0.42	33.2	semi-erect	1	1	beige	round

Cont'd.

Line	Cross/Prog.	DFL no.	DMA no.	PH cm	BYD kg ha^{-1}	SYD kg ha^{-1}	HI	SW g	GH	UM	PD	SC	SS
Lines significantly superior to check in plant height													
67	AE/F$_7$	119	165	53.6	4616	1617	0.36	31.7	erect	2	1	orange	round
38	AE/F$_7$	117	168	53.0	3620	1273	0.37	30.2	semi-erect	2	1	brown	round
130	AE/F$_6$	112	161	52.9	4408	1743	0.41	24.5	erect	1	2	beige	round
118	AE/F$_6$	119	167	52.1	3165	1382	0.46	31.1	erect	2	1	beige	round
Lines significantly superior to check in 100-seed weight													
90	RA/F$_6$	110	162	28.0	2691	1329	0.48	36.9	semi-erect	2	1	beige	owl
82	RA/F$_6$	121	158	37.6	4460	2284	0.50	36.5	semi-erect	1	1	beige	owl
41	AE/F$_6$	119	171	46.9	5431	1931	0.41	35.8	semi-spreading	2	1	beige	owl
51	AE/F$_7$	114	161	51.6	3407	1188	0.38	33.85	erect	1	2	beige	round
81	AE/F$_7$	115	159	55.4	5531	2392	0.42	33.2	semi-erect	1	1	beige	round
111	AE/F$_6$	118	163	59.1	3659	1595	0.40	32.0	semi-erect	2	1	orange	round
126	AE/F$_6$	102	161	48.0	4275	1949	0.46	31.8	semi-spreading	1	1	orange	round
2	AE/F$_7$	108	158	42.8	5388	2033	0.36	31.8	semi-erect	1	1	orange	round
18	AE/F$_7$	111	158	39.2	4539	2102	0.48	31.7	semi-spreading	1	1	brown	round
67	AE/F$_7$	119	165	53.6	4616	1614	0.36	31.7	erect	2	1	orange	round
Parents													
A		105	156	41.8	4559	1882	0.44	27.2	erect	1	1	beige	owl
R		119	158	23.4	1307	202	0.16	11.6	spreading	2	2	brown	angular
E		121	158	11.1	758	126	0.12	11.7	spreading	2	2	brown	owl
S.E.		1.89	1.98	5.13	563.1	262.7	0.04	2.152					
C.V.		1.48	1.14	11.99	13.60	17.70	11.64	7.52					

A = *C. arietinum* (ILC 482), R = *C. reticulatum* (ILWC 36), E = *C. echinospermum* (ILWC 35), AE = *C. arietinum* (ILC 482) × *C. echinospermum* (ILWC 35) and AR = *C. arietinum* (ILC 482) × *C. reticulatum* (ILWC 36). DFL = days to flowering, DMA = days to maturity, PH = plant height, BYD = biological yield, SYD = seed yield, HI = harvest index, SW = 100-seed weight, GH = growth habit, UM = uniformity in maturity (1 = uniform and 2 = non-uniform), PD = pod dehiscence (1 = non-dehiscent and 2 = dehiscent), SC = seed color and SS = seed shape n.a. = datum not available, s.e.d. = standard error of differences, C.V. = coefficient of variation.

pod dehiscence and had kabuli type seeds.

In conclusion, the results of the 9 × 9 diallel cross have shown that among the eight annual wild *Cicer* species only *C. reticulatum* and *C. echinospermum* are readily crossable with the cultigen. Although sterility and semi-sterility were observed in cross-combinations involving *C. echinospermum*, this wild species showed great potential in increasing the variability of the cultigen, producing transgressive recombinants for traits of agronomic importance such as seed yield, 100-seed weight, plant height and biological yield. Selection carried out in F_2 generation for plants phenotypically similar to the cultigen, has been effective in generating lines with better agronomic attributes compared with those of the cultigen. It is concluded from this study that seed yield in chickpea can be increased by introgression of genes from wild *Cicer* species.

2.1.6.2. Cyst nematode

No sources of resistance to cyst nematode were found in 10,000 accessions of cultivated species. But when wild *Cicer* species accessions were evaluated 22 accessions of *C. bijugum*, five of *C. pinnatifidum* and one of *C. reticulatum* were found resistant. Since cyst nematode is a serious problem in chickpea growing areas in WANA and no sources of resistance were found in the cultigen, crosses were made during 1990/91 to transfer resistance genes from the wild *Cicer* species. The materials evaluated during 1993/94 included 1166 F_2 plants, 223 F_4 plants and 3052 F_5 plants from different crosses (Table 2.1.20) involving cyst nematode resistant *C. reticulatum* accession ILWC 119 as one of the parents. Eight plants were identified

with no nematode infection (0 rating) and 62 plants with low infection (1 rating on 0-5 scale). After the examination of roots these selected plants were transplanted to let them grow to maturity to produce seeds. Several selected plants produced no seeds when transplanted. Of the remaining, many had undesirable seed characters and were rejected. Rusts were advanced in the off season nursery. Five new crosses were also made. **K.B. Singh, M. Di Vito, N. Greco and M.C. Saxena.**

2.1.7. Protein Quality

It is our endeavor to develop cultivars with the same or higher protein content as the check cultivar. To meet this objective we evaluate newly developed lines for protein content. During 1993/94, 350 newly developed lines were grown at Tel Hadya, Jindiress and Terbol in winter and spring seasons. Since earlier studies had indicated no significant effect of seasons on protein content, only newly developed lines grown at Tel Hadya during winter were tested. The protein contents of the newly developed lines were at par with the check cultivar. **K.B. Singh.**

Table 2.1.20. Reaction of plants from interspecific crosses in F_2, F_4, and F_5 generations to cyst nematode in the greenhouse at Tel Hadya, 1993/94.

Generation	Cross no.	Parents	Scale[1]						Total
			0	1	2	3	4	5	
F_2	X 93TH161	ILWC 119 X FLIP 84-15C	0	6	27	59	95	31	218
	X 93TH162	ILWC 119 X FLIP 85-5C	0	7	30	82	364	135	718
	X 93TH163	ILWC 119 X FLIP 88-85C	3	9	15	49	116	38	230
	Total		3	22	72	190	575		2041166
F_4	X 91TH214	ILC 846 X ILWC 119	2	5	14	47	0	0	68
	X 91TH215	ILC 863 X ILWC 119	0	5	14	65	8	0	92
	X 91TH318	FLIP 84-92C X ILWC 119	0	6	7	49	1	0	63
	Total		2	16	35	161	9		0223
F_5	X 90TH571	ILC 482 X ILWC 119	2	19	79	1624	501	110	2335
	X 90TH572	FLIP 87-69C X ILWC 119	1	5	23	457	190	41	717
	Total		3	24	102	2081	691		1513052

1/ Scale 0 = no cyst formation on roots, 5 = $\geq$ 50 cysts on roots.

2.2. Molecular Techniques and Tissue Culture in Chickpea

2.2.1. Study on Biodiversity in Chickpea using DNA Markers

2.2.1.1. DNA fingerprinting and RAPD analysis of 83 *Cicer arietinum* accessions

Conventionally diversity is estimated based on morphological and physiological traits. These traits are highly influenced by environment making precise diversity estimation difficult and unreliable. Isozyme analysis has been suggested as an alternative method for estimation of biodiversity and germplasm evaluation. Since isozymes are limited in number and are not polymorphic in chickpea, their usefulness is limited. Recently, DNA markers have been used for biodiversity studies and germplasm evaluation in many crop species. Since DNA markers detects variation directly at DNA level, they are not influenced by the environment and they are unlimited in number, making them highly useful for their application in chickpea improvement.

To study the genetic diversity within kabuli chickpeas, 83 accessions collected from 26 different countries and carrying a wide range of variability for agronomic traits, were selected from the germplasm bank. Equal quantity of leaf sample from 10 individual plants of each accession was pooled and DNA was extracted by following CTAB procedure. The pooling of the leaf samples was done to detect the predominant alleles within an accession. The extracted DNA was used for studying genetic variation using RFLP (DNA fingerprinting) and PCR (RAPDs). RFLP analysis using the restriction enzyme *EcoRI* and the probe (GATA)$_4$ revealed high degree of polymorphism (Figure 2.2.1) whereas RAPDs did not reveal any polymorphism (Figure 2.2.2). Therefore only the results

of RFLP analysis was further analyzed using the computer program NTSYS-pc to calculate genetic distance (Neis' genetic distance) and to perform cluster analysis (Figure 2.2.3).

The genetic distance (dissimilarity index) ranged from 0.00865 (between ILC 5587 and ILC 5588) to 0.09521 (ILC 3401 and ILC 200) indicating existence of a high degree of genetic diversity among the accessions analyzed. The cluster analysis classified 83 accessions into 83 distinct genotypes, indicating that no duplicates existed in the analyzed germplasm collection. Most of the accessions, even from the same country of origin, clustered separately except two accessions each from France (ILC 5587 and ILC 5588), Egypt (ILC 546 and ILC 560), Pakistan (ILC 5649 and ILC 5652), Palaestine (ILC 2299 and ILC 2300), Mexico (ILC 3715 and ILC 3743), USA (ILC 2514 and ILC 2517) and the former USSR (ILC 200 and ILC 2665) which clustered together. The results of the present study could be useful in deciding the cross combinations in chickpea breeding programs and also for targeting future germplasm collection missions. **F. Weigand, S.M. Udupa and L.D. Robertson.**

2.2.1.2. Microsatellite-primed polymerase chain reaction (MP-PCR) analysis of *Cicer* species

Oligonucleotide motifs complementary to microsatellite sequences (e.g. $(GACA)_4$) can be used as single PCR primers to analyze the length variability of inter-repeat regions in the chickpea genome. In order to analyze intra-as well as interspecific variability, single plant DNAs of ILC 482, ILC 1272, ILC 3279 and ILC 1929 representing the cultivated chickpea, ILWC 123, ILWC 137 representing *C. reticulatum* and ILWC 179 representing *C. echinospermum* were selected and analyzed using $(GCGT)_4$ and $(GACA)_4$ as
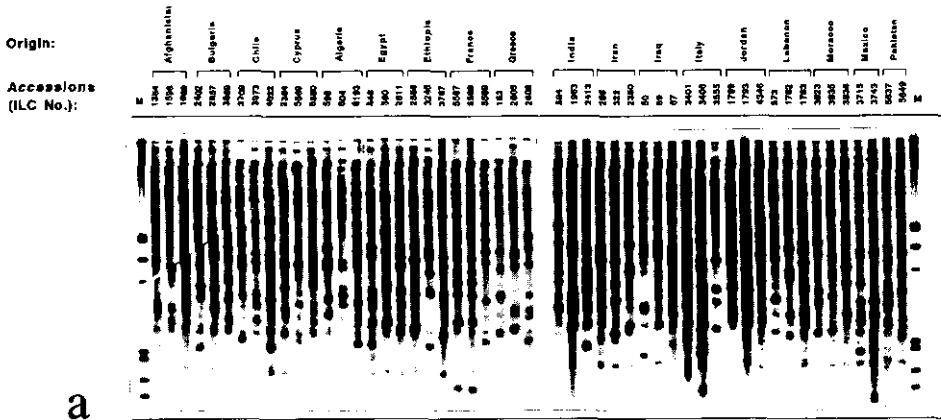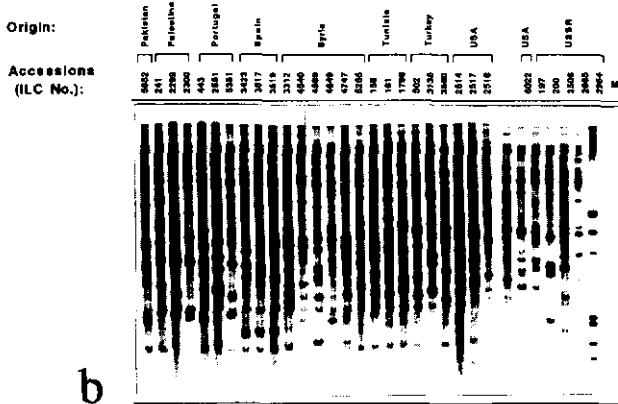
Figure 2.2.1a and b. RFLP analysis of 83 accessions of kabuli chickpea using the enzyme EcoRI and probe (GATA)$_4$.

primers. A single primer typically generated 4-14 detectable bands. The inter- and intraspecific polymorphism was modest and low, respectively (Figure 2.2.4). In conclusion, some microsatellite motifs can be used for the evaluation of genetic diversity between *Cicer* species. **F. Weigand (ICARDA), B. Hüttel and G. Kahl (Franfurt University, Germany).**

21 Different Accessions of Chickpea

Figure 2.2.2. RAPD analysis of 21 accessions of kabuli chickpea using the primer OPI-10.
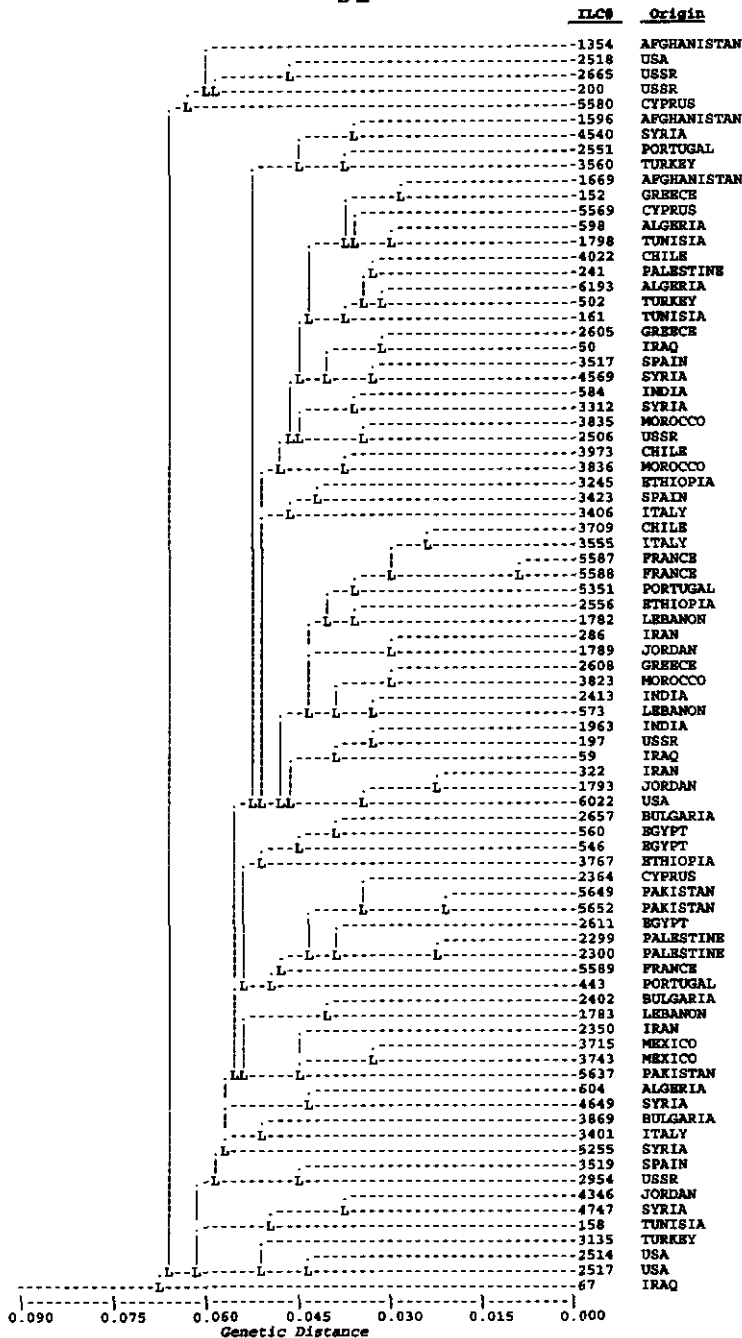
Figure 2.2.3. Dendrogram of 83 accessions of kabuli chickpea based on the UPGMA method using the Neis' genetic distance.
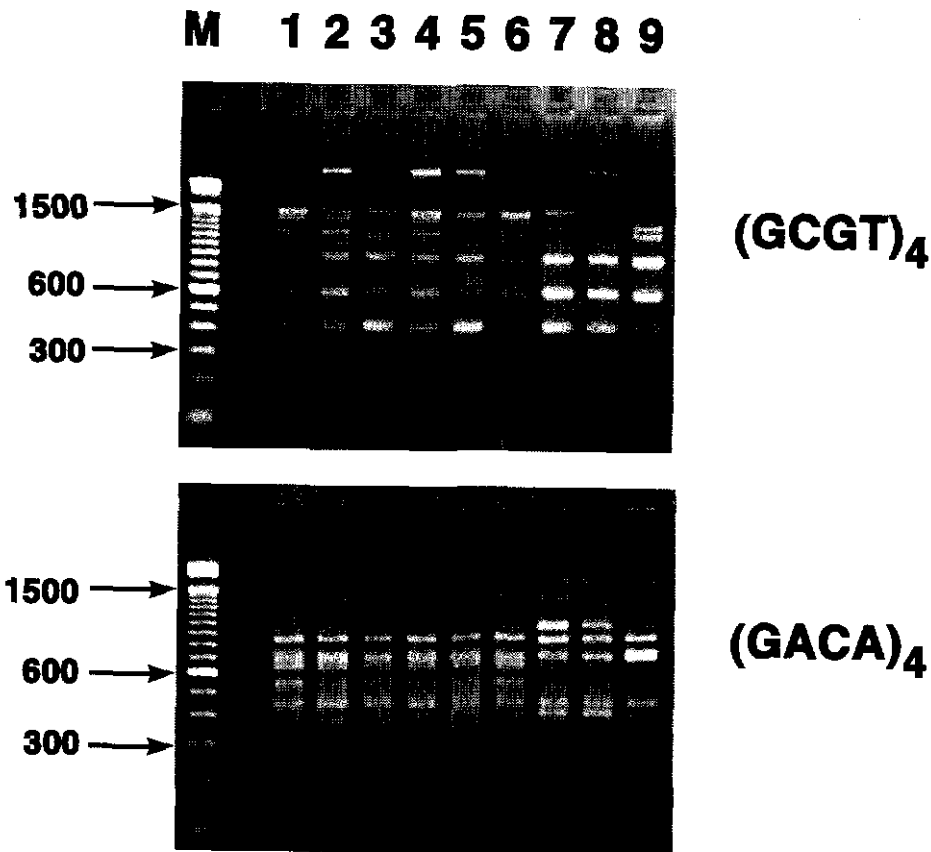
Figure 2.2.4. Microsatellite-primed polymerase chain reaction (MP-PCR) analysis of *Cicer* species using $(GCGT)_4$ (upper panel) and $(GACA)_4$ (lower panel). The amplified DNAs of single plants of ILC 482 (lane 1), ILC 1272 (lanes 2 and 3), ILC 3279 (lanes 4 and 5), ILC 1929 (lane 6), ILWC 123 (lane 7) ILWC 137 (lane 8, both representing *C. reticulatum*) and ILWC 179 (lane 9, *C. echinospermum*) were separated by agarose gel electrophoresis and visualized by ethidium bromide staining. Molecular weights in bp are indicated by arrows.

2.2.2. Generation of Sequence-tagged Microsatellite Site Markers

Application of microsatellites in a RFLP (DNA fingerprinting) approach revealed considerable intra- as well as interspecific polymorphism in *Cicer* species. These polymorphisms has been exploited in genetic diversity and linkage studies. Application of DNA fingerprinting might be limited, especially in linkage studies, because it is a dominant marker system, detecting too many undistinguishable loci at the same time, and a limited number of informative oligonucleotides are available. In order to increase the number and the level of informativeness of microsatelitte loci the ICARDA/University of Frankfurt collaborative research was focused on the generation of sequence-tagged microsatellite site markers (STMS). The STMS markers are codominant and allow the discrimination between homozygous and heterozygous individuals in breeding populations. This marker system includes the advantage of PCR, e.g. it is an automatized procedure and requires only low amounts of DNA. It is, therefore, useful for application in plant breeding.

For the generation of STMS marker the following steps are involved:
(i)   cloning of chickpea DNA in *E. coli*;
(ii)   screening of DNA libraries with repetitive oligonucleotides;
(iii)   isolation of repeat-containing fragments and
(iv)   sequencing of repeat-containing fragments.

Suitable STMS primers are constructed according to the sequencing information of the repeat flanking unique parts of the fragments.

Within the collaborative research, size-selected (250-600bp) genomic DNA libraries were constructed. The libraries consist of approximately 30,000 recombinant clones which cover nearly 1.5% of the chickpea genome. These clones were hybridized to a variety of repetitive probes [e.g. $(GA)_8$, $(GT)_8$, $(TAA)_5$] and 270 microsatellite-containing clones have been isolated. Some of the clones were sequenced and primer pairs flanking repetitive sequences were synthesized. The results of amplification of chickpea DNA using two primer pairs are given in Figure 2.2.5. STMS-mediated polymorphisms can only be scored if
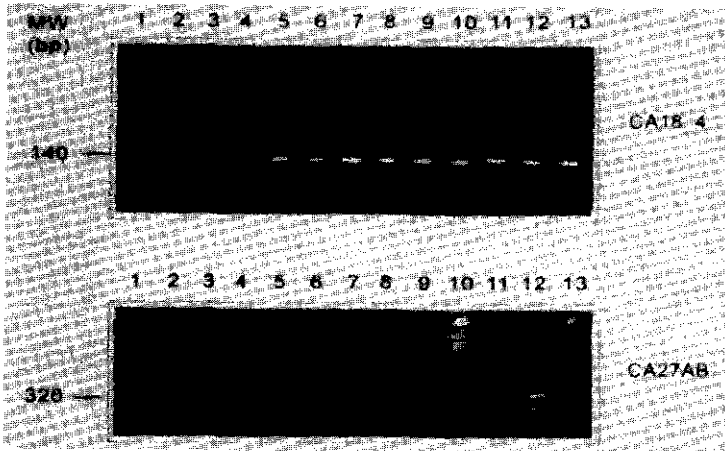
Figure 2.2.5. Evaluation of polymorphism in chickpea using sequence tagged microsatellite site markers. Individual chickpea DNA (1: ILC 482, 2: ILC 1272, 3 and 4: ILC 3279, 5: ILC 190, 6: ILC 191, 7: ILC 200, 8: ILC 202, 9: ILC 4978, 10: ILC 5513, 11: ILC 6298, 12: ILWC 123, 13: ILWC 179) was analyzed by PCR using primer pairs flanking repeat containing fragments. The amplification products were separated on 4% NuSieve agarose gels, stained with ethidium bromide and photographed under UV light.

small electrophoretic mobility differences are detectable. Such a difference, for example is detectable between the amplified fragments of ILC 5513 and ILC 6298 (upper part of Figure 2.2.5). **B. Hüttel, P. Winter, G. Kahl (Frankfurt University, Germany), S.M. Udupa and F. Weigand (ICARDA).**

2.2.3. Genetic Characterization of *Ascochyta rabiei*

Polymerase Chain Reaction based techniques (RAPDs and MPs) and RFLP were used for genetic characterization of *A. rabiei* isolates of Syria and Tunisia, to help in describing an efficient control of ascochyta blight disease of chickpea.

2.2.3.1. Genetic diversity, genetic structure and distribution of *A. rabiei* isolates of Syria as revealed by Random Amplified Polymorphic DNA (RAPD) analysis.

RAPD analysis was performed to: (a) study genetic diversity, genetic structure and distribution; (b) monitor migration, mutations and evolution in the pathogen population; and (c) characterize geneticaly different pathotypes prevailing in chickpea growing areas of Syria. Fifty three isolates collected during 1982, 1991, 1992 and 1993 (see LP Annual Report, 1993, pp: 50-54 for details regarding place and years of collection of the isolates) were subjected to RAPD analysis using 10 base primers of arbitrary sequence. The result of the 10 primers which amplified polymorphic fragments were analyzed for genetic distance and phylogeny using a computer program NTSYS-pc.
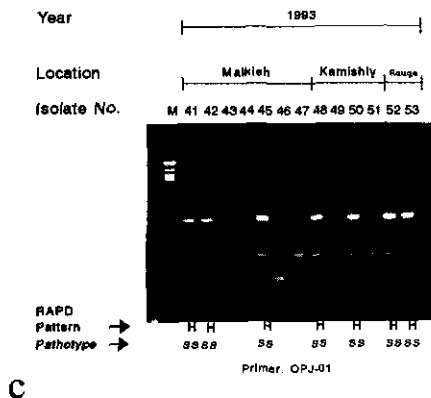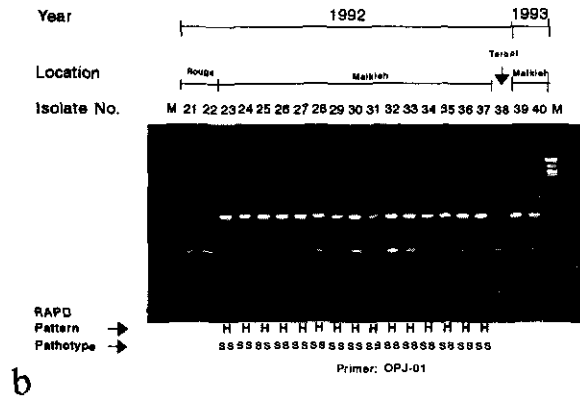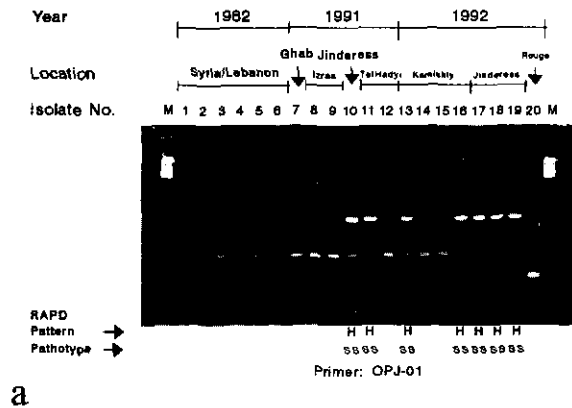
Figure 2.2.6a,b,c. Random Amplified Polymorphic DNA analysis of *Ascochyta rabiei* isolates from Syria using the primer OPJ-01. Lane M contains molecular weight marker (lambda *Eco*RI digested DNA).

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Genotype	Pathotype	Isolate #
A	W	1
B	W	2
C	W	3 & 12
U	W	51
E	W	5
J	W	14 & 15
M	W	22
Q	SS	43
I	S	9
G	SS	8
S	S	46 & 47
O	W	38
K	W	20
L	W	21
F	SS	7
H	SS	
N	SS	34
P	SS	42
R	SS	44
T	W	49
D	S	4 & 6

10,11,13, 16-19, 23-33,35-37, 39-41,45,48, 50,52 & 53

0.072 0.060 0.048 0.036 0.024 0.012 0.000
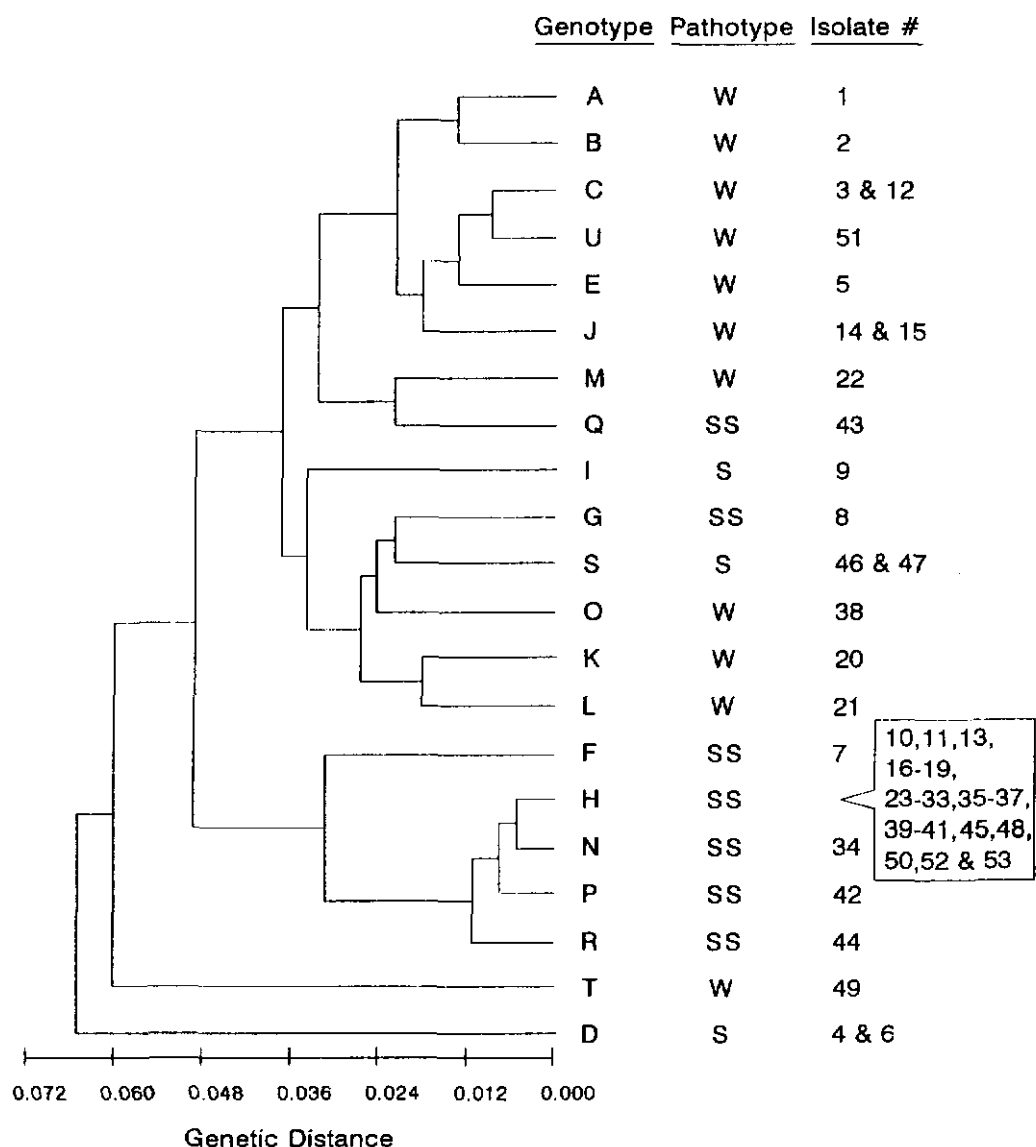
Genetic Distance

Figure 2.2.7. Dendrogram of 53 isolates of *Ascochyta rabiei* of Syria based on the UPGMA method using the Neis' genetic distances. The Neis' genetic distances were calculated based on RAPD pattern.

The isolates could be grouped into 21 genotypes, A to U (Figures 2.2.6 and 2.2.7). The genetic distance ranged from 0.00425 (between genotypes H and N) to 0.07371 (between genotypes D and R, D and T). The genetic group H was predominant in all the chickpea growing regions. This group consists of isolates belonging to pathotype III (super strong) and were frequently sampled in blight epidemic areas of north - east of Syria.

The results indicated that there is a considerable degree of genetic variation in *A. rabiei* within and between chickpea growing regions and over the years (Table 2.2.1). Genetic diversity as indicated by diversity index is decreasing in the year 1992 and 1993 as compared to 1982 and 1991. Within the different pathotypes, genetic diversity was the highest in pathotype I (weak, diversity index 0.90) followed by pathotype II (strong, diversity index 0.64) and pathotype III (super strong, diversity index 0.39) was the lowest. Based on these results we conclude that **adaptive mutations**[*] in the pathogen and its selection over the generations could help the pathogen to break resistance in the released cultivars. On the other hand, the existence of pathotype III for many years at a low frequency and therefore not being detected during previous surveys, can not be excluded. Occurrence of a single genotype H over a large area indicates a founder effect. It reflects the preference of the pathogen for asexual mode of reproduction under natural conditions.

Some of the primers which did not reveal polymorphism within *A. rabiei* were used as an additional test, to confirm the identity of *A. rabiei* (to avoid confusion about pathogens causing similar disease symptoms, such as *Phoma*) and to test purity of

the culture used for pathogenicity tests. Figure 2.2.8 shows one such example with primer OPI-10. The A. rabiei isolates showed distinct monomorphic pattern which was different from A. fabae f.sp.lentis indicating the identity and purity of the cultures used for pathotyping and genotyping.

* Adaptive mutation is a process that appears to produces useful mutations only in the presence of selection for those mutations and in the absence of cell growth (Science, vol. 264, pp: 224-225 and 258-260, 1994). **F. Weigand and S.M. Udupa**.

Table 2.2.1. Genetic characterization of Ascochyta rabiei isolates of Syria.

Year collected	Genotypic groups			Diver. index
	Pathotype I	Pathotype II	Pathotype III	
1982	A(1),B(1) C(1),E(1)	D (2)	-	0.77
1990	C(1),	I(1)	F(1),G(1) H(2)	0.77
1992	J(2),K(1) L(1),M(1) O(1)		H(19),N(1)	0.41
1993	T(1),U(1)	S(2)	H(8),P(1) Q(1),R(1)	0.67
Total #				
Geno.	11	3	7	
Isol.	13	5	35	
Diver. index	0.90	0.64	0.39	

[a] The number in parenthesis indicates number of isolates belonging to the genotype.

A.rabiei isolates (#41-53) A.f.lentis

M
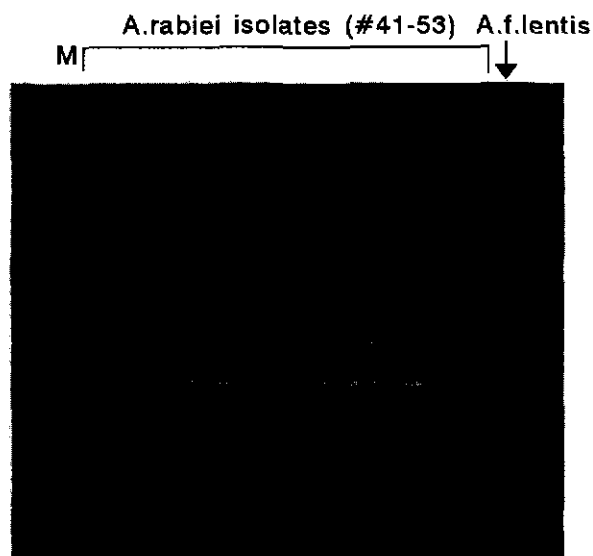
Figure 2.2.8. Random Amplified Polymorphic DNA analysis of *Ascochyta rabiei* isolates (41-53) and *Ascochyta fabae* f.s.*lentis*. Lane M contains molecular weight marker (lambda *Eco*RI-*Hind*III digested DNA).

2.2.3.2. Distribution of *A. rabiei* genotypes in Tunisia

About 400 *A. rabiei* isolates, hierarchically sampled all over Tunisia, have been radioactively finger printed and 34 different fingerprint genotypes detected amongst them. An *A. rabiei* distribution map has been completed for the Beja region, the main chickpea growing area of Tunisia. Around 150 isolates

were tested from this particular region and 17
distinct genotypes were detected and compared to four
distinct genotypes from Syria using band sharing data
in UPGMA cluster analysis (Figure 2.2.9). The
analysis was based on the presence or absence of bands
derived from *Hinf*I-digested DNA and the probes (CA)$_8$,
(CAA)$_5$, (CAG)$_5$ and (GATA)$_4$. **F. Weigand (ICARDA), H.
Morjane, M. Harrabi (INAT, Tunis, Tunisia), J.
Geistlinger, G. Kahl (Frankfurt University, Germany).**

Figure 2.2.9. Dendrogram based on UPGMA cluster
analysis of fingerprints of 21
Ascochyta rabiei genotypes and one
genotype of *Ascochyta fabae*. Seventeen
genotypes were collected in Tunisia
(TAr1 to TAr17) and 4 were collected in
Syria (A, B, C and D).

2.2.3.3. Microsatellite-primed PCR analysis of A. rabiei

Microsatellite-primed PCR (MP-PCR) has been applied to
A. rabiei. The primer with the highest degree of
informativeness found so far is (GAA)$_5$. This primer
can distinguish between eight predominant genotypes
from Beja (Tunisia) and one genotype (isolate no. 6)
from Syria (Figure 2.2.10). An amplification fragment
produced from (GGGTTT)$_3$ as primer was isolated and
cloned into a sequencing vector. The isolated locus
contained a sequence, which represents a cluster of
many different microsatellite motifs. By using this
sequence as a probe in Southern hybridization a RFLP
with a high degree of polymorphism was displayed
(Figure 2.2.11). **F. Weigand (ICARDA), J. Geistlinger,
G. Kahl (Frankfurt University, Germany).**

2322 bp
2027 bp

564 bp

M a b c d e f g h j

a = Syrian isolate 6

b-j = Tunisian isolates of Beja

M = Hind III-digested lambda-DNA
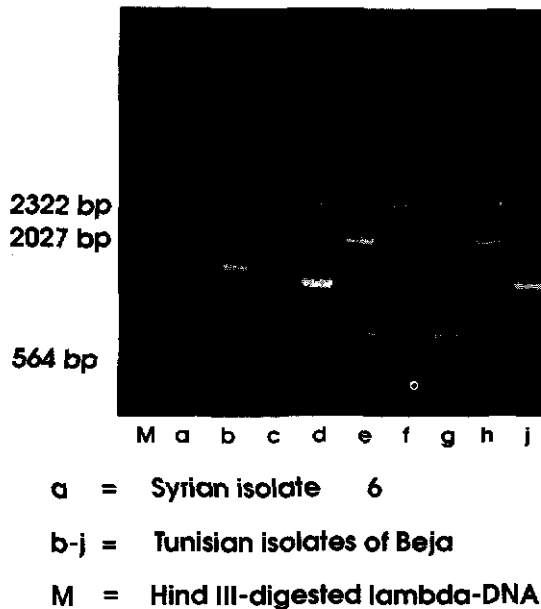
Figure 2.2.10. Microsatellite primed PCR analysis of
Ascochyta rabiei using (GAA)$_5$ as
primer.

564 bp -

a b 1....... 34

a, b = Syrian isolates   2 and   6

1 - 34 = Tunisian isolates
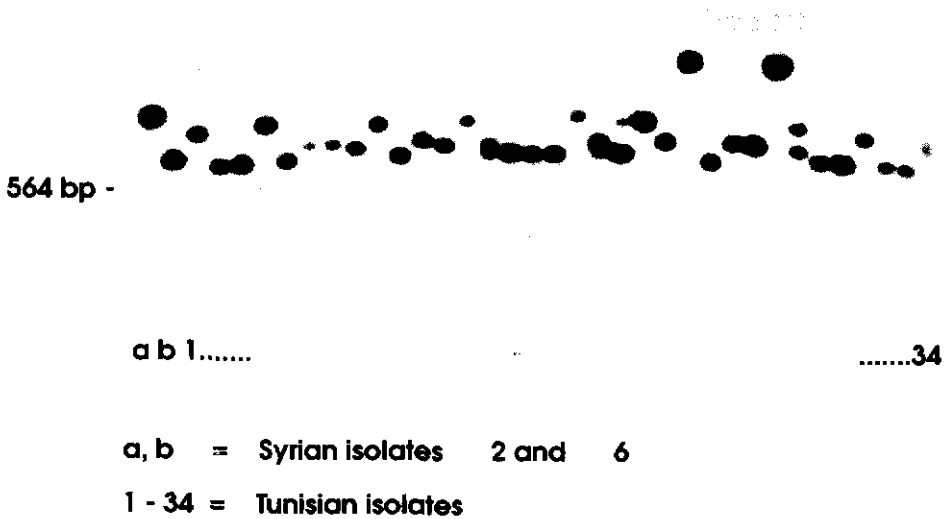
Figure 2.2.11. Use of a cloned (GGGTTT)$_3$ -flanked
sequence as an RFLP probe in *Ascochyta
rabiei*.

2.2.3.4. Isolation of aggressiveness-related genes

Differential display of mRNA has been applied to *A.
rabiei*. The technique is based on PCR amplification
of cDNAs derived from mRNA populations of different
pathotypes.   Differences between the pathotype
populations can be displayed by a silver stained
polyacrylamide gel electrophoresis. Plant-sap media
were prepared from chickpea or wheat leaves and spores
of *A. rabiei* a pathotype III (Syrian isolate no. 13)

grown in the different media for 12h. Subsequently mRNA was isolated. After PCR amplification of the corresponding cDNAs the results were displayed on the gel. Around 20 chickpea induced bands, which were not present in the wheat medium, have been isolated from the gel and reamplified to be tested in Northern analysis to confirm their induction. Positive bands will be cloned and characterized to understand aggressiveness in *A. rabiei*. **F. Weigand, J. Geistlinger, G. Kahl (Frankfurt University, Germany), S. Hamze (INAT, Tunis, Tunisia).**

2.2.4. Wide Crosses in Chickpea

The wild annual *Cicer* species carry genes conferring biotic and abiotic stress resistance. Some of these species carry: (a) resistance genes which cannot be found in the cultivated Cicer, (b) multiple stress resistance, and (c) resistance of higher level compared to the cultivated species. Plant breeders would like to make use of these sources of resistance. Unfortuantely, sexual hybrids have so far been reported only between *C. arietinum* and *C. reticulatum* or *C. echinospermum*. In order to test crosscompatibility and existance of pre- or postfertilization barriers crosses between cultivated and wild annual chickpeas were initiated. *Cicer arietinum* lines used included: ILC 482, FLIP 84-15C, ILC 200 (Russia kabuli), ILC 519 (Egypt kabuli), ILC 5359 (Mexico kabuli), ILC 6328 (India desi), ILC 4475 (Iran desi), and Amdoun-1 (Tunisia kabuli). The wild species included: *C. bijugum* ILWC -32, -62; *C. judaicum* ILWC -46, -95; and *C. pinnatifidum* ILWC -236, -171.

Control experiments were first carried out with

selfed flowers to determine the best *in vitro* protocol and best media composition. Subsequently, crosses between the above mentioned 8 cultivar lines and two lines each of the three wild species were initiated. Selfed as well as crosspollinated flowers were handled as follows:

1. The crosses were made with flowers in a stage when the sepals were turning from green to white/yellow. The anthers were taken out, preferably all at once, with extremely care. Pollen from a flower of the wild species (sepals were coloured but the flowers were not yet completely open) were transferred on the pistil. A tag or identification was fixed on one of the lower branches. A drop of hormone solution, containing 8mg/1 gibberilic acid (GA3), 1mg/1 kinetin (KIN) and 1mg/1 napthalene acitic acid (NAA) was applied to the base of the pistil. This was repeated the following two days to enhance pollen tube growth.

2. Up to 10 days there after the flowers could be collected and sterilized (30 minutes in 10% Zephir, a Syrian commercial desinfectant, containing quartemary ammonium salts). They were then rinsed with sterilized water. If necessary petals and sepals were removed with sterilized forceps to leave small pods for further handling.

3. Flowers or small pods were transferred onto pod growth medium (D4B). Flowers or small pods developed further within 1 to 2 weeks. Big pods (>10 mm) were dissected with sterilized forceps on sterilized filter paper. The ovules were transferred onto induction medium D3E.

4. Calli, formed on D3E medium, were transferred to

regeneration medium (+ 1mg/IAA).

5. Developed plantlets were transferred to tubes containing MS medium + 1mg/1IAA + 1mg/1BAP.

Above stages of development occurred in selfed plants. Induced calli from selfed plants showed morphogenesis after a period of about two month. Interspecific crosspollinated flowers also showed the same development to small pods. They grew bigger on induction medium. Applying drops of hormones prolonged the time that pods would stay on the plants and permitted transfer of ovules straight onto D3E. A high percentage of ovaries and later ovules were infected with bacteria. Too stringent sterilization killed the ovary, a less stringent sterilization created contamination problems. Successfully developed ovules had to be transferred to fresh medium every few weeks to keep them alive. The obtained ovules of various cross combinations excluded very strong post- fertilization barriers (Table 2.2.2). Post-pollination barriers can be circumvented by in vitro rescue. Simple media such as MS did not work with small pods or small embryos. Loss of chlorophyll was followed by death. The newer media (D30, D3E, D4B, D4E and D40) showed good chlorophyll development and growth of the smallest pods. Excised proembryos or embryos reached a size of at least 2-4 mm diameter within 2-4 weeks. The rate of recovery proembryos per cultured pod was very high (50-80%).

The application of hormones to enhance pollen-tube growth resulted in bigger pods and ovules. Small pods grew rapidly (7-14 days) to normal podsize. Bigger pods contained ovules that could be transferred to D3E. After 2 weeks calli growth was visible. In the case of calli from selfings transfer of the calli to

regeneration medium showed morphogenesis and development of plantlets. In the case of interspecific crosses calli were formed. The results obtained for planlet regeneration from selfings and the possibility of ovule rescue and calli development from interspecific crosses indicate that it should be possible to regenerate plants from calli of interspecific crosses. **B. van Dorrestein, A. Comeau and M. Baum.**

Table 2.2.2. In vitro performance of various cross combinations between cultivated and wild *Cicer* species.

Cross	Combination	No. of crosses	Ovules		Calli formation
			white	green	
ILC4475	x ILWC236	11	4	2	no
ILC482	x ILW236	9	5	6	yes
Ad-1	x ILW236	9	3	2	yes
L6328	x ILW236	10	3	4	yes
L5359	x ILW236	2	4	-	no
FLIP84-150	x ILW236	2	-	-	no

2.3. Chickpea Pathology

Diseases form a major biotic constraint to the productivity of chickpea and to a large extent, cause instability in chickpea yields. Ascochyta blight caused by *Ascochyta rabiei* is the most serious foliar disease of chickpea in the west Asia and north Africa (WANA) region, particularly where low temperatures (15-25°C) prevail during the crop season. Its occurrence is not regular and is weather dependent. However, a good season for the chickpea crop is often favourable to ascochyta blight. Winter sowing of chickpea provides an opportunity to increase chickpea yield by almost 100%; unfortunately it also increases the risk to ascochyta blight devastation. Therefore, control of ascochyta blight is essential for *increasing chickpea production and yield stability.* Host resistance is the most practical and economic way of managing the ascochyta blight problem yet levels of host resistance have to improve. Fungicides that are effective as foliar sprays have been identified but their use has limited success in that when environmental conditions are favourable for ascochyta blight, even ten applications may not be effective.

Fusarium wilt caused by *Fusarium oxysporum* f.sp. *ciceri* is the most important soil-borne disease. Other soil-borne diseases such as black root rot (*Fusarium solani*), and wet root rot (*Rhizoctonia solani*) that are favoured by high moisture conditions are important in some areas in Ethiopia and irrigated fields in Egypt and Sudan. Dry root rot (*Rhizoctonia bataticola*) occurs throughout and region while collar rot (*Sclerotium rolfsii*) and stem blight (caused by *Sclerotinia sclerotiorum*) have also been reported in the region but overall, they are economically less important than fusarium wilt.

The objective of chickpea pathology is to (1) assist chickpea breeders in the development of high-yielding, disease-resistant cultivars and evaluate breeding material for resistance to the major diseases of chickpea; (2) collect information on disease epidemiology and pathogenic variability and develop disease management strategies for ascochyta blight; (3) collect information and monitor disease incidence and severity in the WANA region in collaboration with the national scientists; and (4) develop research collaboration with national programs on diseases management of ascochyta blight and wilt.

2.3.1. Field Survey of Chickpea Diseases

Disease incidence and severity on chickpea was surveyed in Dara'a, Homs, Hama, Aleppo and Hassakeh provinces in Syria. In total 16 locations, including off-station and on-farm trials, were visited and the disease incidence and severity at the different locations was assessed. The objective of this survey was to evaluate the disease situation in chickpea in Syria and to assess disease reaction of three released varieties (Ghab 1, Ghab 2 and Ghab 3) and three promising lines (FLIP 84-15, FLIP 86-5 and FLIP 86-6) that were in demonstration plots in farmers fields, on-farm trials and on-station trials.

Results are summarized in Table 2.3.1. Unlike last season, all provinces showed high incidence of ascochyta blight followed by *Fusarium* wilt and dry root rot as the dominant diseases. Laboratory tests confirmed the presence of four major pathogens: *Ascochyta rabiei*, *Fusarium oxysporum*, *Phoma medigaginis* var *pinodella*, and *Rhizoctonia bataticola* (Table 2.3.2). In the El-Hassakeh province the

Table 2.3.1. Chickpea disease incidence at different locations in Syria, May 1994.

Location	Genotype	Disease incidence and severity								
		On-farm trials			Demonstrations			Farmers' fields		
		Asc	FW/rt	Sci	Asc	FW/rt	Sci	Asc	FW/rt	Sci
Dan's:										
Darr'a/zone	Ghab 1	5	2-3	-	3	3	3	-	-	-
Nawa/zone	Ghab 2	NP	NP	NP	-	3	2	NP	NP	-
Lara'a	Ghab 3	5	2/3	-	-	NP	2	NP	NP	-
Jellin	FLIP 84-15	7	3/3	-	-	NP	NP	NP	NP	-
	FLIP 86-5	5	3/3	-	-	NP	NP	NP	NP	-
	FLIP 86-6	5	2/3	-	-	7	NP	NP	NP	-
	Local	-	-	-	-		1	-	-	-
Homs:										
Homs/zone 2	Ghab 1	7	-	3	8	-	-	NP	-	-
Homs/zone 2	Ghab 2	NP	-	NP	b	-	-	NP	-	-
Tarcoub/zone 1	Ghab 3	7	-	3	8	-	NP	NP	-	-
El-Ghab	FLIP 84-15	5	-	2	NP	-	-	NP	-	-
	FLIP 86-5	5	-	2	NP	-	-	-	-	-
	FLIP 86-6									
	Local spring									
Hama:										
Hama station	Ghab 1	-	2	-	4	-	-	-	-	-
	Ghab 2	-	2	-	3	-	-	-	-	-
	Ghab 3	-	4	-	4	-	-	-	-	-
	FLIP 84-15	-	4	-	NP	-	-	-	-	-
	FLIP 86-5	-	2	-	NP	-	-	-	-	-
	FLIP 86-6	-	2	-	NP	-	-	-	-	-
Aleppo:										
Kafr Baleg	Ghab 1	7	2	3	NP	NP	NP			
Atareb	Ghab 2	NP	NP	NP	NP	NP	NP			
	Ghab 3	5	2	2	NP	NP	NP			
	FLIP 84-15	7	2	3	NP	NP	NP			
	FLIP 86-5	5	2	1	NP	NP	NP			
	FLIP 86-6	6	2	3	NP	NP	NP			
	Local									
Idleb/	Ghab 1	7	NP	3	NP	NP	NP	7	NP	NP
Aphas	Ghab 2	NP	-	NP	NP	NP	NP	NP	NP	NP
	Ghab 3	5	-	2	NP	NP	NP	NP	NP	NP
	FLIP 84-15	7	-	3	NP	NP	NP	NP	NP	NP
	FLIP 86-5	5	-	1	NP	NP	NP	NP	NP	NP
	FLIP 86-6	6	-	3	NP	NP	NP	NP	NP	NP

Cont'd 2/..

Location	Genotype	Disease incidence and severity								
		On-farm trials			Demonstrations			Farmers' fields		
		Asc[1]	FW/rt[2]	Scl[3]	Asc[1]	FW/rt[2]	Scl[3]	Asc[1]	FW/rt[2]	Scl[3]
Hasakeh:										
Kamishly:										
Tel Hasoud	Ghab 1	7								
	Ghab 2	5								
	Ghab 3	6								
	FLIP 84-15	8								NP
	FLIP 86-5	7/6								
	FLIP 86-6									
Heimo	Ghab 1	7	-	-	8	-	-	NP	NP	
	Ghab 2	NP	-	-	6	-	-	NP	NP	
	Ghab 3	5	-	-	5	-	-	NP	NP	
	FLIP 84-15	8	-	-	NP			NP	NP	
	FLIP 86-5	6	-	-	NP			NP	NP	
	FLIP 86-6	5	-	-						
Malkideh	Ghab 1	7	-	-	NP			NP		
	Ghab 2	NP	-	-	NP			NP		
	Ghab 3	5	-	-	NP			5		
	FLIP 84-15	8	-	-	NP			NP		
	FLIP 86-5	6	-	-	NP			NP		
	FLIP 86-6	5/6	-	-						

1. Ascochyta blight on a 1-9 scale for locations indicated as a,b, or c.
2. Wilt/root rot (W/rt) on a 0-5 scale.
3. Sclerotinia blight (Scl) on a 0-5 scale.
4. NP = not planted.

Table 2.3.2. Disease incidence on promising chickpea lines grown in on-farm and on-station trials in 1993/94 in Syria.

Province	Location	Syrian production zone	Trial	Disease	Pathogen isolated	Frequency of isolation %
Dara'a	Izra'a	B	On-station	Ascochyta blight	A. rabiei	44
				Wilt	Fusarium oxysporum	44
				Charcoal Rot	Rhizoctonia bataticola	37
				Root Rot	R. bataticola	37
					R. oxysporum	30
				Collar rot	R. bataticola	7
					F. oxysporum	30
					Neocosmospora	14
	Nawa	B	On-farm	Charcoal rot	R. bataticola	37
				Collar rot	F. oxysporum	47
					Phoma medicaginis	7
					R. bataticola	27
				Root rot	R. bataticola	4
					F. oxysporum	4
				Root rot	R. bataticola	57
					F. oxysporum	20
	Jellin	A	On-station	Collar rot	R. bataticola	43
					F. oxysporum	20
					Phoma medicaginis	20
Tartous	Tartous	A	On-station	Charcoal rot	R. bataticola	82
				Root rot	R. bataticola	40
					F. oxysporum	40
					F. solani	27
				Collar rot	F. oxysporum	88
					R. bataticola	53
				Wilt	F. oxysporum	47
				Ascochyta blight	A. rabiei	4
Lattakia	Jableh	A	On-station	Wilt	F. oxysporum	93
				Root rot	F. oxysporum	40
					R. bataticola	40
					F. solani	27
				Collar rot	F. oxysporum	73
					R. bataticola	13
				Ascochyta blight	A. rabiei	7
Homs				Stem rot	S. sclerotium	67
				Wilt	F. oxysporum	33

Province	Location	Syrian production zone	Trial	Disease	Pathogen isolated	Frequency of isolation %
Hama	Hama	B	On-station	Charcoal rot	R. bataticola	29
				Root rot	R. bataticola	33
					F. solani	33
					F. oxysporium	24
					Pratylenchus thornei	50
					A. rabiei	4
					F. oxysporium	4
	Al Ghab	A	On-station	Ascochyta blight	A. rabiei	52
				Charcoal rot	R. bataticola	28
				Collar rot	R. bataticola	42
					F. oxysporium	38
					F. solani	12
					Phoma medicaginis	13
					F. oxysporium	33
					R. bataticola	28
					Phoma medicaginis	4
					F. oxysporium	14
					Sclerotinia sclerotorum	13
Idleb	Tel Sandel	A	On-station	Ascochyta blight	A. rabiei	69
					Phoma medicaginis	13
				Wilt	F. oxysporium	32
				Collar rot	A. rabiei	86
					Alternaria with chlamydospores	2
						9
					Phoma medicaginis	5
	Afess	B	On-farm	A. blight	A. rabiei	93
				Stem rot	P. medicaginis	13
				Root rot	Sclerotinia sclerotorum	53
					F. oxysporium	67
					F. solani	27
					Phoma medicaginis	7
					S. sclerotorum	60
					F. solani	13
Aleppo	Kafr Halab	B	On-farm	Ascochyta blight	A. rabiei	67
				Root rot	F. oxysporium	80
				Root-lesion nem.	P. thornei	67
				Collar rot	F. oxysporium	67
					S. sclerotorum	20
					A. rabiei	13

Province	Location	Syrian production zone	Trial	Disease	Pathogen isolated	Frequency of isolation %
	Atareb	B	On-farm	Wilt	*F. oxysporium*	20
				Stem rot	*S. sclerotorium*	13
				A. blight	*A. rabiei*	47
				Stem rot	*S. sclerotorium*	27
				Wilt	*F. oxysporium*	20
				Root rot	*R. bataicola*	54
					F. oxysporium	24
					R. bataicola	27
				Collar rot	*P. oxysporium*	20
					S. sclerotorium	9
	Breda	B	On-station	A. blight	*Phoma medicaginis*	60
					A. rabiei	47
				Collar rot	*A. bataicola*	44
					Phoma medicaginis	40
				Wilt	*F. oxysporium*	14
					Algernaria sp.	4
					F. oxysporium	20
					R. bataicola	10
	Breda	B	On-station	Charcoal rot	*F. oxysporium*	56
				Wilt	*R. bataicola*	53
				Charcoal rot	*A. rabiei*	46
				A. blight	*Ph. medicaginis*	38
	Afrin	A		Root rot	*F. oxysporium*	82
					R. solani	12
				Root-lesion nem.	*P. thomei*	77
				A. blight	*A. rabiei*	3
					P. medicaginis	4
					Mycosphaerella	48
					F. oxysporium	69
					R. solani	55
					F. oxysporium	4
					Phoma medicaginis	
					P. thomei	
					Nematode	
Hassakeh	Heimo	A	On-station	Ascochyta blight	*A. rabiei*	56
					Phoma medicaginis	2
					Neocosmospora sp.	23
				Root rot	*R. bataicola*	27

8

Province	Location	Syrian production zone	Trial	Disease	Pathogen isolated	Frequency of isolation %
					F. solani	30
					F. oxysporium	17
					Phoma medicaginis	13
				Collar rot	F. oxysporium	27
					R. bataticola	35
					Phoma medicaginis	13
					F. solani	3
				Charcoal rot	R. bataticola	9
				Wilt	F. oxysporium	9
				A. blight	R. rabiei	53
					P. medicaginis	4
				Collar rot	P. oxysporium	22
					Phoma medicaginis	20
					Alternaria sp.	43
					R. bataticola	12
				Wilt	F. oxysporium	3
				Pleospora stem	Pleospora sp.	3
	Kamishly		On-farm	Ascochyta blight	A. rabiei	55
				Root rot	F. oxysporium	62
					Phoma medicaginis	10
					R. bataticola	6
					F. solani	5
				Root-lesion nem.	Pratylenchus thornei	43
				Collar rot	F. oxysporium	20
					Phoma medicaginis	5
					F. solani	

following are highlights of the situation of ascochyta blight:

1. On-farm trials, planted in the El-Malkieh area showed that, as of May 8, 1994, FLIP 84-15, FLIP 86-5, FLIP 86-6, Ghab 1 and Ghab 3 had a disease rating of 7-8, 6, 5-6, 7-8 and 5 on 1-9 scale, where 1 = no disease, 9 = whole plant killed by disease.

2. Kamishly (Tel Hasoud) (Zone 1) had more disease than last season: FLIP 84-15 rated 7-8, FLIP 86-5 rated 6-7, FLIP 86-6 rated 6, Ghab 1 rated 7 and Ghab 3 rated 6. Some 100 lines planted on Jan 26 as screening nursery had very little disease even on the susceptible check and is thought to be due to late planting and escape from the favourable weather. Rains stopped in March, thus the genotypes were exposed to only 4-6 weeks of favourable rainfall.

3. Heimo station also had more disease than last season: FLIP 84-15 rated 8, FLIP 86-5 rated 5-6, FLIP 86-6 rated 6, Ghab 1 ratted 7, Ghab 3 rated 4-5 in the on-farm trials. Larger plots of demonstration trials for Ghab 3 rated 5, Ghab 2 rated 6, and Ghab 1 rated 8.

4. Disease readings on CIYT winter for the Mediterranean region at Heimo station showed that 7 out of 24 lines (FLIP 88-82, 88-85, 89-79, 90-76, 90-77, 90-149* and 82-150) had moderate infection from Ascochyta blight with a rating of 4-5 on the 1-9 scale. The CIABN trial at this location had 14 out of 41 lines showing moderate resistance to Ascochyta blight (FLIP 88-83*, FLIP 88-89, FLIP 89-78, FLIP 90-76, FLIP 90-85, FLIP

90-112*, FLIP 91-14, FLIP 91-150, FLIP 91-196*, FLIP 92-16, FLIP 92-113, FLIP 92-52, FLIP 92-159* and FLIP 92-189*). These lines show good potential as sources of resistance to the highly aggressive pathotypes found in north-east Syria. The lines marked with * were rated 3-4 while the others were rated 5 in the 1-9 scale.

5. It should be noted that most of the rainfall in Syria occurred in Dec-early March and there was no rain from mid March to May when the survey was done and thus the blight infection severity reflects early infection during the winter months and spring planting escaped infection. This is contrary to the 1992/93 season when disease favourable weather started in mid April and persisted for the rest of the season and affected both winter and spring planted crop particularly in the Malkieh area. The disease reaction of the promising lines and the three cultivars was more or less the same in the 1992/93 and 1993/94 seasons (Table 2.3.1), the disease severity in May 1993 was slightly lower than that recorded in May 1994 when the survey was done. This indicates that the epidemic in north-east Syria (Malkieh area) in 1992/93 was caused by the combination of favourable weather and uniformly susceptible cultivars grown over large area thus increasing the vulnerability of the crop to an epidemic outbreak. Persistent rainfall (not necessarily excessive as in 1992/93) can favour an epiphytotic level of ascochyta blight if temperatures is cool (10-25°C).

6. Some 100 genotypes planted at Malkieh and Heimo on Jan 26 did not develop much disease. Similarly, 2 rows of Ghab 1 that were planted at

Tel Hasoud in mid January to replace some missing lines also escaped infection. This confirms last years observation at Malkieh of a lower disease severity in plots planted 2 weeks later. This indicates the potential of manipulating the planting dates within the winter months to reduce the risk of ascochyta blight and yet take advantage of winter rains to increase yields.

Eventhough *P. medigaginis* closely resembles *A. rabiei* in spore morphology, and is associated with some stem and leaf lesions, its pathogenicity and taxonomic status has been confirmed under greenhouse conditions and was found to be distinct from ascochyta blight. Since the different pathogens are frequently isolated from the same field and often the same plants, investigation on the interaction between different pathogens would be beneficial to show the indirect role of the pathogens in crop damage. **M.T. Mubaga, M. Bellar, and NARS scientists from Syria.**

2.3.2. Ascochyta Blight

Host resistance is the backbone of ascochyta blight disease management at ICARDA, thus screening for blight resistance is a large component of chickpea pathology. Evaluation of the resistant lines against individual and multiple races is essential in identifying race-specific and race-nonspecific resistance.

2.3.2.1. Evaluation of breeding lines for resistance to individual and mixed races

This trial consisted of 225 lines that include 185 lines tested in the 1992/93 seasons and 40 other breeding lines selected for superior resistance in the 1992/93 advanced yield trial (AYT) at Tel Hadya. The 225 lines therefore are the most resistant breeding lines developed between 1980-1990. These lines were tested against individual and a mixture of six races in the greenhouse to reconfirm the 1992/93 results and assess the reaction of some newer lines that were not tested in the 1992/93 seasons. These lines were also tested against two field isolates from Hassakeh (Heimo and Malkieh areas), to identify resistance to the Hassakeh pathotypes which had devastated the chickpea crop in 1992/93. The experiment was replicated three times and each replicate (pot) had five test plants and a susceptible check in each pot.

The reaction of the lines to different races is presented in Figure 2.3.3. The relatedness of the disease reactions from the mixed races and the individual races was determined by using a multiple regression analysis. The results showed a low coefficient of determination (R^2) value and low regression coefficients for all the races. This indicates that reaction to the mixture of races did not relate with that of any individual race. There was slight change in the ranking of the genotypes to the six races but there was no shift from resistance to susceptibility. Such slight shift of reaction is not surprising considering that precision in environmental conditions is not available and the disease is greatly affected by the environment. Results from this season in general confirmed results of last year tests.
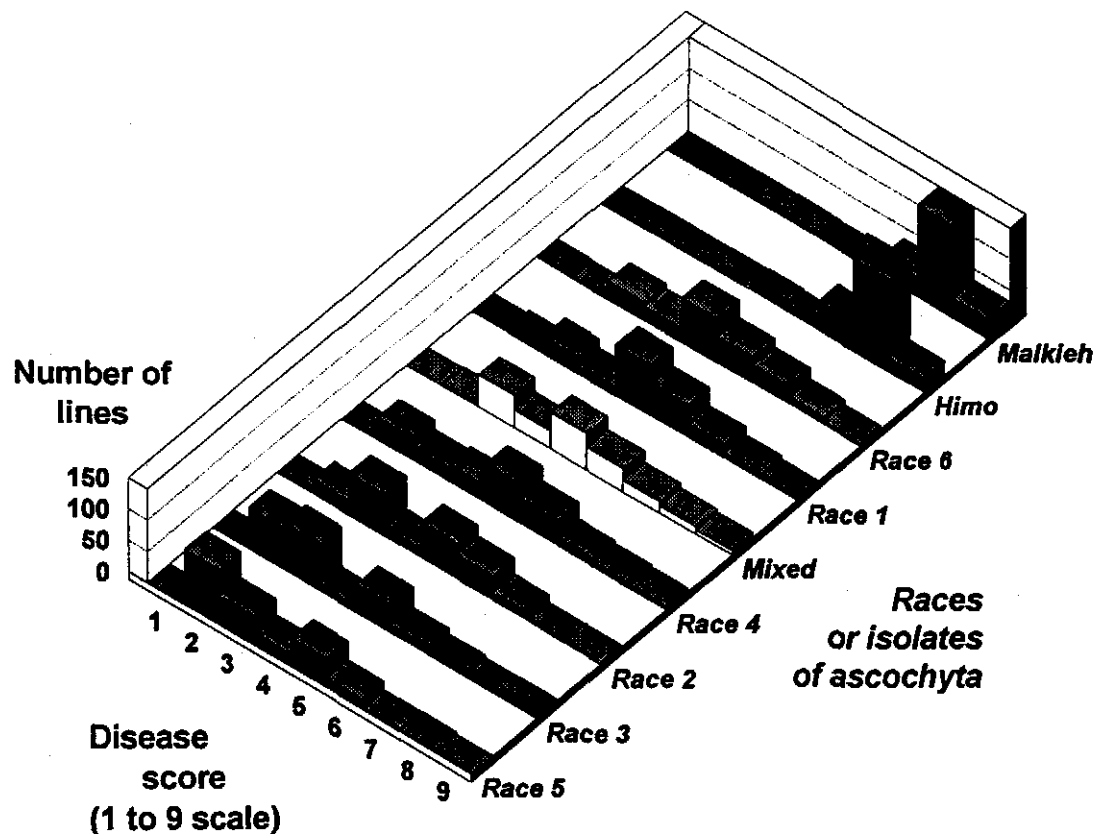
Figure 2.3.3. Reaction of chickpea breeding lines to different races/isolates of Ascochyta blight fungus in the greenhouse.

Lines that were resistant to the six races individually or in different combinations were however susceptible to the field isolates from Hassakeh and only 6 lines had a rating of 6 or less (Fig. 2.3.3). The two field isolates from Hassakeh were similar and were distinctly different from the six races individually or in a mixture (Fig. 2.3.3). Single-spore isolates have been developed and their characterization on a set of differential cultivars will be discussed separately under Section 2.3.2 on pathogenic variability.

Reaction of the lines in the field was very different from last years results. Only 10 lines (FLIP 88-83, 88-87, 89-62, 91-150, 90-112, 91-28, S 91168, S91150, ILC 4475 and ICC 12004) had a reaction of 4-5 in the two replicates with ICC 12004 being the best with a 4 rating in both replicates. Eleven other lines were rated 5 and 6 in the two replicates (FLIP 90-27, 90-76, 91-149, 91-184, 91-192, 91-196, S91139, ILC 7795, ILC 7374, ILC 5926, and ILC 3866). All these lines were rated 6 or more to one or more races and Hassakeh field isolates. All the other lines were susceptible with a rating of 6 and above.

In addition, to the 225 lines screened in the greenhouse and in the field, about 210 other lines selected in 1992/93 because of their rating of 3-4 were included in the field evaluation to confirm their resistance to ascochyta blight. About 16% of the 435 lines tested had a disease rating of 4-5 and about 72% of the lines were rated 7-9 (Tables 2.3.3., and 2.3.5). The reconfirmation trial was devastated as a result of unusually high disease pressure on the trial generated by inoculating the plants with infested debris in January, one month

Table 2.3.3. The best ascochyta blight resistant chickpea lines in the field.

Genotype	1992/93	1993/94	Genotype	1992/93	1993/94
FLIP 82-151^C	NT^a	4,5^b	FLIP 88-83*	3,3	4,5
FLIP 90-10^C	NT	4,4	FLIP 90-27*	4,4	4,5
FLIP 90-9'/^{C}	NT	4,4	FLIP 92-179	NT	4,4
FLIP 92-194^C	NT	4,4	FLIP 91-28	NT	4,5
FLIP 91-131^C	NT	4,5	FLIP 81-161	NT	4,5
FLIP 90-112^C*	3,4	4,5	FLIP 92-175	"	4,5
FLIP 91-149^C	NT	4,5	FLIP 92-178	"	4,5
FLIP 82-32^C	NT	4,5	ICC 4188	NT	3,5
ICC 76	NT	4,3	ICC 2165	"	3,5
ICC 13629	NT	3,5	ICC 2270	NT	3,4
ICC 2342	NT	3,4			
ICC 2364	NT	4,3	ICC 3912	NT	3,3
ICC 3918	NT	3,3	ICC 3919	NT	4,5
ICC 3940	NT	3,3	ICC 3991	NT	3,3
ICC 4000	NT	3,4	ICC 4020	NT	3,3
ICC 4030	NT	3,3	ICC 4045	NT	4,3
ICC 4241	NT	4,5	ICC 4616	NT	3,3
ICC 11932	NT	3,3	ICC 11933	NT	3,3
ICC 12004	NT	3,2	ICC 13292	NT	4,3
ICC 13718	NT	3,2	ICC 13729	NT	3,3
ICC 13754	NT	3,3	ICC 13903	NT	3,3
ICC 4475	NT	3,5	ICC 6373	NT	3,5
ICC 9189	NT	3,5	ICC 4187	NT	3,5

^{a}/ NT = not tested, ^{b}/ disease rating in 2 replicates (1-9 scale).

Table 2.3.4. Reaction of F_2-F_7 generation lines of chickpea to ascochyta blight at Tel Hadya 1992/93.

Generation	Ascochyta blight reaction on 1-9 scale[*]									
	1	2	3	4	5	6	7	8	9	Total
F_2 (RXR) bulk	0	0	1	1	3	0	0	0	0	5
F_2 bulk	0	0	0	10	31	43	28	14	1	127
F_4 bulk	0	0	0	16	61	54	22	2	0	155
F_4 bulk	0	0	0	2	2	1	0	0	0	5
F_4 (RXR) bulk	0	0	0	2	2	1	0	0	0	5
F_5 & F_6 (RXR)	0	0	19	207	58	11	0	0	0	468
F_5 prog. (large) winter	0	0	0	1	26	111	39	1	0	178
F_5 prog. (early) winter	0	0	0	90	468	589	150	12	0	1309
F_5 prog. winter	0	0	0	250	1596	3755	1076	315	107	7099
Total	0	0	193	577	2245	4564	1315	344	113	9346

Disease rating 1-3 = resistant (R), 4 = moderate resistance (MR), 5 = intermediate (I), 6 and above = susceptible.

earlier than the usual (February) time. This decision was based on the prevailing weather conditions: temperatures (10-15°C) favourable for infection establishment and persistent rainfall. The development of high disease pressure during the seedling stage severely damaged the entries by the disease.

Temperatures favourable for disease spread (15-25°C), usually start in the month of March but the rainfall declines or stops around March-April. Therefore, disease development necessitate the dependence on mist irrigation for 3 h a day. However, December and January winter months usually have few scattered days when the temperatures are relatively warm (i.e. above 6°C) and could allow the infection establishment of *A. rabiei* and when temperature increases the spread of infection can be quite rapid if moisture is available. Despite the high disease pressure in the reconfirmation trial, different levels of disease could still be distinguished (Table 2.3.5) and there was no high level of resistance found in the surviving material. In addition, the genotypes that stood the disease pressure at seedling stage remained fairly stable when inoculum in the form of spore suspension was provided at the flowering/early pod stage (a highly susceptible stage) when cool and wet weather occurred. The section of the nursery that was inoculated in early February (the normal time) developed less disease and some of the resistance identified in April, was not maintained at flowering/early pod stage.

This year's results have pointed out that (a) the disease pressure commonly used at the nursery may be high enough to eliminate highly susceptible

Table 2.3.5.   Retesting of lines selected for resistance to Ascochyta blight.

| Lines	Ascochyta blight reaction on 1-9 scale									Total
	1	2	3	4	5	6	7	8	9	
Demonstration	0	0	1	1	3	0	0	0	0	5
Desi[*] R lines	0	0	0	10	31	43	28	14	1	127
PYT and AYT	0	0	2	115	215	190	30	4	4	560
New generation	0	0	0	20	35	48	105	97	227	514
CIABN	0	0	0	7	19	17	7	0	0	50
Reconfirmation[*]	0	0	0	16	57	46	107	118	91	435
Total	0	0	10	148	286	264	148	101	232	1189

[*] Inoculated with infested debris in January; all other trials inoculated in February.

material, but may not be that high to separate the best resistance sources from the moderately resistant and the moderately susceptible lines; (b) it is also possible that some of the resistance is temperature sensitive and it breaks down at cool temperatures. This is not unique and requires attention especially as epidemics with ascochyta blight have often been associated with fairly cool temperatures. If stable resistance is required, the stability of the resistance at different temperatures must be determined, particularly for winter planting where the disease can occur during the winter or spring months.

2.3.2.2.   Evaluation of F_2-F_7 generations

The reaction of the F_2-F_7 segregating populations to ascochyta blight is presented in Table 2.3.4. About 8.2% of the 9,346 lines were R-MR with a disease rating of 3-4 and about 24% had intermediate (I) reaction of a rating of 5. The F_5 R X R crosses were mostly (85%) R (resistant)-MR (moderately resistant). The F_2 bulk had 7.8% MR. and 24% I reaction, quite similar to last season F_2. The F_4 bulk which was generated from last years F_2 bulk had 10% MR and 39% I. The F_5 progeny-winter had 3% MR and 22.4% I (compared to last years <1% R, 18% MR and 42% I). Likewise the F_5 progeny (early) winter had 6.8% MR, and 35.7% I (compared to last years 24% MR and 46% I). The lower recovery of resistance during this season is because of a more favourable weather especially during the winter months when temperature was warm and precipitation was not heavy but persistent and cool and wet weather during pod filling stage (Fig. 2.3.4). This indicates that the screening procedures should be refined to avoid
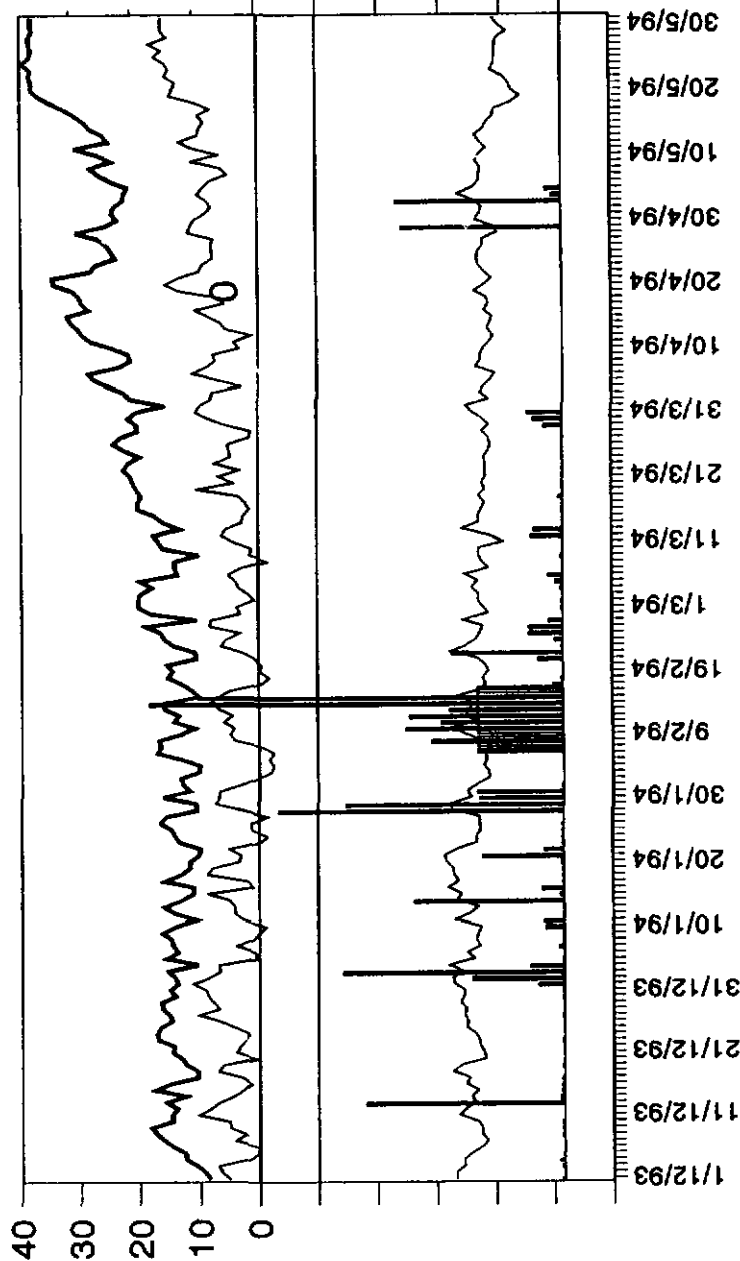
Figure 2.3.4. Tel Hadya weather conditions in the 1993/94 growing season.

disease escape and make use of winter rains in screening. **M.T. Mibaga and K.B. Singh.**

2.3.2.3. Confirmation of resistance

Chickpea lines that had been selected for ascochyta blight resistance in the 1992/93 season, were planted in the ascochyta blight nursery in 1993/94 for confirmation of the resistance. Results are shown in Table 2.3.5. About 50% of the pre-selected resistant desi lines were confirmed to be R to MR, 25% rated I and only 25% succumbed to the blight with a S rating. About 20% of the PYT, AYT, and IYT were confirmed R-MR, while 38% were I with a rating of 5. The CIABN had 14% MR and 38% I with fewer lines showing resistance compared to last year (55% MR and 28% Intermediate). **M.T. Mibaga and K.B. Singh.**

General observations on the nursery revealed that disease symptoms on seedlings were widespread by 1 March before an inoculum with spore suspension was made. Infection development from spore suspension depended on mist irrigation as April and most of May were dry. Spore suspension was subsequently applied 7 times and this included also one application, in case of F_5 & $_6$, during pod filling stage at a time the weather was cool and wet. Infection increased at this stage and many lines which were otherwise healthy succumbed to the disease. Infection at pod filling stage was more uniformly distributed this year compared to last year most likely because of more uniform inoculation in the field. This confirms last years observation on the need to inoculate at the flowering/pod filling stage.

Steps needed to improve the efficiency of resistance screening include: 1) ensuring disease development at flowering and pod filling; 2) increasing the uniformity of disease spread during flowering by providing uniform inoculum in the form of spore suspension, increasing the frequency of susceptible checks between the test entries, and maintaining the susceptible check at a disease rating of about 7 or 8 by using more than one check line giving broader range of disease reaction so as to provide source of secondary inoculum and reference points for disease reading; (3) timing of inoculum should take advantage of favourable weather forecast/or observations instead of following a routine pre-determined timing. Inoculation with debris before February would be desirable to allow early infection establishment and provide 2-3 months of cool and wet weather which would reduce the dependence on mist irrigation for disease development; and (4) making a baseline disease reading at seedling stage if favourable weather has allowed early disease development. **M.T. Mnbaga**.

2.3.2.4. Pathogenic variability of *A. rabiei*

Pathogenic variability of *A. rabiei* is the main cause for inconsistency in resistance ratings that has been frustrating the efforts to control ascochyta blight with host resistance. While quantifying the variability is important, results so far available can not be easily compared. Race identification must be based on use of standard differentials, environmental conditions and disease rating scales.

The objectives of this study were to test the virulence pattern of single spore isolates collected

from north-east Syria (Hassakeh area) and compare it with the six races previously described at ICARDA. To standardize methodology used in race identification a set of differential cultivars that different researchers have used in the literature was used. Plants were maintained at 20-22°C, spray inoculated with 500,000 spores per ml on 10 day-old plants, incubated for 72h at 100% relative humidity and disease reading (using a 1-9 scale) done 14 days after inoculation. Since studies on pathogenic variability require homogeneity of the differential varieties, the seed source was derived from single plant selections. These results are expected to complement the work on molecular-marker assisted study on variability in the ascochyta blight fungus (Section 2.2.4).

The virulence patterns of 19 single spore isolates collected from north-east Syria in 1992-1993 were compared with the six races on a set of 26 differential lines. Reverse ranking order of genotype reaction was evident (Table 2.3.6) and the analysis of variance showed significant genotype x isolates interaction (Table 2.3.7). A physiologic race is defined as "a sub-specific group of parasites characterized by specialization to different cultivars of one host" (Johnson and Booth, 1983). According to this definition, *A. rabiei* populations can be characterized into races if the procedures used in the characterization are standardized. The overall aggressiveness of the isolates can be computed from the mean disease score on all the genotypes which clearly show differences in aggressiveness (Table 2.3.6 Fig. 2.3.5). Differences in aggressiveness of the "races" also exist. The Hassakeh isolates were very aggressive compared to the other races. Race 6 is aggressive

Table 2.3.6. Virulence characteristics of 13 isolates of *Ascochyta rabiei* on a set of 16 differential genotypes selected on the basis of broad resistance in multiple locations.

Differential genotypes[1]	Isolates[2] and mean disease score													
	R1	R2	R3	R4	R5	R6	C	D	E	G	M	N	U	Mean
ILC 72	2	2	2	8	–	2	7	2	5	2	6	2	2	3.7
ILC 200	2	2	1	1	2	2	7	3	6	2	2	2	3	2.7
ILC 215	6	6	7	9	3	7	8	8	7	6	7	3	7	6.4
ILC 482	–	2	2	8	2	8	7	8	7	7	7	3	6	6.0
ILC 1929	8	8	8	8	8	8	8	8	6	7	7	8	8	7.4
ILC 2956	2	2	2	3	6	2	6	8	7	7	6	4	3	4.6
ILC 3856	7	2	2	2	–	2	7	8	7	6	6	3	2	4.1
ILC 5894	3	–	1	1	–	3	7	3	5	6	6	3	5	3.9
ILC 6260	5	7	6	5	6	8	7	8	6	7	5	3	3	5.6
ILC 7374	2	2	1	–	2	2	2	7	3	3	4	2	2	3.0
ICC 3996	2	2	2	2	4	2	2	2	2	2	2	2	2	2.3
ICC 4475	2	5	2	2	1	7	2	3	2	2	2	2	2	2.4
ICC 9189	2	–	1	3	2	3	2	4	1	2	2	2	2	2.0
ICC12004	2	2	1	3	2	7	2	4	2	2	2	2	2	2.4
F8	2	6	3	8	3	7	7	7	7	7	5	2	5	4.4
PCH 15	5	6	2	2	2	7	2	7	7	8	2	2	4	3.2
Mean	3.0	3.4	2.7	4.0	2.7	4.6	5.3	6.0	5.6	4.7	4.7	2.7	3.7	4.08

[1] Isolates C to U are representatives of 20 isolates for north-east Syria collected in 1992-93 from an epidemic situation, the selection of the isolate C to U was based on the relatedness of the isolates shown a dendrogram in Fig. 2.3.6a.

[2] Selection of 16 differential lines is from 26 lines, based on their selectiveness for their reaction to 26 isolates of A. rabiei as shown in Fig 2.3.6b.

but different from the Hassakeh isolates. Mild pathotypes also occurs at Hassakeh (Fig. 2.3.5).

Table 2.3.7. Analysis of variance of disease score of 26 genotypes of chickpea, each infected with one of 26 isolates of *Ascochyta rabiei*.

Source of variation	df	Mean square	Varianc ratio	P
Main effects:				
Genotypes of chickpea	25	140.1	179.46	<0.001
Isolates of ascochyta	26	108.8	139.40	<0.001
Interaction:				
Genotype x Isolate	635	6.732	8.62	<0.001
Residual	1274	0.781		

These results provide evidence that *A. rabiei* isolates differ in both aggressiveness and in their specific virulence patterns on a set of differential lines. The occurrence of a complex pathogenic variability in *A. rabiei* is not surprising since the pathogen has a sexual stage, is heterothallic (Kaiser 1992) and can generate new genetic recombinants and broad virulence spectrum. The choice of differential lines, however, determines the degree of variability that can be detected in such studies. Standard international differential lines have to be effective to separate the pathogenic groups with a clear-cut separation of resistance and susceptibility over broad geographical areas. While it is important to
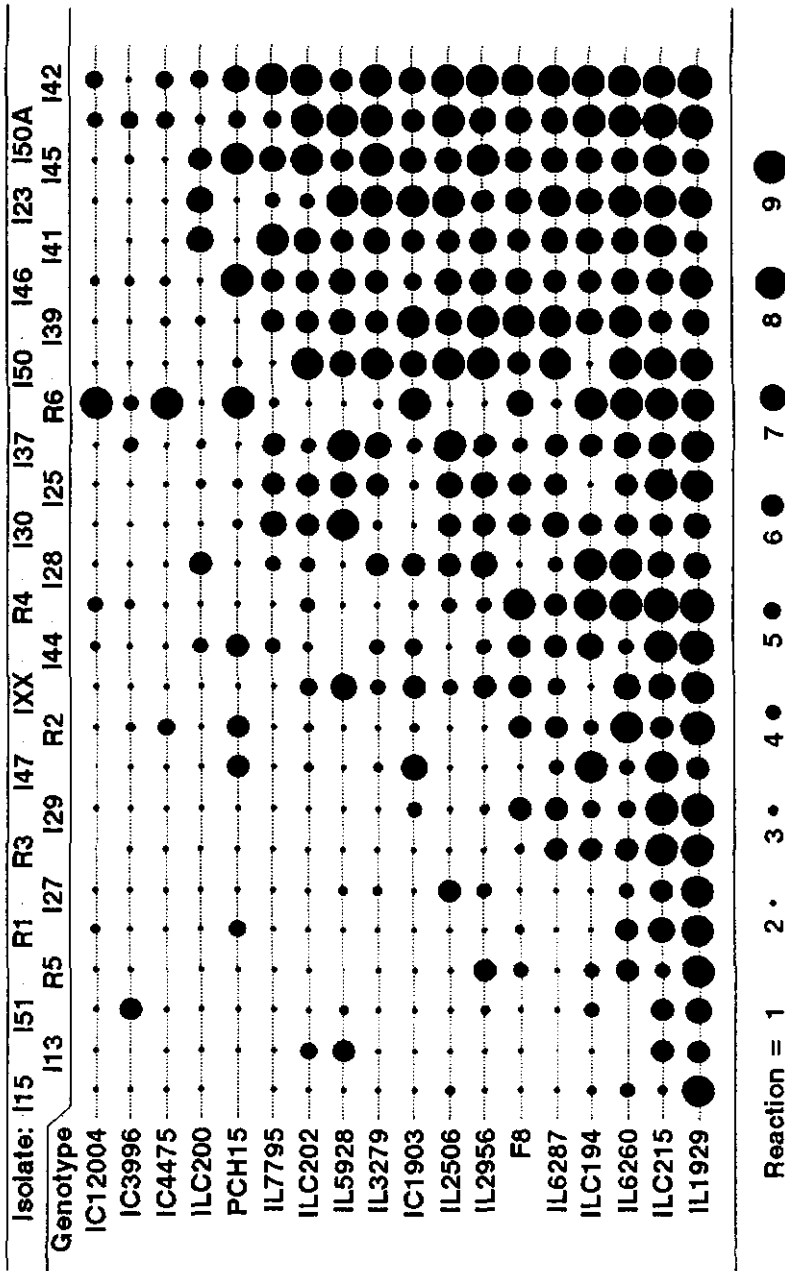
Figure 2.3.5. Reaction of 18 differential chickpea lines to different isolates of *Ascochyta rabiei.*

understand the extent and dynamics of the variability, it is equally important to focus our research efforts on studies, that guide resistance breeding and identify new virulence in the pathogen that match the available resistance, before its population becomes economically important.
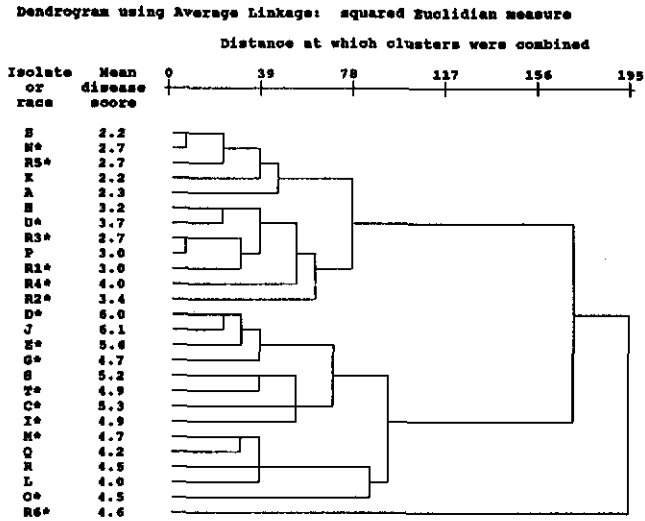
The relatedness of the pathotypes is presented in a dendrogram in Figure 2.3.6a. The Hassakeh isolates seem to have a large component of highly aggressive pathotypes that differ distinctly from the six races. However mild pathotypes are also found in the area. These results indicates that the pathotypes in *A. rabiei* can be defined by both their virulence pattern on a set of differential lines i.e. races, and also in their aggressiveness which is demonstrated in Figure 2.3.5. Such results facilitate the decision making on representative pathotypes of the different groups that may be used to identify specific resistance groups to be combined through breeding. Similar analysis was done on the differential lines (Fig. 2.3.6b). Due to the multiplicity of complex and subtle host-pathotypesand pathotype-pathotype interactions, and use of other more detailed statistical analysis of the data such as principle component analysis need to be explored (Shane 1987). The role of the weak pathotypes in generating aggressive pathotypes through accumulation of virulence and genetic recombination is not yet understood and needs to be explored. **M.T. Mnbaga and K.B. Singh.**

2.3.2.5. The role of teliomorphic stage of *A. rabiei* in Syria

The dispersal of *Ascochyta rabiei* from plant to plant is mostly short distance through water splashing, but small spore-bearing water droplets can be carried a long distance through air currents. Discharge of ascospores from pseudothecia in chickpea debris can be a source of long distance movement and source of primary inoculum in areas where chickpeas are grown for the first time. During the 1992/93 season seedlings grown from fungicide treated seeds and placed at 1 to 30 km from chickpea growing areas developed typical ascochyta blight infection after two weeks exposure to open air. The objective of this study was to assess the potential of the teliomorphic stage as a source of primary inoculum and long distance movement of *A. rabiei* in the air.

To assess the long distance movement of *A. rabiei* and air-borne source of primary inoculum biological indicators and sticky slides for trapping spores were placed at various distances from chickpea growing areas. Susceptible plants were exposed to open air for 2-10 weeks starting from 26 January. Disease reading was done using a 1-9 scale after 14 days. Plants from some locations often had infection before they were provided with high humidity (100% RH) and the disease severity was therefore noted (Figs. 2.3.7 a & b) to compare it with that obtained after incubation for 48 hr in 100% RH (Figs 2.3.8 a & b). Slides were exposed to open air for 24 h every two weeks. Observation of different forms of inoculum trapped in the sticky slides was done and representative samples were grown in culture to confirm the identity of the isolate.

(a)

Dendrogram using Average Linkage: squared Euclidian measure

Distance at which clusters were combined

(b)

Dendrogram using Average Linkage (Between Groups)
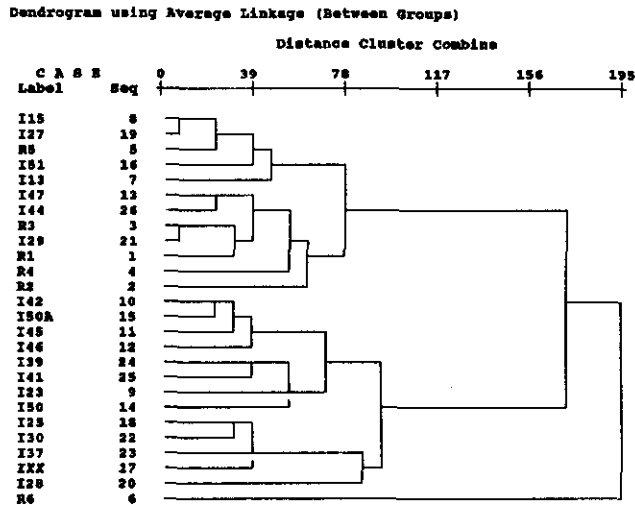
Distance Cluster Combine

Figure 2.3.6. Cluster analysis to show (a) the similarity in virulence of single-spore isolates of *Ascochyta rabiei* on a set of differential cultivars; (b) the similarity of differential cultivars in their reaction to single spore isolates. R_1 to R_6 represent races 1 to 6 and I refers to single spore isolates from Hassakeh.

All plants exposed to open air at 0.5-35 km
from the nearest chickpea fields (Tel Hadya)
developed stem lesions of Ascochyta blight. Plants
placed at some locations developed more disease than
others regardless of the distance from chickpea
fields (Fig. 2.3.7 & 2.3.8). The amount of
infection increased with the period of plant exposure
to open air (Fig. 2.3.7b and 2.3.8b). Plants
exposed to inoculum for two weeks had low infection
and confirmed last years observation and hypothesis
that more than two weeks were needed to provide more
inoculum level for a high level of disease
development. Different forms of air-borne inoculum
trapped on slides were minute pieces of debris that
carried *A. rabiei*, and large clusters of
conidiospores. All these were isolated and purified
in culture and gave rise to morphologically typical
A. rabiei cultures. Pathogenicity tests confirmed
their role as sources of air borne inoculum. These
results partly explain why sudden disease outbreaks
occur after 10 or more disease free years where the
teliomorph does not occur to provide source of
inoculum from ascospore. The results further
indicate that a regional approach on the ascochyta
blight disease management would be desirable. **M.T.
Mubaga**.

2.3.2.6. The spread of ascochyta blight from the infection focus

Infested debris and seed are the two most important
sources of primary inoculum for ascochyta blight,
producing several infection foci from where the
disease spreads. The frequency of seed transmission
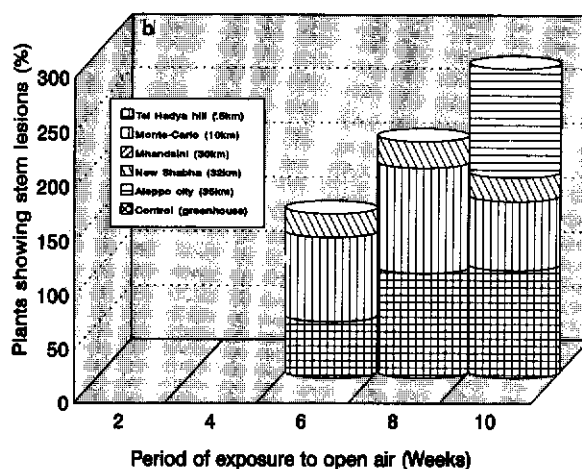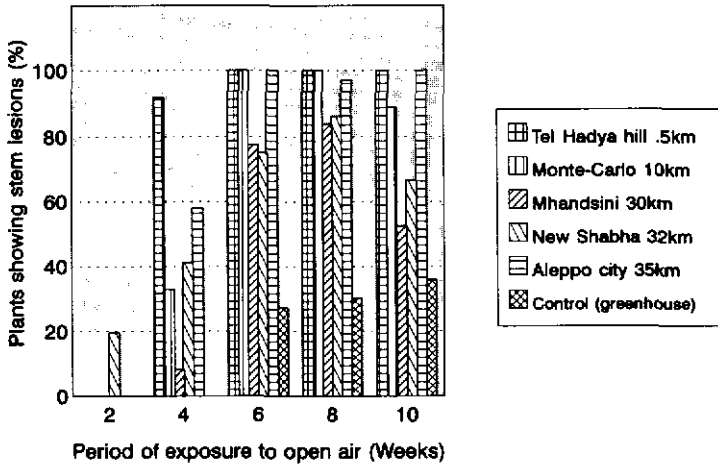can be low. Seed transmission ensures a random

(a)
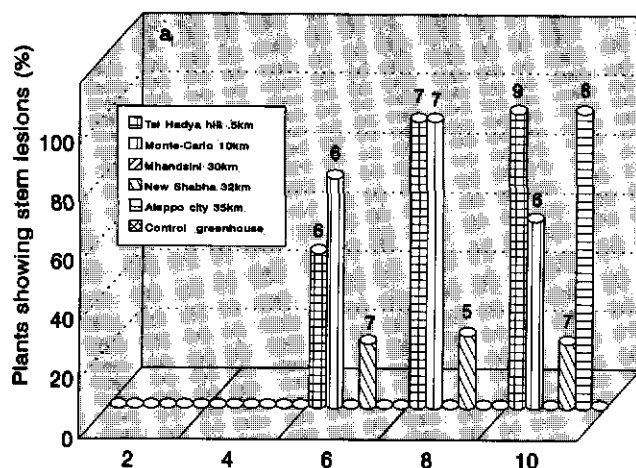
(b)

Figure 2.3.7. Effect of period of exposure of disease-free chickpea plants to open air at various distances from the chickpea production site at Tel Hadya, on disease development in 1993 before incubating the plants for 48 hrs in 100% RH: (a) Percentage of exposed plants showing stem lesions at each location; (b) Cumulative total of percentage of exposed plants showing stem lesions.
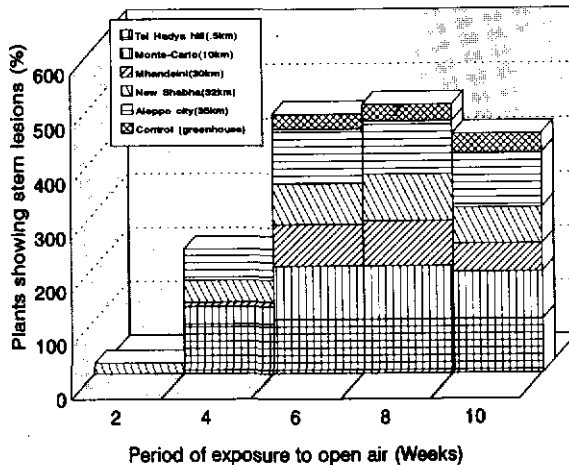
(a)

(b)

Figure 2.3.8. Effect of period of exposure of disease-free chickpea plants to open air at various distances from the chickpea production area at Tel Hadya on the disease development in 1993/94, after incubating the plants for 48 hrs at 100% RH: (a) Percentage of exposed plants showing stem lesions at each location; (b) Cumulative total of percentage of exposed plants showing stem lesions.

distribution of the pathogen in a field, that provides many primary infection-foci from which the pathogen can spread. The objectives of this study was to determine the distance of spread of secondary inoculum from the infection focus and to estimate the frequency of infection foci necessary for epiphytotic disease development under the Tel Hadya conditions.

Two infection foci, $2m^2$ each 26 m apart, were developed in a field planted with a blight susceptible cultivar ILC 1929, as last year. Plants in the marked infection-foci areas were inoculated with blight-infested debris of *A. rabiei* on 2 January. The spread of infection from the infection-foci was assessed, ones a week, by following the infection development on non-inoculated plants, and by measuring the direction and distance of spread. Infection development on non-inoculated plants was first assessed by a preliminary survey of the distance and direction of spread. The results are presented in Figure 2.3.9. The infection severity at different radial distances from the infection focus was evaluated on a 1-9 scale. Disease spread started after approximately one month from the inoculation date and spread rapidly (Fig. 2.3.9).

Temperature and moisture conditions were favourable for disease development (10-25°C) in January to the last week of March while April was dry (Figure 2.3.4). Infection spread over time included the formation of numerous secondary infection foci that increased in number and size and covered the entire field over a period of one month and disease spread stopped when dry conditions became limiting (Figure 2.3.4). The number of plants killed by blight increased radially from the

infection foci over time and the spread was farthest following the predominant wind direction. Another period of favourable weather occurred briefly in mid May and resulted in very high pod infection over the entire field. These results confirm last year's results that an integrated approach that reduces the rate of disease spread is necessary to control ascochyta blight. The results also indicate that management of ascochyta blight requires education of farmers on the importance of using clean seed. **M.T. Mnbaga**.

2.3.3. The Fusarium Wilt-sick Plot

To screen chickpea germplasm and breeding material for root rot and wilt a sick plot is needed. Efforts have been underway for last 2 seasons to develop such a plot.

The sick plot was mapped out according to the status of development at the end of the 1992/93 season and the 0.6 ha sick plot will be ready for use in spring 1995. Plans were carried out with off-season planting using artificially infected seed. Section A was assessed for uniformity of inoculum in the soil in spring 1994 and mapped out for areas that still needed inoculum reinforcement. This was done in Sept 1994 and the section can be used for preliminary screening work in spring 1995 as initially planned. In addition, section B (0.7 h) has high wilt incidence and portion of it could also be used for preliminary screening. Wilt incidence in section C (0.7 h) is still patchy and will require inoculum reinforcement before it is assessed for uniformity distribution. The entire 2 h can be ready for use in spring 1996 while at least 1 ha (section A & 50% of section B) can be used in spring 1995 (Fig. 2.3.10). **M.T. Mnbaga and K.B. Singh**.
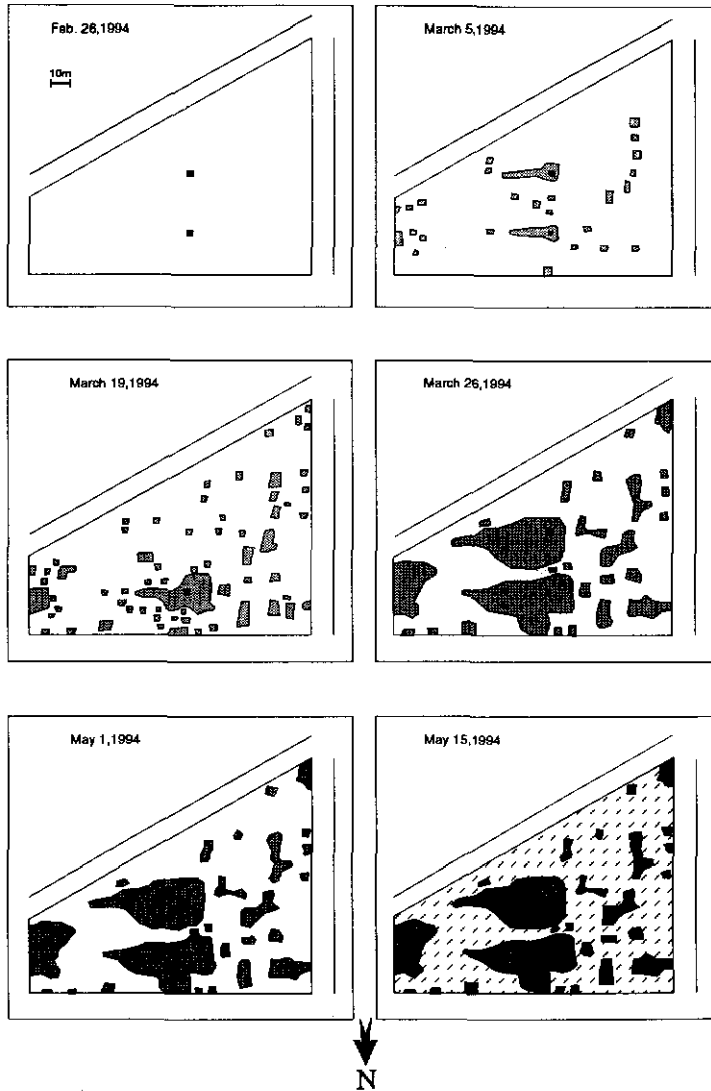
Figure 2.3.9. Spread of Ascochyta blight in chickpea field over time from two infection foci, 'A' and 'B', Tel Hadya, 1993/94.
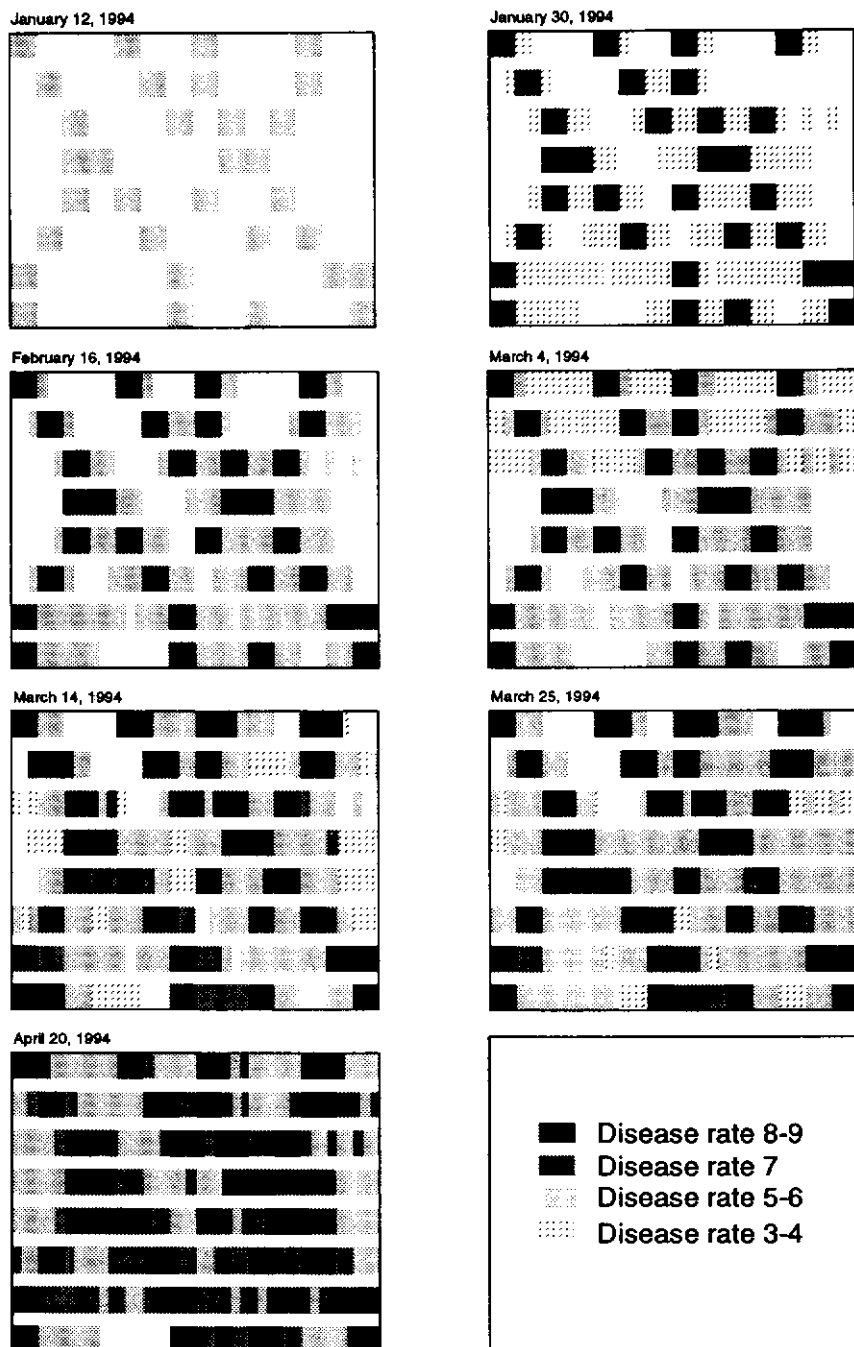
Figure 2.3.10. The wilt and root-rot sick plot at Tel Hadya, January 1994.

2.4. CHICKPEA ENTOMOLOGY

2.4.1. Population Dynamics of Chickpea Leafminer and its Parasitoids

Chickpea leafminer populations were sampled by D-Vac in winter- and spring-sown chickpea plots at Tel Hadya. In winter-sown chickpea leafminer started occurring in late March and reached one peak in late April (Figure 2.4.1). In spring-sown chickpea leafminer appeared slightly later, in mid April and quickly reached a first peak in late April and a second in mid May. The number of leafminer per sample was high reaching 130 and 180 adults per sample in winter- and spring-sown chickpea, respectively. Population of the parasitoids followed the same trend as the leafminer population, but did not build up to higher numbers corresponding to the leafminer population (Figure 2.4.1). **S. Weigand and A. Joubi.**

2.4.2. Monitoring of Podborer (*Helicoverpa armigera*)

The monitoring of podborer populations by pheromone traps was continued. Traps were placed in chickpea fields in Tel Hadya and in farmers fields at 4 locations around Tel Hadya, as well as in Afrin, Alkamiye and Al Ghab area. After chickpea was harvested in late June, the traps were moved to the closest cotton field. The traps were checked every 2 weeks. In general podborer populations were low as compared to 1992 and especially 1993. Inside Tel Hadya the highest number was 51 moths per trap (Figure 2.4.2.A). In the chickpea fields around Tel Hadya pheromone catches also were not high and only increased in cotton at Banus and Kosinya (Figure 2.4.2.A). In chickpea higher podborer numbers were

Winter chickpea
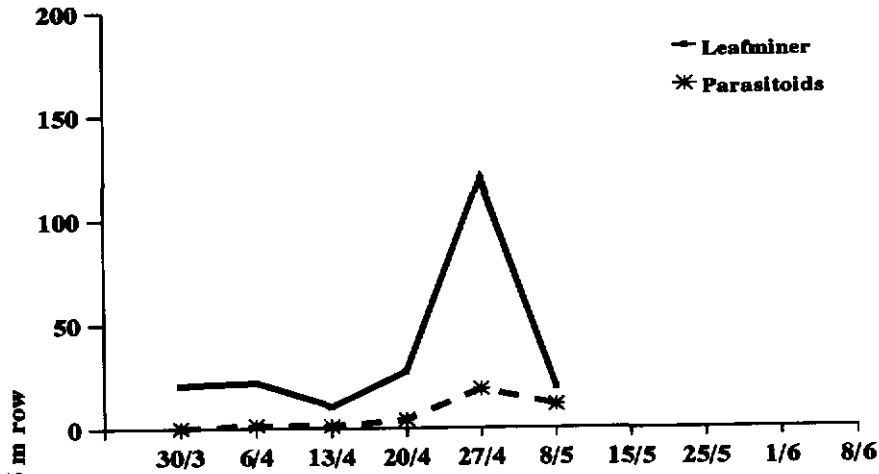
Spring chickpea
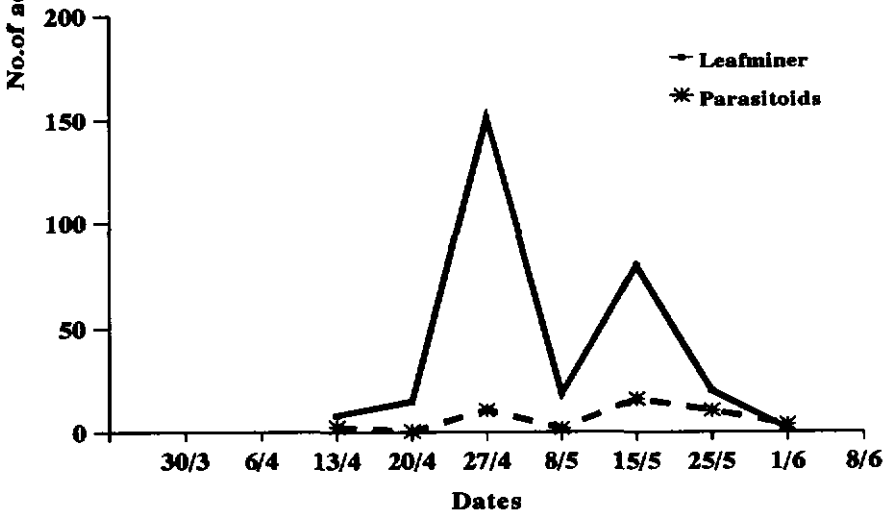
Figure 2.4.1. Development of population of adults of leafminer and parasitoids, as sampled by D-Vac at different sampling dates in winter- and spring-sown chickpea at Tel Hadya, 1993/94.
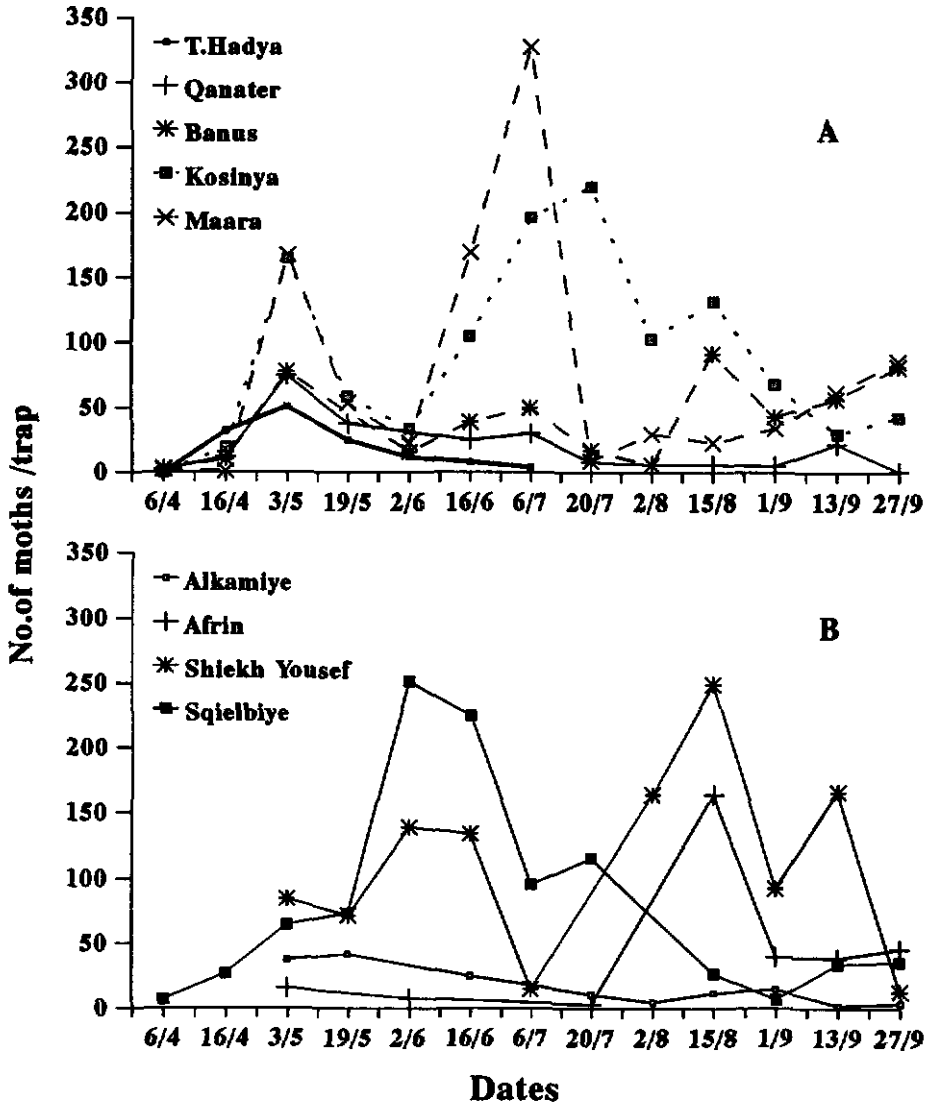
Figure 2.4.2. Pheromone trap catches of podborer, *Helicoverpa armigera*, in chickpea fields in Tel Hadya station, farmers chickpea and cotton fields around Tel Hadya (A) and chickpea on-farm trials and subsequent cotton fields (B), 1993/94.

caught in Sqielbiye and Sheikh Yousef (Figure 2.4.2.B). In northern Syria (Alkamiye and Afrin) podborer numbers were low. These data show the high variation in *H. armigera* populations over regions and years indicating the importance of monitoring. The relative importance of podborer in the regions monitored has been almost the same over the past 3 years, but fluctuations between years have been high. **S. Weigand and A. Joubi.**

2.4.3. Chemical Control of Leafminer and Podborer

In addition to neem (*Azadirachta indica*) seed extract applications a commercial synthetic neem product produced in India (Godrej neem antifeedant "Achook") was tested as a safe chemical for leafminer and podborer control in winter- and spring-sown chickpea at Tel Hadya. In winter chickpea two sprays consisting of 600 g neem seeds per 10 l water at 500 l/ha and two sprays of 100 g Achook Neem powder per 20 l water at 500 l/ha applied in mid April and early May were compared with one spray of Thiodan 35 EC (2 cc/l at 500 l/ha) applied in mid April. This season two check treatments were included, i.e. one untreated check and one check was sprayed with water (500 l/ha) on the same dates as the neem. In spring chickpea the treatments were the same except for an additional third spray of neem extract, neem product and water in mid May. In winter chickpea the leafminer damage was high reaching, a Visual Damage Score (VDS) rating of 5.6 and 6.6 on a 1 to 9 scale in the untreated and water-sprayed check (Table 2.4.1). Compared to the rating of the water-sprayed check both neem extract and neem product reduced leafminer damage significantly, but less effectively than Thiodan. Podborer damage was low (2% in the check) and is

therefore not presented. Seed yield was increased significantly by the neem extract spray and Thiodan, but not by the neem product (Table 2.4.1).

Table 2.4.1. Effect of 3 applications of neem-seed extract, neem product, water and one spray of Thiodan 35EC on leafminer infestation (VDS) and seed yield (kg/ha) at Tel Hadya in winter- and spring-sown chickpea, 1993/94.

Treatment	Winter		Spring	
	VDS (4 May)	Seed yield	VDS (15 May)	Seed yield
Neem extract	4.7	1947	4.3	995
Neem product	5.0	1747	4.3	969
Thiodan	2.4	2093	3.8	987
Water	6.6	1601	4.4	926
Check	5.6	1673	4.9	984
LSD 5%	1.07	230.9	0.92	106.6

In spring-sown chickpea leafminer damage was less severe than in winter, which is unusual, with little differences between treatments (Table 2.4.1). Only the difference between Thiodan and the untreated check was significant. Seed yields were low and not significantly different. There were no significant differences between the untreated and water-sprayed check, showing that the water sprays did not affect the leafminer populations. The experiment will be repeated for one more season to test the neem product on farmers field in Al Ghab area under higher leafminer and podborer infestations. **S. Weigand A. Joubi.**

2.4.4. Host-Plant Resistance to Leafminer

The most promising line ILC 5901 was grown in winter and spring together with the susceptible check ILC 1929 without and with the application of Thiodan 35 EC (2cc/l). Leafminer damage was again higher in the winter- than in the spring-planting. The resistance of ILC 5901 was confirmed with a VDS rating of 3.4 and 2.7 as compared to 8.4 and 5.5 in ILC 1929 in winter- and spring-sowing, respectively (Table 2.4.2). In winter-sowing the spray reduced the leafminer damage significantly, but seed yields did not differ significantly. Thus, in spite of the high leafminer damage in ILC 1929 seed yield was not affected.

Table 2.4.2. Leafminer visual damage score (VDS) and seed yield of resistant (ILC 5901) and susceptible (ILC 1929) winter- and spring-sown chickpea lines with and without one spray of Thiodan, Tel Hadya, 1993/94.

Treatment	Winter		Spring	
	VDS (16 May)	Seed yield	VDS (16 May)	Seed yield
ILC 5901 no spray	3.4	1499	2.7	622
ILC 5901 one spray	2.1	1688	2.2	659
ILC 1929 no spray	8.4	1462	5.5	1010
ILC 1929 one spray	2.6	1405	3.0	1121
LSD at 5%	1.14	474.6	0.59	771

In spring-sowing the spray significantly reduced leafminer damage and increased yield in ILC 1929 (Table 2.4.2). In winter-sowing yields of ILC 5901 were acceptable, but yields of the spring-sowing were very low, since this line is late maturing. **S. Weigand, K.B. Singh and A. Joubi.**

3. LENTIL IMPROVEMENT

Average lentil yields are low because of poor crop
management and the low yield potential of landraces. In
South Asia and East Africa diseases are also a major
constraint to production. Accordingly an integrated
approach to lentil improvement is being pursued at ICARDA
covering the development of both improved production
technology and genetic stocks. A high priority has been
placed on transferring to national programs the results of
research on lentil harvest mechanization systems to reduce
the high cost of harvesting by hand in the West Asia and
North Africa region.

3.1. LENTIL BREEDING

3.1.1. Base Program

3.1.1.1. Lentil adaptation and breeding scheme

The lentil is an under-exploited and under-researched
annual legume. From the onset at ICARDA, we have studied
the variation in the world germplasm collection to
understand factors affecting lentil adaptation to direct
the breeding program. Such diverse factors as winter-
hardiness, efficiency of iron uptake, phenology as related
to the length of the growing season, the sensitivity of
flowering to temperature and photoperiod, response to
irrigation and disease resistance have all contributed to
the pattern of variation found in the world germplasm
collection.

Additional information on the specificity of
adaptation within the crop has come from collaborative
yield trials of common entries selected in different
locations. For example, the North African Regional yield

trial on lentil was established in 1990 to comprise the best lines selected in Algeria, Libya, Morocco and Tunisia. This regional yield trial has revealed that lentils selected in the various countries of N. Africa differ substantially in phenology, indicating the need for specific adaptation to a range of environments in the region. The requirements of Libya, Tunisia, and lowland Algeria are met by the ICARDA West Asian breeding program. But, late-maturing material is required for high altitude areas of Algeria and early-maturing lines are required in Morocco with resistance to rust.

Armed with this understanding of the specific adaptation of the lentil crop and the various consumer requirements of different geographic areas, we have designed the base breeding program as a series of separate, but finely targeted, streams linked closely to national breeding programs.

The three major target agro-ecological regions of production of lentil are 1. S. Asia and E. Africa 2. Mediterranean low to medium elevation and 3. High elevation area. These correspond to the maturity groups of early, medium and late maturity. Within each of these major regions there are specific target areas. Thus, for example, within the Mediterranean low to medium elevation region, specific target areas are 1. the major production area of 300-400 mm annual rainfall, 2. arid areas with < 300 mm annual rainfall, 3. Morocco, where there is the additional problem of rust and 4. Egypt, where lentil is irrigated. Each of these target areas has slightly different blends of key traits for recombination. The target areas/regions and key traits for selection/recombination are tabulated in Table 3.1.1.

Table 3.1.1. Target agro-ecological regions of production of lentil and key breeding aims.

Region	Key traits for recombination
Mediterranean low to medium elevation	
1. 300-400 mm ann. rainfall	Biomass (seed + straw), attributes for mechanical harvest & wilt resistance
2. <300 mm ann. rainfall	Biomass, drought escape thru' earliness
3. Morocco	Biomass, attributes for mechanical harvest & rust resistance
4. Egypt	Seed yield, response to irrigation, earliness & wilt resistance
High elevation	
1. Anatolian highlands	Biomass & winter hardiness
2. N. African highlands	Seed yield & low level of winter hardiness
South Asia and E. Africa	
1. India, Pakistan, Nepal & Ethiopia	Seed yield, early maturity, resistance to rust, ascochyta and wilt
2. Bangladesh	Seed yield, extra earliness & rust resistance

Based on the premise that local selection for adaptation to a specific target area is the most efficient selection method, selection at ICARDA in West Asia is limited to adaptation to the home region - Mediterranean low to medium elevation and for traits where we have a comparative advantage such as vascular wilt resistance. As a result, the breeding program has decentralized to work closely with national programs.

For the home region the breeding program uses a bulk-pedigree system with off-season generation advancement (at Terbol, Lebanon 950 m elevation), single plant selection in the F_4 generation and selection of progeny rows for vascular wilt resistance in the F_5 generation. For the other regions, crosses are agreed with cooperators and made at Tel Hadya; the generations advanced in the off-season and the segregating populations shipped to national cooperators for local selection. We started making specific crosses in 1985 and since then have made specific crosses for Algeria, Bangladesh, India, Jordan, Morocco, Nepal, Syria and Turkey. A total of approximately 200 crosses are made annually. One avenue for the distribution of segregating material is through these country-specific crosses. The international trial network provides another system whereby these crosses can be tested sub-regionally (see section 3.1.1.3). Selections made by NARS are fed back into the international trial system for further distribution. The results from this decentralized system are described in section 3.1.2.

3.1.1.2. Yield trials

Selections from the breeding program for the Mediterranean low to medium elevation region are tested at three locations varying in their annual average rainfall, namely Breda (long-term average annual rainfall total ca 260 mm) and Tel Hadya (ca 330 mm) in Syria and Terbol (ca 550 mm) in Lebanon in preliminary yield trials in the F_7 generation and in advanced yield trials the following season. The lines are also re-tested synchronously for vascular wilt resistance in the wilt-sick plot at Tel Hadya (see Section 3.1.1.4) to ensure that only high-yielding, wilt resistant lines are advanced in the breeding program. The winter of 1993/94 season was relatively mild but temperatures were abnormally high in

the early pod-filling stage at all sites in mid-April. Rainfall in the 1993/94 growing season was below average 478 mm at Terbol, above average at Tel Hadya (373 mm) and Breda (299 mm).

The results of the yield trials are summarized in Table 3.1.2. The percentage of entries yielding significantly more than the best check reached a maximim of 5.3 % at Terbol. But considerably more entries ranked for yield above the best check at all sites. The coefficient of variation was acceptable at 7 to 13 % in Terbol and 8-14 % in Breda, but it was too high at Tel Hadya (10-37 %), where Orobanche infestation occured in some yield trial plots. **W. Erskine**.

3.1.1.3. International nurseries

The lentil international breeding nurseries have evolved in response to the needs of NARSs from the provision of untargeted yield trials to a diversified array of crossing blocks/resistant sources, segregating populations and yield trials for each of the three major target agro-ecological regions of production (Table 3.1.3). Since 1987, for example, we have diversified and targeted the supply of segregating material from two into four different nurseries - Cold Tolerant, Large-seeded, Small-seeded and Early. In the same period, new nurseries of stress resistant material have been launched against rust, Ascochyta blight, Fusarium wilt and cold. This season we launched a new stress nursery - Lentil International Screening nursery for drought avoidance - targeted to dry areas in the Mediterranean Basin (see Section 3.1.1.7. Breeding for adaptation to dry Mediterranean environments through drought avoidance).

Table 3.1.2. Results of the lentil yield trials (preliminary and advanced) for seed (S) and biomass (B) yields (kg/ha) at three contrasting rainfed locations; Terbol (Lebanon), Tel Hadya and Breda (Syria) during the 1993/94 season.

Location	Terbol		Tel Hadya		Breda	
	S	B	S	B	S	B
Number of trials	6	6	11	11	7	7
Number of test entries*	132	132	243	243	155	155
% of entries sig. (P<0.05) exceeding best check**	5.3	3.8	0.4	2.8	0.0	00
% of entries ranking above best check (excluding above)	40.9	37.9	21.0	16.0	20.6	271
Yield of top entry (kg/ha)	2883	9717	2348	6957	1552	3730
Best check yield (kg/ha)	2278	7855	1841	5506	1183	3098
Location mean (kg/ha)	2137	7492	1591	4693	1080	2798
Range in C.V. (%)	7-13	6-10	10-37	7-21	8-14	8-15

*   Entries common over locations.

** Large-seeded checks: ILL 4400 long-term, 78S26002 improved; small-seeded checks: ILL 4401 long-term, ILL 5883 improved.

Table 3.1.3. Lentil international breeding nurseries showing target regions and type of nursery in the 1994/95 season.

Type of nursery	Region		
	Mediterranean	Lower latitudes	High elevation
Resis. source/ crossing block	Large-seeded Small-seeded * Fusarium wilt* Drought escape*	Early* Rust* Ascochyta blight*	Cold tolerant*
Segregating population	Large-seeded* Small-seeded*	Early	Cold tolerant*
Yield trial		Early	

* Launched since 1987.

There is a slow increase in the number of entries in international trials provided by national programs. It is our aim to increase the input of national programs into the international testing program. The line-up of the 1995 international trials included three lines from Pakistan, two from Turkey and one each from Bulgaria, Slovakian Republic, Russia and USA. Other entries have been supplied by NARSs and are in multiplication for inclusion in next season's trials. **W. Erskine and R.S. Malhotra.**

3.1.1.4. Screening for vascular wilt resistance

Vascular wilt caused by *Fusarium oxysporum* f. sp. *lentis* is the major fungal disease of lentil in the Mediterranean region.

Comparison of screening methods

Several screening methods for resistance to lentil

vascular wilt have been developed at ICARDA. A comparison was made of 63 genotypes, spanning the complete range in wilt reaction, using the following methods/seasons: 1. Field sick plot (described in Legume Program Annual Report 1993) in 1993 2. Field sick plot in 1994. 3. Pots (diameter c. 25 cm) with sterilized soil in plastic house. 4. Cone-shaped pots (diameter c. 4 cm, length 14 cm) with sterilized soil in plastic house. 5. Test tubes with semi-solid, Hoaglands medium in the laboratory.

Artificial inoculation with isolate #31 of *F. oxysporum* f.sp. *lentis* was carried out following the procedure described earlier (FLIP Annual Report 1988) in Methods 3-5. Scoring (1-9 scale) was done repeatedly in all methods in the laboratory and the plastic house and as % of wilted plants in the field to monitor changes in susceptibility with plant age.

In all the screening methods, the full range in reaction from highly resistant to highly susceptible was found. A strong correlation ($r = 0.991$) between the final scores in the field of the two seasons clearly indicated the high repeatability of the field screening over seasons (Table 3.1.4). The correlations between early scoring in the field and the final field scores were $r = 0.806$ in 1993 and $r = 0.629$ in 1994 and lower than the correlation in final score between seasons, because the timing of appearance of wilt symptoms varies over genotypes. There was good correspondence of final field results with those from screening in the plastic house (both methods) and the laboratory with $r > 0.7$. Clearly, all tested methods can be used to screen for resistance to lentil vascular wilt. The choice of method depends, however, on seed requirement/availability, the availability of environmental control and the test duration.

Table 3.1.4.   Table of phenotypic correlation coefficients between 63 genotype means for reaction to lentil vascular wilt in five methods/seasons for the first and last disease score (Sc).

Method/season			1	2	3	4	5	6	7	8	9
Field 1993	Sc1	(1)	1.000								
Field 1993	Sc7	(2)	0.806	1.000							
Field 1994	Sc1	(3)	0.844	0.612	1.000						
Field 1994	Sc7	(4)	0.799	0.991	0.629	1.000					
Pots	Sc1	(5)	0.732	0.915	0.604	0.922	1.000				
Pots	Sc5	(6)	0.618	0.722	0.497	0.733	0.859	1.000			
Cones	Sc1	(7)	0.742	0.919	0.589	0.922	0.875	0.685	1.000		
Cones	Sc4	(8)	0.605	0.809	0.483	0.815	0.753	0.584	0.869	1.000	
Test tubes	Sc1	(9)	0.691	0.856	0.605	0.873	0.858	0.704	0.799	0.652	1.000
Test tubes	Sc5		0.610	0.819	0.477	0.826	0.762	0.613	0.785	0.730	0.526

r at P = 0.01 with 60 degrees of freedom = 0.325

Refinement of field screening technique

On farmers' fields the distribution of lentil vascular wilt is patchy. Screening for resistance to lentil wilt in the wilt-sick plot at Tel Hadya is conducted in replicated plots interspersed with a susceptible check, repeated every third plot. The susceptible check can be used to monitor the uniformity/heterogeneity of the experimental area.

In an effort to refine the field screening technique in order to cope with spatial variation in disease incidence, we tested the efficiency of the susceptible check as a local control in adjusting the test plot results in a core collection of 577 accessions of germplasm. Covariance analysis using check values was compared with an analysis of variance of unadjusted values. Different check values were compared in three separate covariance analyses: 1. Nearest check plot value; 2. Check value based on the mean of the two nearest check plots; and 3. Check value based on two-thirds of the nearest check plot value and one-third of the next-nearest check plot value. The efficiency of covariance analysis was gauged by a comparison of 1. the standard errors of genotype means, 2. variance ratio of genotype v. residual in the variance and covariance analyses and 3. the residual mean square of the variance and covariance analyses. Wilt screening was initiated in the second week of April and closed after nine ratings.

There were highly significant differences among genotypes in resistance to lentil vascular wilt, assessed as the percentage of wilted/dead plants, at every scoring date. Over all scores, there was a significant improvement in efficiency through the use of check values as measured by residual mean square, standard error of genotype means and variance ratio (genotype/residual).

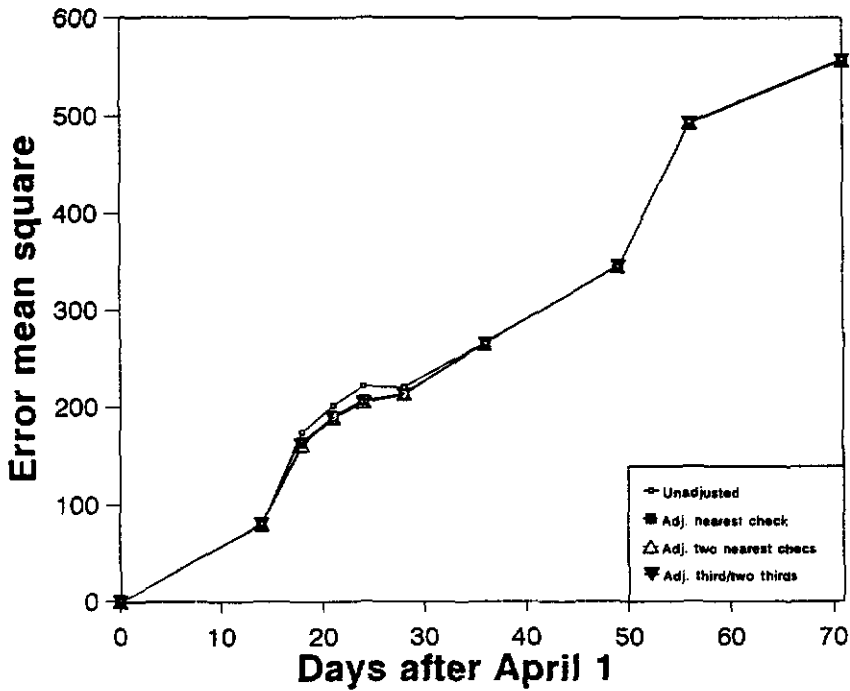**Figure 3.1.** Error mean squares from analysis of variance of percentage wilt scores of unadjusted treatment values compared to adjusted values from covariance analyses based on 1. nearest check value,2. mean of two nearest check values and 3. two thirds of the nearsest check value and one third of the next nearest check value over nine scores in the wilt-sick plot at Tel Hadya in the 1992/93 season.

Although the gain in efficiency was slight at 3% over all scores, it varied through time, as is apparent in Figure 3.1. The susceptible check (ILL 4605) is an 'early wilter' with an overall mean of more than 80 % of plants wilted by score 5 (Figure 3.2); consequently it was more efficient at 'lighting up' spatial variation in wilt inoculum density early in the season, when it showed a high variance, than later in the season. Differences in efficiency among the three check values were non-significant.

The results emphasize the relative uniformity of disease pressure throughout the Tel Hadya wilt sick area. The use of local control through covariance analysis of systematically arranged check values will be of greater use in land which is less uniformly infected.
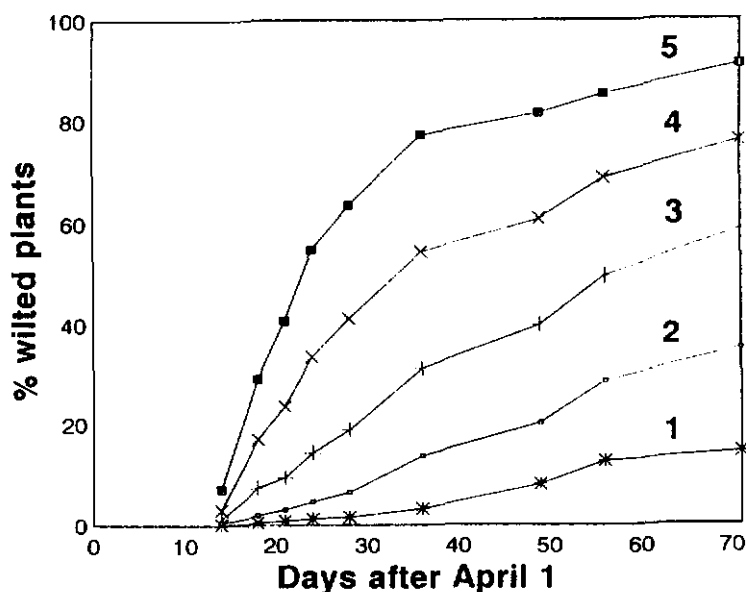

Figure 3.2. Mean and variance of the percentage wilted plants of the susceptible check (ILL 4605) over time in the wilt-sick plot at Tel Hadya in the 1992/93 season.

Different responses to wilt in a core collection of lentil germplasm

In earlier reports (FLIP Ann. Reports 1989, 1990 & 1991), the seedling wilt reaction of a genotype was found to differ from the adult reaction in some genotypes in the plastic house. In the field in North Syria, similar temporal differences in reaction occur. Wilt symptoms only appear after the onset of flowering, but some genotypes wilt early in their period of reproductive development, whereas others exhibit symptoms just prior to senescence. We examined the variation in response to wilt in a core collection of germplasm in order to quantify the different genetic patterns of response.

The core collection of 577 accessions was split into five response groups, by cluster analysis on the basis of Mahalanobis distance, using the wilt ratings in nine scores. The means of the five groups are shown graphically in Figure 3.3. The proportion of variance accounted for in joint regression analyses of linear and exponential models over genotypes varied over groups; the responses to wilt of all groups except Group 3 (the most-resistant group) were better described by an exponential than a linear model (Table 3.1.5). Joint regression analysis of the data of Group 3 was only possible with the linear model, because some highly resistant lines with zero wilt symptoms were impossible to fit into an exponential strait-jacket.

Screening of breeding material for wilt resistance

The screening of breeding material in the wilt sick plot at Tel Hadya continued in the 1993/94 season with a total of 1229 lines tested, an increased volume of material in comparison to the 897 lines screened the previous season.
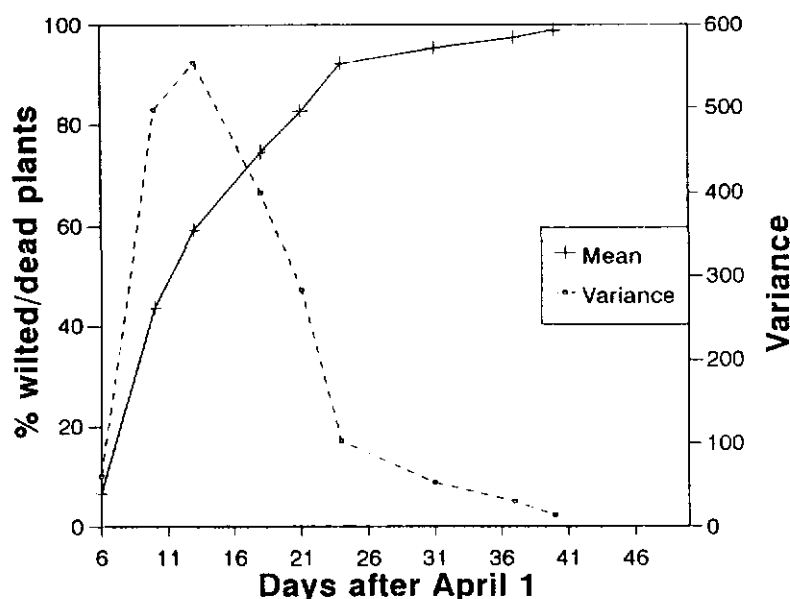
Figure 3.3. Average percentage of wilted plants of five groups of accessions (labelled 1-5 with 1 as the most resistant group) differing in response to vascular wilt scored over time in the wilt-sick plot at Tel Hadya in the 1992/93 season.

The tested lines may be divided into two categories: Cycle I - new untested lines and Cycle II - lines tested previously (primarily those which showed a resistant reaction). On the basis of mean over replicates, the percentage of entries with a highly resistant (0-5% wilted plants, mean over replicates) or resistant (>5-20% wilted plants, mean over replicates) response to lentil vascular wilt were 62.1 % for Cycle I and 89.7 % for Cycle II (Table 3.1.6). However for breeding purposes, we have selected for advancement a specific subset of these lines - those which have a maximum plot score of < 20 % wilted plants in the last score. This screening is a key and integral part of the breeding program; this is elucidated in section 3.1.1.1.

Table 3.1.5. The mean of % wilted plants, percentage of variance accounted for by joint regression analysis and residual degrees of freedom (d.f.) using linear and exponential models of individual genotype responses in five groups (with number of accessions (acc.) in each group) of lentil accessions, grouped on the basis of differing response to lentil vascular wilt in the 1992/93 season.

Group	Mean % wilted plants	Number of acc.	Linear %	Residual d.f.	Exponential %	Residual d.f.
1	14.6	176	85.9	1232	-	-
2	35.4	145	89.2	1015	91.3	870
3	58.9	91	91.1	637	94.6	546
4	76.2	78	83.4	546	95.8	468
5	91.3	87	72.2	609	96.5	522

Table 3.1.6. Results of field screening for resistance to lentil vasular wilt in the wilt-sick plot at Tel Hadya in the 1993/94 season. Cycle I indicates screening for the first time and Cycle II indicates confirmation of a resistant reaction.

Disease category	Cycle I		Cycle II	
	Number of entries	Percentage of entries	Number of entries	Percentage of entries
Highly resistant (0-5% wilted plants)	442	39.7	44	37.9
Resistant (>5-20% wilted plants)	249	22.4	50	43.1
Tolerant (>20-35% wilted plants)	110	9.9	15	12.9
Susceptible (>35-65% wilted plants)	139	12.5	4	3.4
Highly susceptible (>65-100% wilted plants)	173	15.5	3	2.6
Total # screened	1113		116	

Wilt incidence and inoculum density

The causal organism of lentil vascular wilt, *Fusarium oxysporum* f.sp. *lentis*, is a soilborne fungus which can infect plant roots throughout the growing season. The pathogen persists in the soil as chlamydospores which remain viable for an extended period. Being a single cycle disease, the inoculum level in the soil of the pathogen plays a critical role in disease development. A study was made of the relationships between inoculum density, disease incidence and crop losses.

The study was divided into two parts: the first part investigated the association of inoculum/micro-propagule density and wilt incidence both *in vitro* and in the field; the second part examined the relationship between wilt incidence and yield loss in the field.

In the laboratory, two lentil lines, contrasting in their resistance to wilt in the field, were challenged with inoculum concentrations from 0 - 10^6 microconidia/ml. Disease reaction was positively correlated to inoculum density. However, at intermediate concentrations the lines differed in their disease reaction. In the field in two seasons, inoculum density was found to be unrelated to disease incidence on susceptible lentils (FLIP Annual Report 1992). Thus, although *in vitro* studies clearly showed that vascular wilt incidence on lentil increased with the inoculum density of the fungus, in the field there was no evidence of such a relationship with soil samples collected either before sowing or during lentil reproductive growth. This apparent lack of relationship in the field may be for several reasons: the effect of antagonists in the soil, representational error (soil sample to plot) and differences in temperature between the stable laboratory environment and the fluctuating field one.

Wilt incidence and yield loss estimation

Six field trials conducted in northern Syria from 1986 to
1992 were affected by vascular wilt, assessed for yield
and used to relate yield loss to disease incidence. At
Tel Hadya there were two trials comprising genotypes
selected, at random, from the advanced generations of a
lentil breeding program with 30 genotypes in Trial I and
25 genotypes in Trial II. Trials at the other locations
comprised a reduced number of genotypes grown as part of
the on-farm verification of cultivars prior to their
registration.

At Tel Hadya the mean wilt incidence among genotypes
ranged from 0.3 - 95.0 % in Trial I and from 0.7 - 85.7 %
in Trial II. The maximum grain yields attained were over
2 t/ha, whereas the lowest grain yields were below 100
kg/ha in heavily diseased plots.   In Trial I the
regressions of seed yield on to wilt incidence for each
individual genotype over replications were significant at
$P = 0.05$ for 14 out of 30 genotypes. The goodness of fit
of these regressions (R^2) was correlated to wilt incidence
at $r = 0.589$, indicating that wilt-susceptible genotypes
had a better fit of their regressions than wilt-resistant
genotypes, for which the regressions were non-significant.
Wilt-resistant genotypes were dropped from further
analysis.   For the susceptible genotypes, the overall
correlation of wilt incidence with grain yield was $r = -
0.700$ with 136 degrees of freedom (df) In Trial II, the
corresponding correlation among susceptible genotypes was
$r = -0.678$ with 63 df.

The percentage reduction in grain yield (kg/ha) per
percent change in wilt incidence among susceptible
genotypes was $b = -0.859 \pm 0.077$ in Trial I and $b = -0.970
\pm 0.135$ in Trial II.

For straw yield, the percentage reduction per unit change in wilt incidence (%) was considerably lower at b = -0.416 ± 0.056 in Trial I and b= -0.381 ± 0.096 in Trial II.

On farmers' fields the wilt incidence ranged from 0 to 100 % wilted plants at Suran (Hama Governorate) and the lowest range in wilt incidence was at Taftanaz (Idlib Governorate), where the highest wilt incidence was 40 %. The coefficient of determination (R^2) of the effect of wilt on seed yield varied from R^2 = 0.223 at Efes (Idlib Governorate) in 1992 to R^2 = 0.97 at Efes in 1986, showing the variation in grain yield attributable to wilt incidence. The trials at Efes were sown at different sites in the same field, which was not evenly infected with the causal organism.

Over all six trials the percentage reduction in grain yield per unit change in wilt incidence (%) was b = 0.851 ± 0.117 with a range over trials from b = 0.41 to b = 1.22 %.

In Syria, lentil vascular wilt is the key disease limiting lentil production and symptoms appear in the field after the onset of flowering in the month of April. By the time symptoms appear during reproductive growth, most biomass accumulation has already occurred, leaving little scope for compensatory growth by healthy, neighboring plants. Consequently, the incidence of the lentil vascular wilt may be clearly related to the loss in seed yield. This study showed that the percentage change in grain yield per percentage change in wilt incidence (%) was b = 0.851 ± 0.117 over six field trials. This general relationship may be used to predict crop losses from wilt incidence in N. Syria. The model may be useful in other areas of West Asia and North Africa, but care, however, must be taken to confine the use of the model to the

reproductive stage of growth, for example, in India, where symptoms may appear either in early vegetative growth or during reproductive growth.

The distribution of symptoms of lentil vascular wilt in the field is patchy and rarely uniform over a crop. For example, in the two large trials in Tel Hadya the wilt incidence in plots of susceptible varieties varied from 0 % in one replication to up to 100 % in an adjacent replication. Hence, the most practical method of assessing disease loss is to estimate both wilt incidence and its area and then to relate this to the yield of an unaffected, healthy contiguous part of the crop.

Seed transmission of *F. oxysporum* f. sp. *lentis?*

A seed survey was undertaken to establish whether *F. oxysporum* f. sp. *lentis* is seed-borne. Plant samples from genotypes with different reactions to lentil wilt were taken from wilt infected fields. Surface-sterilized stem segments revealed the presence of the causal organism only in the samples of the susceptible genotypes. Seed samples (200 surface sterilized seeds and 200 unsterilized seeds/genotype) from these susceptible genotypes, and also from resistant ones, were incubated for a week on potato dextrose agar. Only three colonies of *Fusarium* sp. were recovered and none were pathogenic to lentil. This confirms our earlier finding that *F. oxysporum* f. sp. *lentis* is not seed-borne.

Seed microflora

The composition of the microflora from the above unsterilized seed samples was investigated. Seeds from the wilt-sick plot in Tel Hadya were predominantly infected with *Rhizopus* sp. Seeds from other infected

areas of the Tel Hadya farm had more diverse microflora with the following frequencies: *Penicillium* spp. (33.9%); *Cladosporium herbarum* (19.5%); *Aspergillus* spp. (14.1%); *Rhizopus* sp. (10%); *Sporobolomyces* sp. (9.4%); *Alternaria alternata* (4%); *Stemphylium* sp. (1.1%); *Fusarium* sp. (1.1%). Other less common (<1%) fungi were *Phoma* sp., *Cephalosporium* sp., *Ostrachoderma* sp., *Ulocladium* sp., and *Gliomastix* sp. Bacterial colonies represented 10.5% of the total colony number. In the surface-sterilized samples only 1.9% of the seed gave rise to fungal colonies, most of which were *Penicillium* spp., *Cladosporium* spp. and *Aspergillus* spp. Surface sterilizaton of seed increased the germination from 85 to 95 %. **B. Bayaa and A. Abbas (Aleppo University) and W. Erskine.**

3.1.1.5. Screening for resistance to rust

Rust caused by *Uromyces fabae* is the major fungal disease affecting lentil in the Indian sub-continent, Ethiopia and Morocco. Systematic screening for resistance was initiated four seasons ago in collaboration with the Moroccan national program in a disease 'hot-spot' at Jemma Sheim. Unfortunately, the winter in Morocco was dry and screening was not possible this year.

3.1.1.6. Screening for resistance to downy mildew

The 1993/94 season at Tel Hadya provided a unique opportunity to screen the breeding material for resistance to downy mildew (*Peronospora lentis*), a disease causing increasing concern in newly-reclaimed and irrigated parts of Egypt. The disease, spread by sporangiospores, is common and serious only under cool and humid conditions. Symptoms are seen on the aerial plant parts with leaves

near branch tips curled, dwarfed and twisted with fine, dirty pinkish tufts of fungal growth of sporangiospores developing on the under-surface of leaves. The extent of damage depends on the duration of cool and wet conditions.

In Syria the disease is relatively unimportant because of the rapid rise in temperature in the spring.

At Tel Hadya in 1994, downy mildew infection was observed on lentil in late March/early April. The preceding period in late February and March, during which disease had developed, was wetter than average, but temperatures were around average with a monthly mean of 7.6 °C in February and 10.9 °C in March (Figure 3.4). Infection was halted in mid-April by the onset of hot weather with maximum daily temperatures crossing 30 °C.
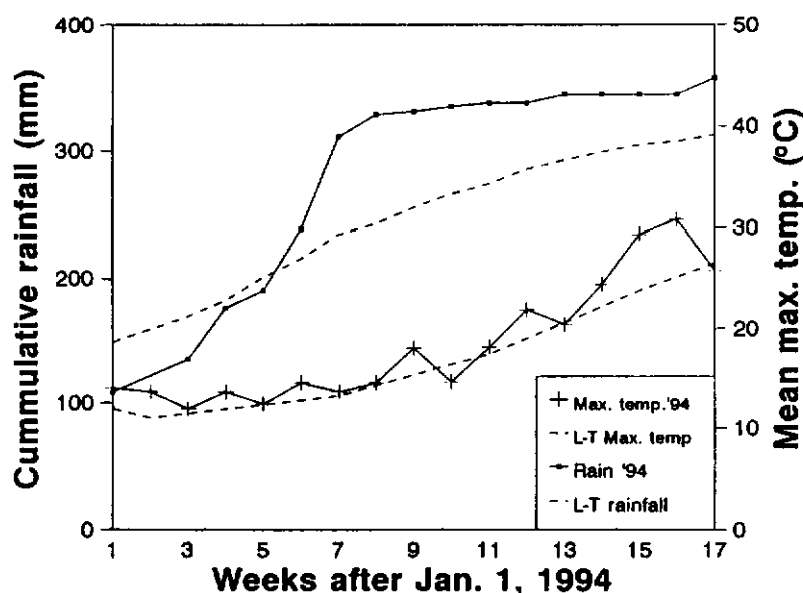

Figure 3.4. Data of the 1993/94 season and the long-term (L-T) average of cummulative seasonal rainfall (mm) and mean maximum temperature (°C) at Tel Hadya, Syria.

The breeding field was scored in early April and found uniformly infected. The disease ratings in different trials of common checks were very similar. There were significant differences among genotypes in their reaction to the disease with the percentage of foliage affected varying over genotypes from 5-94%. A quarter of the tested lines showed 50% or more of the foliage affected by the disease in two trials covering 104 lines. In contrast, 11% of lines showed less than 15% of foliage affected.

Associations of infection rating with other plant traits were examined. The downy mildew ratings were negatively correlated with plant height in both trials indicating the stunting effect of the disease on the vegetative canopy. Correlations between disease rating and grain and biomass yields were non-significant, but disease rating was negatively and significantly correlated with straw yield. The period of cool, wet weather this season in March was interrupted by a hot, dry spell in April, which limited disease spread at flowering and prior to pod formation. In these circumstances damage was confined to the vegetative canopy.

Previous control strategies for this disease are based on chemical control and crop management to reduce canopy humidity levels. This is the first report of genetic variation in reaction to the disease. Although the results have to be confirmed, the results suggest that the control of downy mildew through host-plant resistance is also a possibility for areas with a significant downy mildew problem. **W. Erskine, B. Bayaa (Aleppo University) and N. Abou-Zaid (ARC, Giza).**

3.1.1.7. Breeding for adaptation to dry Mediterranean environments through drought avoidance

In the Mediterranean region lentil is usually sown in the zone with between 300 and 400 mm annual rainfall. For adaptation to _even_ drier areas, drought escape through early flowering and maturity has been clearly identified as the key mechanism. Early material is already being produced in the Southern latitudes stream of the breeding program and this same material is now being exploited in another direction i.e. for arid Mediterranean environments.

In the 1993/94 season we launched a new international screening nursery targeted toward arid Mediterranean conditions of the best early flowering/drought-avoiding lines already in the breeding program (see section 3.1.1.3). Testing of new early lines was undertaken in yield trials at Tel Hadya and Breda in the 1993/94 season. Some selected examples of performance in these trials are given in Table 3.1.7. The early flowering check - ILL 4605 matured one week before the local check.

Table 3.1.7. Time to maturity (d) and grain yield (kg/ha) in Breda and Tel Hadya of selected entries from the drought avoidance trials in the 1993/94 season.

Name	Time to maturity	Grain yield	
		Breda	Tel Hadya
92S72970	120.5	1248	1656
ILL 6025	121	1170	1984
ILL 4605 (early check)	113	1140	1075
ILL 4401 (local check)	125	1003	1543
LSD $_{P=0.05}$	4.0	184	471

In the wetter site Tel Hadya, the local check ILL 4401 yielded more than the early check, but at the dry site - Breda the earlier check yielded more. Some early lines exceeded the yields of the best checks in both sites. The screening of early lines in dry areas will be continued. **W. Erskine**.

3.1.1.8. Screening for winter hardiness

Lentil is currently sown in spring in Turkey at elevations above c. 850 m elevation on c. 250 000 ha. Research in Turkey has indicated that yields may be increased by up to 50 % by early sowing in late autumn with winter hardy cultivars. However, the use of such cultivars is not yet widespread in Turkey, because at high elevation the level of winter hardiness is inadequate in cold winters and winter hardiness has not yet been transferred from germplasm sources into acceptable cultivars.

A major program to recombine yield with the necessary winter hardiness is underway at The Central Research Institute for Field Crops in Turkey through field screening at Hymana. Crosses and early generation material are being produced by the ICARDA base program in Aleppo, Syria and segregating populations are sent to Hymana for selection under severe winter conditions (see scetion 3.1.1.1).

Last season the winter cold did not kill the winter-susceptible checks in the trials at Hymana and no progress was made in screening for winter-hardiness. However, some selection for seed yield was possible in the winter crop. The spring crop of 1994 failed completely at Hymana because of inadequate spring rain, indicating once again the potential of winter-sowing. The material has been resown this year for screening at Hymana and also at

another site in Sivas area with a colder climate. I.
Kusmenoglu (Central Research Institute for Field Crops,
Ankara, Turkey) and W. Erskine.

3.1.1.9. Use of phenology model for highland lentils

To support the research to identify superior winter-hardy
lentils for the highlands (see above), we have examined
the consequences of the change from a spring-sown,
highland lentil cropping system to a winter-sown system
using a model of flowering, as part of an ODA-sponsored
'downstreaming project involving Reading University,
Turkey and ICARDA.

We previously developed and verified a simple
descriptive model of flowering, which is governed by
temperature and photoperiod, in lentil as follows:

$$1/f = a + bT + cP,$$

where f is the time (d) from sowing to first flowering, T
and P are the respective values of mean temperature and
photoperiod in the same period, and a, b and c are
genotypic constants.

In a survey of patterns of morphological variation in
the world germplasm collection of lentil, phenology was
found as the key to the adaptation of the crop on a macro-
geographic scale. Dissemination of the lentil crop
following domestication in West Asia to the lower
latitudes such as Ethiopia and India has depended on
selection for intrinsic earliness and reduced sensitivity
to photoperiod. Movement from West Asia to the higher
latitudes accompanied by spring-sowing has resulted in a
modest reduction in photoperiod sensitivity and an
increase in temperature sensitivity.

The flowering model has also been used to study the consequences of the change from a spring-sown, highland lentil cropping system to a winter-sown system (Keatinge *et al.*, 1995). Definitions were made of the first date of 'safe' germination in the autumn (receipt of 15 mm of rain within a five day period after September 1) and the latest autumn sowing date (date on which probability of average air temperature falls below 4 ^{0}C first exceeds 10%) to define the window of opportunity for autumn germination and emergence. Additional definitions were made in spring of the first 'safe' sowing date and the first and last 'safe' flowering dates, juxtaposed between the threats of late frosts and the onset of drought. The definition for the first 'safe' sowing date in spring was the inverse definition of that used to define the end of the season in winter i.e. the date at which air temperature averaged > 4 ^{0}C in at least nine years out of ten. The first 'safe' flowering date in spring was defined as the date of the last recorded frost in the 40 year data set at the target location. The last 'safe' flowering date was defined as the date on which the weekly probability of rain declines to below 25%.

Meteorological data (40-year runs) at three representative Anatolian sites - Çorum (798 m a.s.l.), Sivas (1285 m a.s.l.) and Erzurum (1869 m a.s.l.) were analyzed to establish the timing and duration of the autumn germination window and the spring flowering window at each location. Simulating various sowing dates, the model was able to clarify appropriate periods for each site. This nullified the need for expensive field experiments of date of sowing across highland Turkey, providing a major cost saving to the national program.

Then the model was used to examine the consequences of sowing a range of cold-tolerant germplasm differing in response to temperature and photoperiod (Figure 3.5).
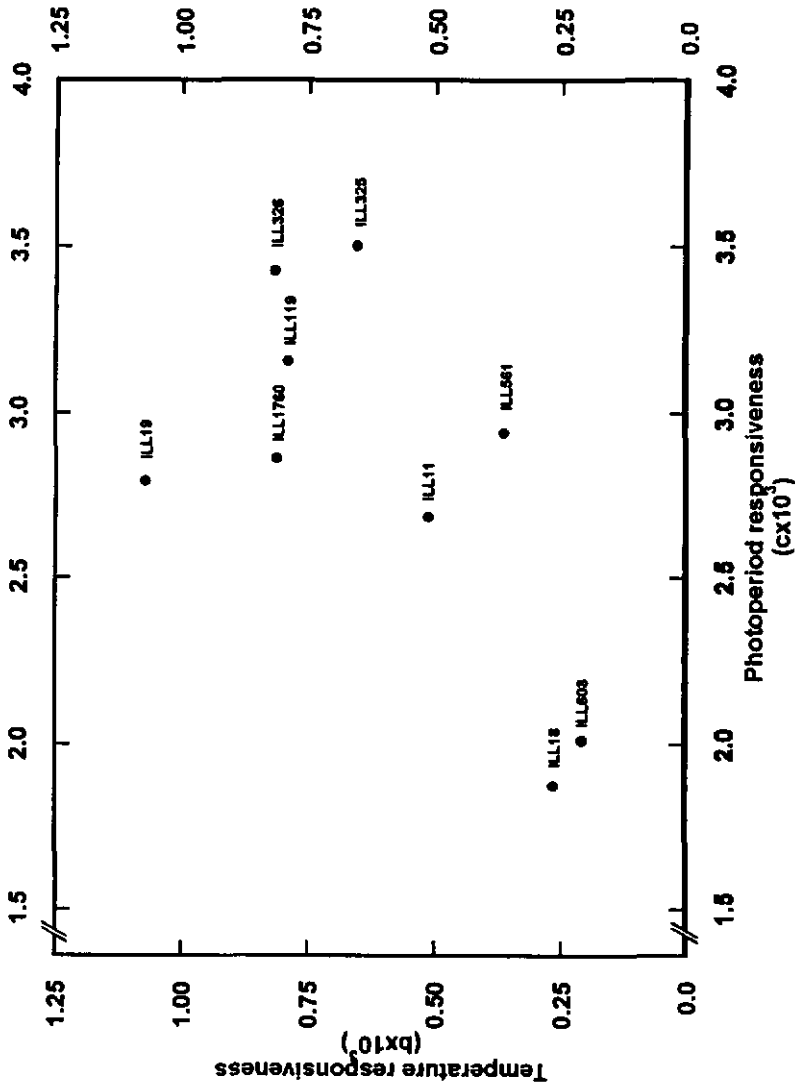
Figure 3.5. Temperature- and photoperiod-response constants (*b* and *c* of Equation 1) of nine cold-tolerant lentil genotypes for rate of progress from germination towards first flowering (1/*f*).

Flowering times were calculated for a range of genotypes spanning the variation present in response to temperature and photoperiod among winter-hardy material. These flowering dates were then superimposed on the 'safe' flowering windows defined above for each location.

In Figure 3.6 the area defined by the two vertical and the single horizontal broken lines (representing the 'safe' germination window and 'safe' first flowering date, respectively) indicates that all genotypes at Erzurum, with the exception of very photothermally-sensitive material such as ILL 326, will flower too early if germinated within the 'safe' window of opportunity in early winter. In contrast, as a result of the comparatively milder environments at both Sivas and Çorum, all genotypes, with the exception of very photothermally-insensitive material such as ILL 18, can flower safely if germinated within the 'safe' window of opportunity. Sowing photothermally-insensitive accessions such as ILL 18 and ILL 603 would be an extremely risky strategy at all locations and would frequently risk complete crop failure from frost damage, because they were unable to synchronize flowering into the 'safe' spring window, despite their considerable tolerance to winter cold. The most useful photothermal response for a winter-sowing at high elevations was a highly photoperiodic response, which could synchronize flowering within a particular 'safe' window, almost regardless of sowing date. **Keatinge, J.D.H., Aiming, Qi, Kusmenoglu, I., Ellis, R.H., Summerfield, R.J., Erskine, W. and Beniwal, S.P.S.**
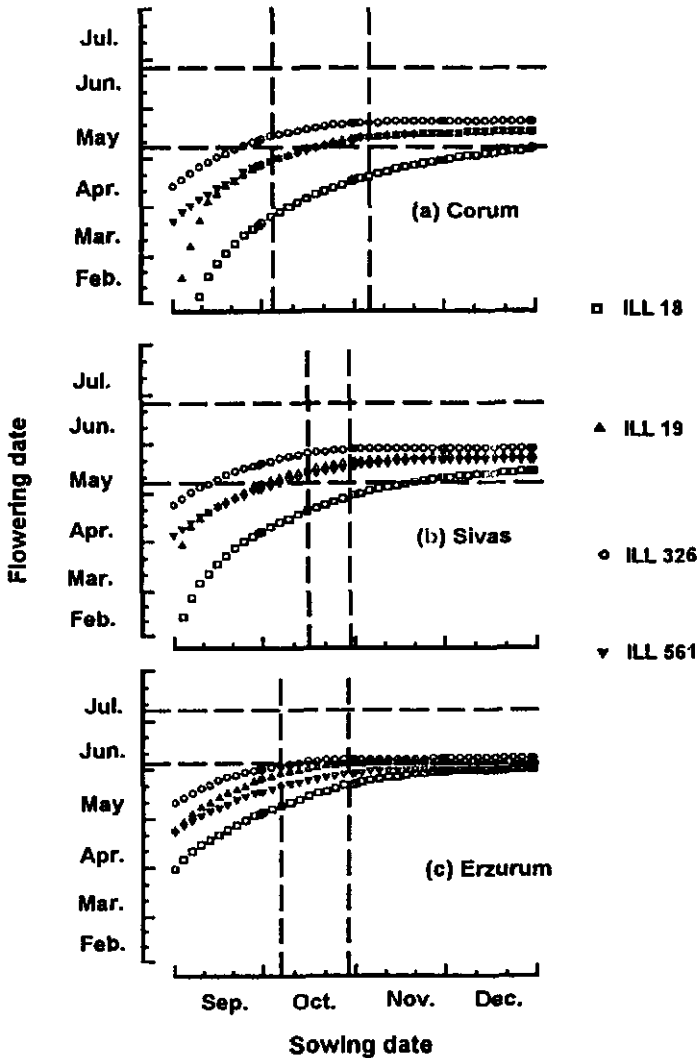
Figure 3.6. The mean first flowering date over 40 years of four accessions of lentil at Çorum, Sivas and Erzurum with winter-sowing dates from 1 September to 31 December. The two broken vertical lines represent the safe germination window for winter-sowings and the broken horizontal line represents the first safe flowering date in spring.

3.1.1.10. Exploitation of wild lentils for yield improvement

Soon after the foundation of ICARDA a few exploratory crosses were made between the cultivated lentil and its putative wild progenitor *L. culinaris* ssp. *orientalis*. In 1984 we selected 10 lines for distribution worldwide in the Lentil International Nursery Program from bulk segregating populations of these wild x cultivated crosses. Among these selections containing genes introgressed from the wild, the small-seeded ILL 5700 ranked 3rd, 1st, 7th and 2nd for average yield among 24 entries of the Lentil International Yield Trial tested in 13-15 countries from 1985 to 1988. This selection with wild parentage has since been widely used in crossing to introgress wild genes into the cultivated plant. Recently, further crosses of wild x cultivated lentil have been made to study more systematically the agronomic potential of this wide hybridization.

A line x tester mating scheme was used with three diverse cultivated lines (ILL 2501 - ex India; ILL 5582 & ILL 5674 - ICARDA) and seven male parents of *L. culinaris* ssp. *orientalis*. To ensure diversity in the wild parental population, we selected from the most diverse origins possible within the distribution of the wild material vis. Cyprus, Jordan, Syria, Turkey & former USSR. In the 1992/93 season, the parents and crosses in the F_2 generation were grown at Tel Hadya, Syria and, in the following season and generation, they were studied at the University of Amman, Jordan (within the context of a MSc thesis) and in Egypt. To date, results are only available from Syria.

The experiment of parents and crosses in the F_2 generation was grown in a randomized block design with three replications. Plots were separated by a uniform

border row of ILL 2126 and nylon mesh was placed on the soil to catch dehisced seed.

Biomass yield varied among the cultivated parents from 151 to 343 g m^{-2} and among the wild parents from 4 to 98 g m^{-2}, indicative of the wide gulf between the yields of wild and cultivated lentil (Table 3.1.8). Heterosis from the mid-parent was high at 51.3 % on average. Heterosis above the better parent was only present in crosses with the low yielding cultivated lentil line ILL 2501. Heterosis above the better parent was not found in crosses with the currently recommended cultivar ILL 5582, consequently it had a low general combining ability (GCA). With substantial heterosis (deviations from the model of the mid-parent mean) for biomass, specific combining ability (SCA) was of greater importance than general combining ability, indicating the importance of non-additive gene effects.

For grain yield, general combining ability effects were of greater importance than specific combining ability effects with the ratio of variances GCA/SCA = 0.74, and, although heterosis from the mid-parent mean was significant, it was smaller in magnitude than for biomass.

Clearly, for grain yield additive gene effects were of more importance than non-additive gene effects. The ratios of the variances GCA/SCA were 0.35 for straw yield, 0.27 for time to maturity and 0.82 for time to flower, showing that additive gene effects were of prime importance for time to flower and non-additive effects of greater significance for straw yield and time to maturity. **A. Hamdi and W. Erskine.**

Table 3.1.8. Biomass means (g/m^2) of parents, with their general combining abilities (GCA), and crosses in the F$_2$ generation of a line x tester mating system of three cultivated lentil lines and seven *L. culinaris* ssp. *orientalis* testers. Accession numbers of cultivated (ILL) and wild (ILWL) accesions are given.

Testers (ILWL)	Lines ILL 2501	ILL 5582	ILL 5674	Parent mean	GCA
1	165	71	118	13	-36
11	141	268	242	26	63
80	217	58	149	11	-14
89	212	145	82	70	-8
120	202	153	168	98	21
177	181	65	144	8	-24
325	204	77	173	4	-3
Parent mean	151	343	293		
GCA	35	-35	0		

SED treatment means   47.8 g/m^2
SE line GCA values   13.2 g/m^2
SE tester GCA values   28.6 g/m^2

3.1.1.11. Mutation breeding

Despite the antiquity and the large area (1.49 million ha) of lentil cultivation in South Asia, the genetic variation of lentil is low because of a photothermal bottle-neck, which has precluded the growth of all but phenologically adapted material. The spread of the crop into India was accompanied by strong selection pressure toward increased sensitivity of flowering to temperature and a reduced sensitivity to photoperiod (FLIP Annual Report 1988). As a result, the widening of the genetic base of the crop in S. Asia is an important breeding goal. This can be achieved by crossing landraces with exotic material and/or through mutation breeding. ICARDA is undertaking substantial

wide crossing for South Asia, but, for the mutation breeding approach, we have cooperated with the Nuclear Institute of Agriculture and Biology (NIAB), Faisalabad, Pakistan, which has particular expertise in this domaine.

As a prelude to mutation breeding, an experiment was undertaken at NIAB to establish not only optimum procedures for lentil irradiation, but also to test whether predictions could be made of genotypic sensitivity to irradiation from a wide range of morphological and cytological attributes. Eight adapted, but diverse, lines were treated with gamma irradiation at 0, 10, 20, 30 and 40 kr.

Response to irradiation was gauged by percentage survival (lethal dose$_{50}$ (LD$_{50}$)) in the M$_1$ generation and the frequencies of chlorophyll and morphological mutants in the M$_2$ generation.

Survival percent was adequately described by joint linear regression on to irradiation dose, which accounted for 96.4 % of variance. The effects of dose and the interaction variety x dose were highly significant, showing that the lines differed in their response to irradiation. The mean LD$_{50}$ over lines was 24.1 kr (Figure 3.7). The highest LD$_{50}$ was 52 kr with ILL 4605, which was nearly double that of the next highest LD$_{50}$ (30.4 kr in ILL 5782); the lowest LD$_{50}$ was 16 kr with ILL 6024.

Joint linear regression analyses on to irradiation dose of the frequencies of chlorophyll and morphological mutants only accounted for 20.8 % and 14.5 %, respectively, of variance and, clearly, the linear model did not fit the response to irradiation. However, joint regression with a quadratic model

accounted for 79.6% of the variance of the frequency of chlorophyll mutants and 70.4% of the variance of the frequency of morphological mutants (Figure 3.8). There was a significant effect of irradiation dose and the lines differed significantly in their response to dose. With the quadratic model $y = a + bx - c^2$, the optimum irradiation dose (x) was calculated for each genotype as $-b/2c$. There was good agreement in the average optimum irradiation dose for the production of chlorophyll mutants (21.8 ± 0.52 kr) and that for the production of morphological mutants (21.4 ± 0.65 kr).
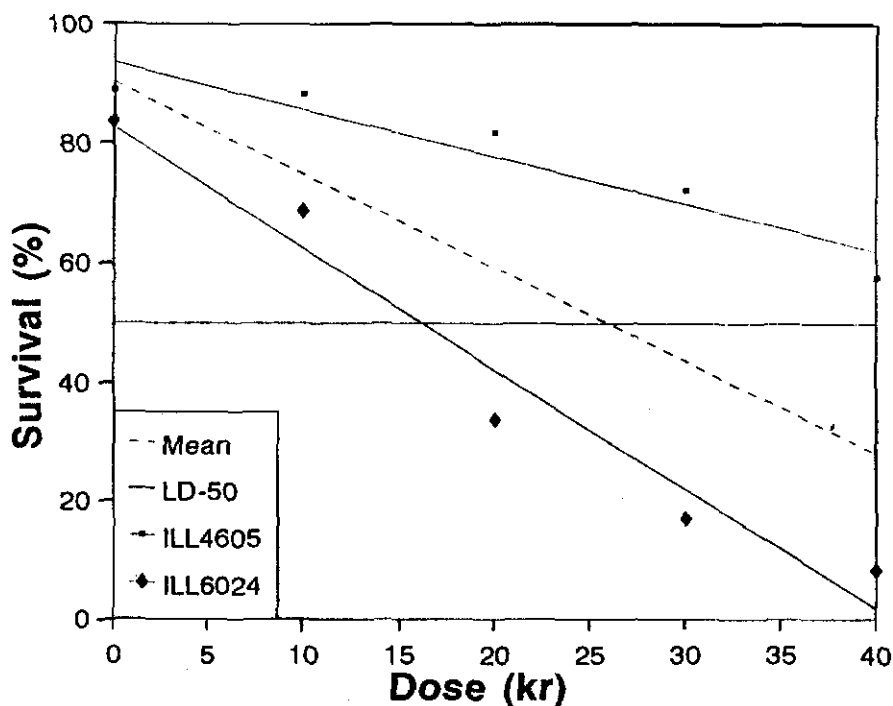
Figure 3.7. Survival % of the mean of eight lentil genotypes and the most (ILL 6024) and least responsive (ILL 4605) genotypes as affected by dose (kr) of gamma ray irradiation. The lethal dose (LD_{50}) at 50% survival is also shown.
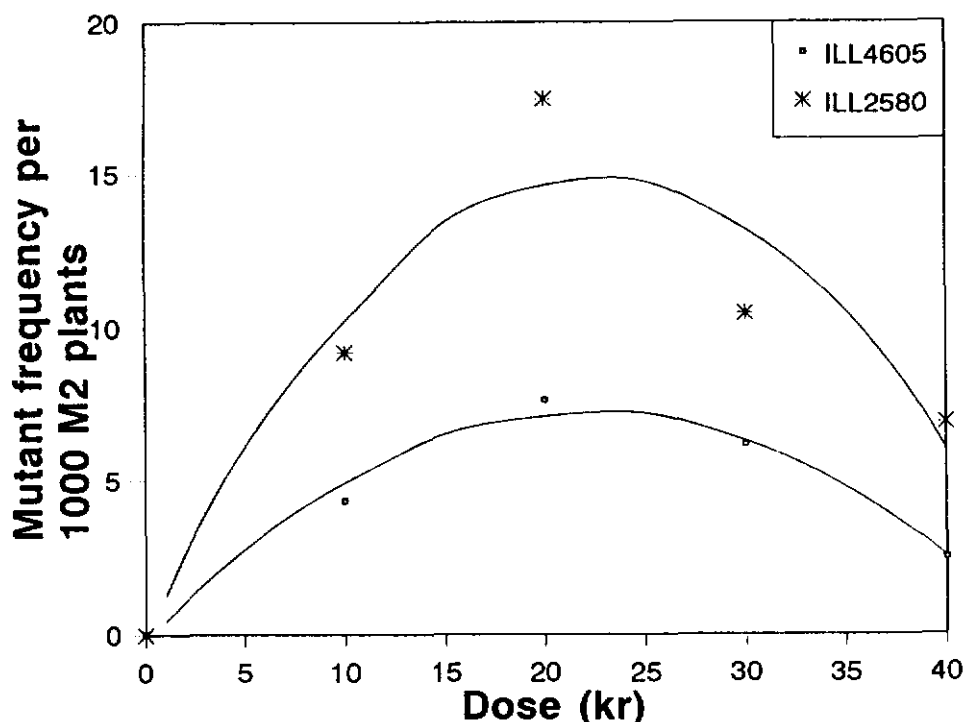
Figure 3.8. Frequency of chlorophyll mutants per 1000 M_2 plants as affected by dose (kr) of gamma ray irradiation in two contarsting genotypes - less responsive ILL 4605 and more responsive ILL 2580. Quadratic curves are shown for each genotype.

Correlations were made between radiation sensitivity, gauged by LD_{50} and the optimum radiation dose for the production of chlorophyll and morphological mutants, and a range of cytological, morphological, phenological and developmental traits: nuclear diameter and volume at interphase, and pollen fertility in the laboratory, plant sterility (% of surviving M_1 plants), seedling emergence, time to

flower, plant height, average seed mass, the numbers of seeds per pod and pods per plant, and grain yield in the M_1 generation in the field and M_1 seedling root and shoot growth in a growth chamber.

Most of the correlation coefficients between characters measuring radiation sensitivity and other traits were non-significant. But for LD_{50} there was a strong correlation ($r = -0.930$; $P = 0.007$) with time to flower, largely because Precoz (ILL 4605) was both earlier to flower and also had a higher LD_{50} than the other lines. The optimum dosage for chlorophyll mutant production was significantly correlated ($r = 0.860$; $P = 0.028$) with the number of seeds per pod. It remains to be seen if these associations have a general predictive value for radiation sensitivity beyond the lines tested. **I.A. Malik (Nuclear Inst. of Agric. & Biology, Faisalabad, Pakistan) and W. Erskine**.

3.1.1.12. Seed-size effects on lentil yield potential and adaptation to temperature and rainfall in West Asia

The major lentil-producing countries in the Mediterranean basin are Syria and Turkey, where production is approximately 75% of small-seeded, red-cotyledon lentil and the remainder is of the larger seeded, yellow cotyledon type. In this area, small-seeded lentils, typically 3.5 g/100 seeds, are primarily consumed in soup following post-harvest dehulling and splitting; whereas large seeds, usually > 6 g/100 seeds, are eaten whole with rice or cracked wheat as *mujadarah* with post-harvest processing confined to seed cleaning. Outside West Asia, national lentil production is exclusively of either

one or the other seed-size type.

A study examined the adaptation to temperature and rainfall of the two seed-size groups in West Asia, firstly in germplasm from Syria and Turkey evaluated in Syria and assessed for cold susceptibility in Turkey, and, secondly, in breeding lines over a decade at three sites in West Asia.

Seed size is known to be associated pleiotropically with flower and pod size. This study revealed other, non-pleiotropic, associations with seed size within the genetic base examined. In the breeding material, the large-seeded group produced taller plants and more straw. In both germplasm and breeding material, the large-seeded group consistently had a longer period of reproductive growth than the small-seeded group, probably due to the greater seed mass within each pod of the large-seeded group; the average seed mass in a pod of small-seeded germplasm accessions was 49 mg compared to the 72 mg/pod found in large-seeded germplasm. It takes longer to fill the seeds of large-seeded lentils than those of small-seeded lentils.

Cool and wet seasons allowed a protracted period for growth. In turn, this allowed the requirement of large-seeded lentil for prolonged reproductive growth to be fulfilled and led to the advantage in grain yield of large-seeded lines over the small-seeded at the two wetter sites.

Temperature was a major factor in differentiating the adaptation of the seed-size groups of germplasm and breeding lines. In the germplasm evaluation, the large-seeded accessions were, as a group, consistently less susceptible to winter cold than the small-seeded

group. Additionally, among the breeding material
there was a significant correlation between the
difference in grain yield between the seed-size groups
and the number of frost nights. Allied to the higher
tolerance of the large-seeded group to winter cold is
their response to cool conditions throughout the
growing season. Among the breeding material, the
significant regression of the difference between
large- and small-seeded groups for grain yield on to
mean temperature from January to April indicated an
advantage of the large-seeded group over the small-
seeded group in cool growing seasons, and the converse
at higher temperatures with the cross-over at 10 ^{O}C
(Figure 3.9).
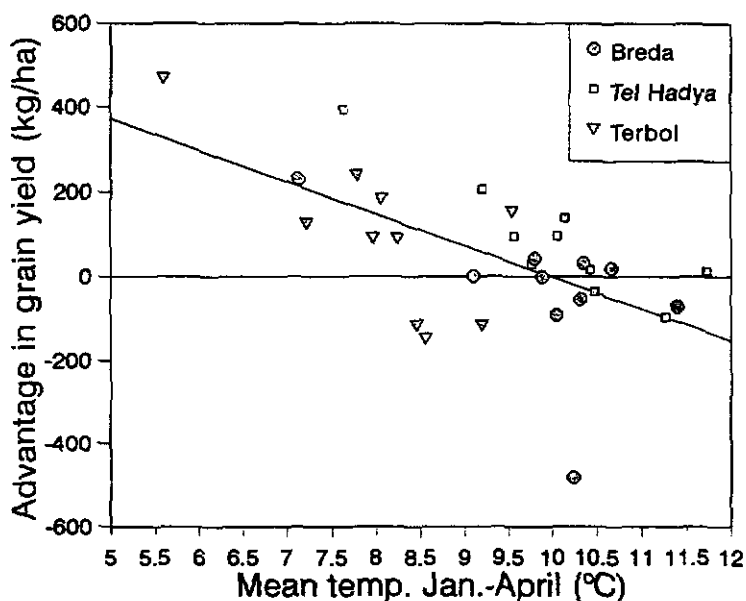
Figure 3.9. Average advantage in seed yield of large-
seeded lentil breeding material over
small-seeded material is plotted, with
its regression, on to the mean
temperature from January to April (^{0}C) at
three sites (Breda, Tel Hadya and Terbol)
over ten seasons.

Some principles of importance to lentil breeding arise from the study. For example, drought escape through early flowering and maturity was confirmed as an important mechanism to cope with dry environments (FLIP Annual Report 1990). In the search for large-seeded lines adapted to dry, warm environments it will be most practical to select for early flowering, because an extended period of reproductive growth may be required by all large-seeded lentils. Interestingly, selection within the germplasm collection has already revealed such an early-flowering, large-seeded line (ILL 4605 - Precoz), which has recently been released to farmers for warm and dry environments of the North coast of Sinai (Egypt) and the Chaoui region of Morocco.

In parts of North Africa, the environmental conditions for lentil production are similar to those in West Asia, but only large-seeded lentil is currently grown. For such warm and dry areas, it may be relatively easy to select small-seeded lentil cultivars. A similar approach of selection for small-seeded cultivars might be usefully explored in other areas outside the Mediterranean basin, where exclusively large-seeded material is currently grown, such as Canada, the USA and South America. However, in these areas new markets for a small-seeded lentil crop require exploration. **W. Erskine**.

3.1.1.13. Relationship of pod numbers per peduncle with grain yield

The number of flowers on a lentil inflorescence varies considerably from one flower per peduncle to four flowers per peduncle, with up to seven recorded from the glass-house. This results in different numbers of

pods per peduncle at maturity. There is variation within an individual plant in the flower numbers per inflorescence, between plants within a genotype, between genotypes and a strong environmental effect on this trait. This MSc thesis at the American University of Beirut aimed to quantify the variation due to these different factors and to understand the significance of this variability to the final yield.

Last season the nodal position, date of opening and subsequent fate (flower or pod abortion) of every flower was studied in the plastic house and the field on two genotypes and sowing dates to provide an overall picture of the floral biology of the lentil (Legume Program Annual Report 1993). This data was used to design a sampling method of the average number of pods per peduncle per plot for different genotypes and environments. The selected sampling method utilized the basal two primary branches on five plants per plot.

This season 81 genotypes, representing a wide range of diversity in the lentil, were sown at the American University Farm, Beqaa, Lebanon and at Tel Hadya, Syria in a 9x9 lattice design with four replications.

The data are still being analysed. However, a restricted suite of six traits from the trial at the American University Farm will be discussed herein.

The overall seed yield was low at 867 kg/ha, primarily because of a period of hot weather in mid-April, coincident with the early podding growth stage. Empty pods contributed 53 % of the overall number of pods and the average number of seeds per pods was also low at 0.57. Both these traits reflect a high level

of seed abortion and a reduction in yield potential resulting from the stress conditions in the early podding growth stage. The average number of pods per peduncle was 2.07. Under such stress conditions seed yield was positively correlated with both the number of pods per peduncle and average seed mass. **H. Tambal, R. Baalbaki (American University of Beirut) and W. Erskine**.

3.1.2. Use of Lentil Germplasm by NARSs

3.1.2.1. Advances for the Mediterranean region

The ICARDA base program provides segregating populations and breeding lines to national programs in North Africa and West Asia for elevations below 1000 m around the Mediterranean Sea. To date, more use has been made of lines than segregating populations and few lentil crosses are made outside ICARDA in North Africa and West Asia.

Table 3.1.10. lists lentil lines released as cultivars and Table 3.1.11. gives those lines selected for pre-release multiplication and/or on-farm trials by NARSs.

In Syria the red-cotyledon line ILL 5883 will soon be submitted to the variety release committee following its testing in on-farm trials over the last five years, where it yielded significantly more grain than the local check in all geographic regions and rainfall zones. Additionally, it has improved standing ability for harvest mechanization over the local check and resistance to vascular wilt disease, the most important disease of lentil in Syria. The spread in Syria of the earlier registered line

Table 3.1.10. Lentil cultivars released by national programs

Country	Cultivar name	Year of release	Specific features
Algeria	Syrie 229	1987	Yield, seed quality
	Balkan 755	1988	Yield, seed quality
	ILL 4400	1988	Yield, seed quality
Argentina	Arbolito (ILL 4605x-4349)	1991	Yield, tall & early
Australia	Aldinga (FLIP84-80L)	1989	Yield
	Digger (FLIP84-51L)	1993	Yield, red cotyledon
	Cobber (FLIP84-58L)	1993	Yield, red cotyledon
	Matilda (FLIP84-154L)	1993	Yield, yellow cotyledon
Bangladesh	Falguni - BARI Masur 2 (Sel. ILL4353xILL353)	1993	Rust res. & yield
Canada	Indianhead (ILL 481)	1989	Green manure
Chile	Centinela (74TA470)	1989	Rust res.& yield
China	FLIP87-53L	1988	Yield in Qinghai Province
Ecuador	INIAP-406 (FLIP 84-94L)	1987	Rust res. & yield
Egypt	Precoz (ILL 4605)	1990	Intercropping in sugarcane
Ethiopia	R 186	1980	Yield
	ILL 358	1984	Rust res. & yield
	NEL 2704	1994	Rust res. & yield
	FLIP84-7L	1993	Rust res. & yield
Iraq	Baraka (78S26002)	1994	Yield, standing ability
Jordan	Jordan 3 (78S 26002)	1990	Yield, standing ability
Lebanon	Talya 2 (78S 26013)	1988	Yield, standing ability
Libya	El Safsaf 3 (78S26002)	1993	Yield, st. ability in E. Libya
Morocco	Precoz (ILL 4605)	1990	Rust res. & yield
Nepal	Sikhar (ILL 4402)	1989	Yield
N Zealand	Rajah (FLIP87-53L)	1992	Yield, red cotyledon
Pakistan	Manserha 89 (ILL 4605)	1990	Ascochyta & rust res.
Sudan	Aribo 1 (ILL 818)	1993	Yield in Jebel Mara
Syria	Idleb 1 (78S 26002)	1987	Yield, reduced lodging
Tunisia	Necr (ILL 4400)	1986	Large seeds & yield
	Nefza (ILL 4606)	1986	Large seeds & yield
Turkey	Firat 87 (75Kf 36062)	1987	Small seeds & yield
	Erzurum 89 (ILL 942)	1990	Spring sowing & yield
	Malazgirt89 (ILL 1384)	1990	Spring sowing & yield
	Sazak 91 (NEL 854)	1991	Winter sowing, red cotyledon
U.S.A.	Crimson (ILL 784)	1991	Yield in dry areas

Table 3.1.11. Lentil lines in pre-release multiplication or on-farm testing by NARSs.

Mediterranean region	
Algeria	ILL 468
Egypt	FLIP84-51L, FLIP84-112L
Iraq	ILL 5883, FLIP87-56L
Lebanon	ILL 2126, FLIP84-59L, FLIP86-2L, FLIP87-56L
Morocco	FLIP86-15L, FLIP86-16L, FLIP87-19L, FLIP87-22L
Syria	ILL 5883
Tunisia	78S26002, FLIP 84-58L,
Turkey	ILL 1939
High elevation	
Iran	ILL 842, ILL 949
Pakistan	FLIP84-4L, FLIP85-7L
S. Latitudes	
Bangladesh	sel from L5xFLIP84-112L
Ethiopia	FLIP84-78L, FLIP86-41L, FLIP87-74L
Nepal	ILL 2580
Sudan	ILL 813, FLIP88-43L
Yemen	ILL 4605, FLIP84-14L

Idlib 1, which has good standing ability abd yield, is currently being monitored through an impact study on lentil harvest mechanization being conducted jointly with the Syrian General Organization of Agricultural Mechanization and the American University of Beirut.

In Lebanon preliminary results from an adoption study indicate that Talya 2 is starting to spread in the Beqaa valley and that yellow cotyledon is the preferred seed type in the South of Lebanon. Accordingly, demonstration of FLIP 86-2L (ILL 5988), a yellow cotyledon line outyielding Talya 2 in on-farm trials, is planned for the Sour area next year.

In South-East Turkey, where winter red lentil is widely grown, ILL 1939 is in the last year of regional registration trials.

In Iraq the large-seeded, yellow cotyledon line 78S26002 was registered in 1992 as Baraka. We are supplying seed of Baraka to assist in seed production because there are plans to sow 10,000 ha in the 1994/95 season. The red cotyledon lines ILL 5883 and FLIP87-56L (ILL6246) will be tested in on-farm trials in Iraq in the 1994/95 season as they performed well in the last two seasons and there is a demand from Iraqi consumers for both red and yellow cotyledon lentil.

In North Africa, the lines 78S26002 and FLIP84-58L (ILL5728), identified by the Tunisian program as promising in three previous seasons, continue to perform well despite the severe drought conditions of the 1993/94 season in Tunisia. Late planting of early maturing lines was tried in Tunisia since it is a practice of some southern farmers. FLIP88-41L (ILL6465) produced 1231 kg/ha when sown on January 20, 1994 in a low rainfall area. Further testing of such early material will be done under late sown conditions.

In Libya the line El Safsaf 3 (78S26002), released in 1993 for cultivation for the East of the country, continues to perform well in the East but also has given high yields under central-pivot, irrigated conditions in Central Libya at Meknosa.

Lentil production and area continue to decline in Algeria but the lines ILL 468, ILL 4400, LB Redjas, Setif 618 and Balkan 755 are in seed production for future use by farmers.

In Morocco there are several lentil lines in catalogue trials FLIP86-15L (ILL6001), FLIP86-16L (ILL6002), FLIP86-19L (ILL6005), FLIP86-21L (ILL6007), FLIP87-19L (ILL6209) and FLIP87-22L (ILL6212), all with resistance to rust.

The North African Regional yield trial on lentil was established in 1990 to comprise the best lines selected in Algeria, Libya, Morocco and Tunisia. This regional yield trial has revealed that lentils selected in the various countries of N. Africa differ substantially in phenology, indicating the need for specific adaptation to a range of environments in the region. The requirements of Libya, Tunisia, and lowland Algeria are being met by the ICARDA West Asian breeding program. However, late maturing material is required for high altitude areas of Algeria and specific crosses are being made at ICARDA for this area. In Morocco early-maturing lines are required with resistance to rust. A specific joint program of crossing is being undertaken to target this environment. In Tunisia late sowing with early maturing lines needs further testing.

In Egypt the early lines FLIP84-51L (ILL5722) with small seeds and FLIP84-112L (ILL5782) with large seeds are both in pre-release multiplication. The line Precoz (ILL 4605) is becoming popular in the north Sinai region, where it is known commonly as 'Shami' (from Damascus), because its early maturity avoids drought stress under the low prevailing rainfall conditions. These three early lines all have potential for inclusion in the cotton rotation in the Nile Delta region, in contrast to later maturing landraces. **National Agricultural Research Systems.**

3.1.2.2. Advances for southern latitude region

This region comprises the sub-continent of India and Ethiopia where an early flowering habit is required together with resistance to rust, ascochyta blight and wilt. The importance of foliar pathogens contrasts with other major areas of lentil production.

There are three strong lentil breeding programs in Pakistan with two in Faisalabad and the remaining program in Islamabad. Over the last five years ICARDA has worked closely with these programs in joint selection as the focus of a thrust to broaden the genetic base of lentils in South Asia. In Faisalabad a line 88503 is being released with ICARDA parentage by Punjab authorities and the mutation breeding program at NIAB is based on ICARDA supplied material (see section 3.1.1.11). Three relatively salt tolerant lines - ILL 6451, ILL 6788 and ILL 6793 were identified at Bahauddin Zakariya University, Multan.

The major production problem in Bangladesh addressable through breeding is rust. We have been making targeted crosses for Bangladesh of rust resistance sources with the local susceptible cultivar 'L5' in the base program at Tel Hadya. Selections have now been made in Bangladesh of adapted rust resistant plants from segregating populations. As a result, Falguni (BARI Masur 2) was released in 1993 as the first rust resistant lentil cultivar in Bangladesh. Another rust-resistant line (ILX 87247), locally selected from the cross of L5 x FLIP84-112L (ILL5782), is in the pre-release stage, having been submitted to the National Seed Board. It also has resistance to stemphylium blight and an erect plant stature suitable for intercropping in sugarcane.

India has a strong lentil breeding program coordinated under the All India Coordinated Pulse Improvement Project of the Indian Council of Agricultural Research (ICAR). In India, the genetic base of the crop has been widened by the use of Precoz in crossing. Now 15% of entries in All-India Coordinated Lentil trials have Precoz as a parent. Rust resistance, selected in Morocco, was holding in Kanpur. We have established cooperation with Pantnagar Agricultural University on screening for rust resistance in breeding lines, the wild germplasm and the possibility of collaboration in the search for markers for rust resistance. Our vascular wilt resistance lines are being widely used as source parents within India.

Nepal grew around 170,000 ha of lentil spread from the Terai area adjacent to India to the lower Mid-Hills last season. ICARDA has been requested for specific targeted crosses by Nepali program. ILL 2580 is among entries being considered for release.

In Ethiopia NEL 2704 was released in the 1993/94 season for lowlands and recommended for drought-prone areas. Three other lines will shortly be submitted to for registration FLIP84-78L (ILL5748), FLIP86-41L (ILL6027) and FLIP87-74L (ILL6264). Ada and Akaki are the areas where the released line NEL 358 is becoming very popular and a study on its impact is underway.

In Sudan Aribo 1 (ILL 818) was released for cultivation in the Northern Province and Jebel Mara Region in the 1993/94 season on the basis of yields from 11-34 % more than the local check and good seed quality for splitting and dehulling. ILL 813 is in pre-release seed multiplication and the program identified FLIP88-43L (ILL6467) as promising in the

1993/94 season in the Northern Province. **National Agricultural Research Systems**.

3.1.2.3. **Advances for high altitude region**

The high altitude region primarily consists of those regions of Afghanistan, Iran, Pakistan and Turkey where lentil is normally grown as a spring crop because of the severe winter cold. This season at Ankara the national program of Turkey has again demonstrated that winter-sown lentil has a higher yield potential than the spring-sown crop. (see section 3.1.1.8). The spring cultivar Erzurum '89 was tested in farmer managed trials/demonstrations in the Sivas area on the eastern margin of Anatolian plateau. The mean yield of the local was 478 kg/ha compared to Erzurum '89, which yielded 738 kg/ha in the 1994 spring season.

In Iran the lines ILL 842 and ILL 949 are promising and due for testing on farmers' fields.

In Balochistan (Pakistan) the Provincial Technical sub-committee gave its approval for the release of two lines Masoor-931 (FLIP84-4L) and Masoor-932 (FLIP85-7L) for highland Balochistan, subject to the approval of the Provincial Seed Council. The lines were selected at the Arid Zone Research Institute, Quetta, on the basis of their cold tolerance and a larger seed size than the local cultivar. The final release is pending. **National Agricultural Research Systems**.

3.1.2.4. Advances in other areas

The New Zealand Institute for Crop and Food Research registered lentil FLIP87-53L (ILL 6243) as Rajah during 1993. It is a red cotyledon line which has out-performed the commercial standard by 15% and is well received by the lentil trade for use either whole or split. It can be harvested 2-3 weeks before the commercial standard and is more tolerant to Ascochyta blight.

In Australia there is now considerable interest in lentil. Prior to the testing of germplasm from ICARDA, lentil assessment in Australia was limited to a few lines representing phenological extremes - extra early and extra late flowering and maturity. ICARDA Mediterranean-adapted material has fitted in well into the vacuum. In Victoria seed of the red lentils FLIP84-51L (ILL5722) and FLIP84-58L (ILL5728) and the green lentil FLIP84-154L (ILL5823) were handed over to a private company for commercialization by the Victorian Institute for Dryland Agriculture on the basis of their consistent yield advantage. It is hoped that these lines will be the basis for a viable lentil industry. In New South Wales the lines FLIP84-51L (ILL5722) and FLIP86-16L (ILL6002) are in multi-location testing following their selection at Tamworth over several seasons. The most promising new selection in South Australia is FLIP84-61L (ILL5731). In West Australia an earlier maturity is required and early-flowering selections such as FLIP86-16L (ILL6002) are well adapted phenologically. **National Agricultural Research Systems.**

3.2. Application of Molecular Techniques in Lentil Improvement

3.2.1. Lentil Mapping Project

This is a collaborative research program between ICARDA and Washington State University. The objective of this study is to evaluate the usefulness of DNA-marker analses for marker assisted selection in lentil breeding programs.

Ascochyta blight and *Fusarium wilt* are the most important diseases in lentils in the region. In order to develop molecular markers for resistant gnes for teh two diseases, two segregating mapping populations of lines originating from *Lens culinaris* and *Lens orientalis* have been constructed. Two of the four parents were derived from the line ILL 5588 of cultivated lentils. They provide the sources of genes for rsistance to *Fusarium wilt, Ascochyta blight* and rust (F. Muehlbauer), pers. comm). Pure lines of the two other parents were developed from *Lens orientalis*. One F_1 individual hybrid plant each was selfed to produce a F_2 population. One F_2 population of each cross was used as the mapping population, namely cross 1 (X92L-010) and cross (X92L-013). The F_2 populations were developed under controlled environmental conditions in a greenhouse. Green plant material from F_2 plants was collected for RAPD-and isozyme analysis during the growing season.

Single Seed Descent (SSD) method was used to advance F_2 individuals to F_7 recombinant inbred lines. Seeds were planted in 6.5 cm plastic pots in peat moss in a growth chamber. An average yield of 10 seeds per F_2-derived line per generation was sufficient to allow the population development under growth chmaber

conditions. Non fertile plants during population development were replanted from the parental stocks in each generation. A generation time of 10 to 12 weeks allowed to develop the F_7 recombinant inbred lines within 2 years. F_6 seeds were planted in the plastic house for sed multiplication to provide sufficient material for disease screening and application of DNA-markers.

Total genomic DNA was isolated from fresh leaves of 100 F_2 individuals form each cross. RAPD-analysis was conducted on thermocycler Perkin Elmer 9600 system. Parents were screened with 320 primers, each primer was scored fro either amplification or polymorphism (Table 3.2.1). Nearly 23% of the primers showed no amplification, 47% showed no polymorphism and 30% showed polymorphism. Polymorphic primers were then applied on 8 to 10 F_2 individuals and compared with the parents for segregation. Primers segregating on the F_2 samples were used for segregation analysis of 40 F_2 individuals (Figure 3.2.1.a,b).

Three oligonucleotide probes were screened on parental and F_2 material to detect RFLPs. The oligonucleotide probe (GATA)4 revealed a high degree of polymorphism between the parents of cross 2 in genomic digests with the restriction enzyme Hind III (Figure 3.2.2). Alltogether 9 different RFLPs could be detected between the parents from which 8 could be mapped in the segregating F_2 and integrated into the map of lentils. Different from the cereals, the oligonucleotide DNA sequence seems to have a high degree of homology with DNA sequences in the lentil genome.

Table 3.2.1. Screening of parents with 16 Operon primer kits 920 primers per kit).

Kit ID	No amplification	Polymorphism no	yes
OPF	6	10	4
OPG	5	6	9
OPH	0	11	9
OPI	1	11	8
OPJ	5	11	4
OPK	1	14	5
OPN	0	17	3
OPO	2	10	8
OPP	16	2	2
OPQ	12	3	5
OPR	6	10	4
OPS	2	7	11
OPT	3	15	2
OPU	4	9	7
OPV	6	8	6
OPW	9	8	3
OPX	1	11	8
OPV	3	6	11

As cross 1 (L92 - 010) showed about 60% of the markers being skewed the cross was not further used for linkage analysis. Cross 2 showed only a very low percentage of skewnes of markers (39%) and was therefore used for further analysis. The preliminary testing of the F_2 individuals for RAPD segregation provided a potential for screening more primers. Fifty primers (31.49% of the total primers tested) produced a polymorphic product between the parents of this cross. The polymorphic primers revealed 67 markers which were scored in 40 F_2 individuals (Table 3.2.2). All the markers showed a Mendalian segregation except for marker 015c and OPM18a (Table 3.2.3). The number of RAPD-markers assorted independantly was 61 in cross 2 (X92L - 013). Thiry four markers (including RAPDs, morphological and

a

b
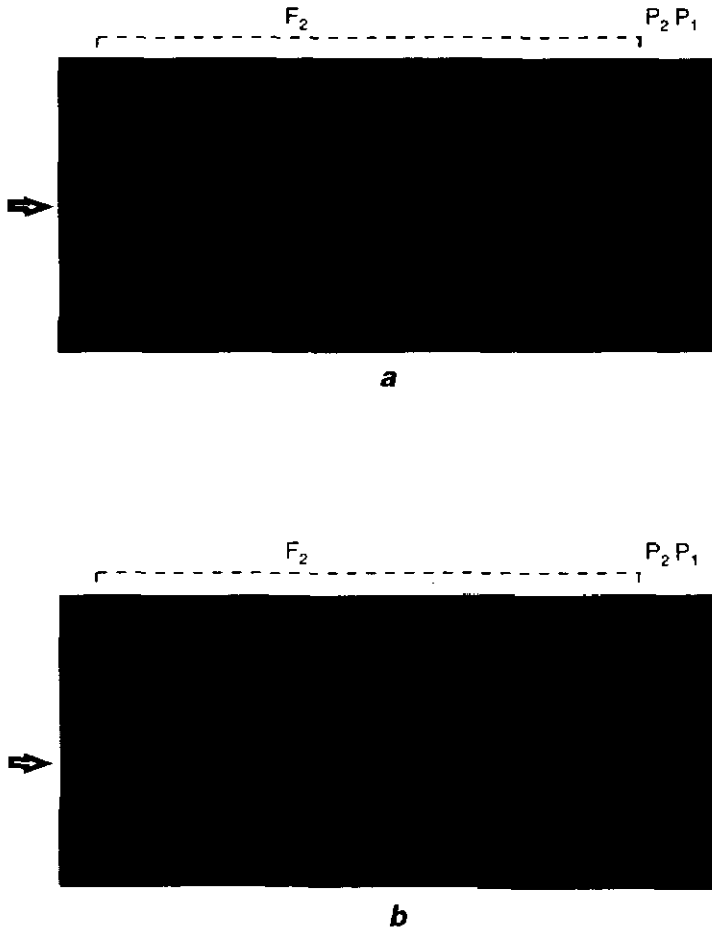
Figure 3.2.1.a,b. Segregation analysis of RAPD-marker (a) OPU1 and (b) OPS14 in the segregating F_2 population. from right to left: Amplification of the RAPD-marker with genomic DNA of parent 1 (lane 1) and with parent 2 (lane 2) and with F_2 individuals (lanes 3-22).
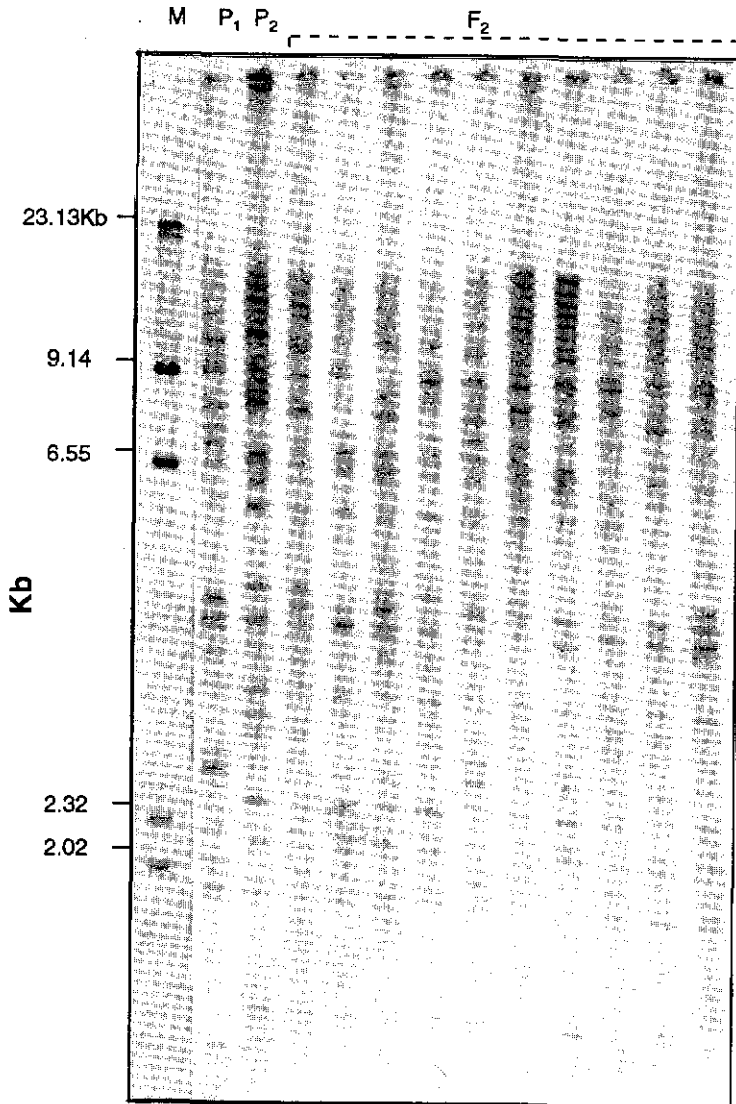
Figure 3.2.2. Colorimetric signal development after hybridization of oligonucleotide probe (GATA)4 with total genomic DNA of parent 1 (lane 1), parent 2 (lane 2) and 10 F_2 individuals (lanes 3-10). Segregating markers are indicated by arrows.

Table 3.2.2.   Table 3. Data file of 67 markers (RAPD, morphological and oligonucleotide) for MAPMAKER 1.9 software. Designation of the markers are given on the left, segregation of polymorphic bands with 40 individuals on the right. Symbols were given A = homozygous pattern for parent 1, B = homozygous for parent 2, C = homozygouis for parent 2 or heterozygous, D = homozygous for parent 1 or heterozygous, - = missing data.

*GS	CCACCCCAAAACACCCCCACCCCCCACCCCCAACCCACCA
*SCP	CACCCCC-CAACCCACCCCACCCCACCCCCCCCCCAC-CCC
*W07	DDBDBDDDDBDDDDDDDBDDBDDDDDBBDDBBDDDDDDDDD
*N19	D-BDBDDDDBDDDDDDDDBDDDDDBDBDDDBDDDDDDDDDD
*O13	BDDDDBBBBDBDDDDDDBDDBDDDDDDDDDDDDDBBDDDDD
*O11	-DDBDBDBDBDDBDDDDBDDDDB-DDDDDBBBDB-DBDDDD
*P03	CCCCACCCCCACCCCACACACC-CCCCCA-CCAAACCACC
*W15	HHHBBHBBA-BHHBHAABH-AHHBHHAAHHHAHAHHHBBH
*T01	DDDDDBDBBDDDBDDDDDDDDDDBDDBDDDDDBDDDD-DDB
*S01a	CCCCCACCCACCCCCC-AACCCCCCCCCCCACCCAAAAACC
*S01b	BDDDDBBBBDBDDDDDD-DDBDDDDDDDDDDDDDDBBDDDDD
*S01c	BDBDDDDDBDDDDDDD-DDDDDBDDDDDDDDBDDBDDDBDD
*O06	CAC-C-CACCCCCCCAACACC-ACCACCCCCCCACCC----
*O08	CCACCCCCCCC-CA-ACCAACCCCCACA-CCAACCACACC
*S04	BAHB-H-HHABHHAHHHBH-BHHHH-BA-A--HBHAHHA-
*S03	--ACCCACCCCCACCCCCAACCCCC-CCCAACCCCCACA-
*S08a	CCCCCACACCCCCCCCAACCACCCCCCCCCCCACCACCCCCC
*S08b	CCCCCACACCCCCCCCAACCACCCCCCCCCCCCACCACCCCCC
*O20a	BDBBBDBDDDBDDDDDDDBDDDDDDDBBDBDDD-D-DDDDB
*O20b	DBDBDBBDDDBDDBBBDBBDBDDDDDDDDDDB-D-DDDDB
*O20c	CACACAACCCCCCACAACACCAACACCCCCCC-C-CCCCA
*O20d	DDDDDBBBBBBDDDDDDDDDBDDDDDDDDDDBB-D-DDDDB
*Q03	BDDDDBDBBDDDBDDD-DDDDDBDBDDBBDBDDDDDDDDB
*Q14	CCCCCAACC-ACAACCCCACCCCCCACCCCCAACCCACCA
*S16	BDDDDBBBBDBDDDDDDBDD-DDDDDDDDD-DDDBBDDDDD
*O15a	CCCCCCCCCACC-CCCAACCCAACCCCAACCCACCCCACCA
*O15b	BBBDBDDDBDD-DDDBDBDBDDBBDBDDDDBBDDDDDBDD
*O15c	CACACCCACCC-CCACACACAACCCACCCCCCCCACCCACAA
*W16a	CCCCCACACCCCCCCAACCACCCCCCCCCCCAAAACCCCCC
*W16b	BDDDDDDDDDDBDBDDDDDDBBDBDBDDDDDBDDDDDDDD
*S14a	CACACCCACCC-CCAC-CACCACCACCCCCCCCC-CCAAC
*S14b	ACCCCCCCAACACCCACCCCACCAC-CCCCC-CCCC-CAA
*Y11	DDBDDDBDDDBDDDBDDBD-DDBDDDDDDDDDDDBBDBBD
*R09	CCACCAACCCCCACCCCCCACCCCCAAACCCAAACCACAA
*Y16	DDBDDDDBDD-DDBBDBDDDDDBBDBBDDDDDDDBDDBDD

*S10	CACCACCCAACCCACCCCCCACCCACCCCCCCC-CCCCC
*V08a	CCCCCCCCCACAACACCCCCCCCCC-ACCCC-AACCCACCA
*V08b	HBHHBHHHHAHHBBHHABAHHHHH-HBHHH-HHHHBHHHH
*V08C	BDDBDDDBBDDDDBBDDDBDDDDBDDDBDDD-DDDBDDDDB
*Y07a	DBDBDDDDDDDDDDDDDDDDBBDDDBDDDDDDBDDDDDDRDD
*Y07b	DBDBBDDBBDDDDDDBBDDDBBBBBBBDDDDDBDDDDDDBBD
*S05	CCCACACCCCCCCCACAAACACCACCCACCCCCAAAAACC
*X04	CCCCCCCCACCACCCAACCCCCCCCCCCCACCACACCCCCA
*S07	DDDDDDDBDDDDBDDDDDDDDDDBDDBDDBDDDDDDDDDDB
*Y10	BDDDDBBBDBDDDDDDBDDBDDDDDDDDDDDDDDBBDDDDD
*U01	DDBDBDDDBDDDDDDDDBDDDDDDDDBBDDD-DDDDDDDDDD
*U02	BDDDDBBBDBDDDDDDBDD-DDDDDDDDDDDDDDBBDDDDD
*H07a	CACA-C---CCCA-ACACACAACCACCCCCCCCACCCACAA
*H07b	DDBD-D---DDDD-DBDDDBDDDDDBDDDDDBDDDDDDDDDD
*V12	CACACCCAA--CCCA--ACCAACCACACCC-CACCCCCAA
*H19	BDDDDBBDBDDDBDDDDDBDBDDB-DBDDDBDDDBDDDBD
*V01	C-CCCCA-CCACCACCAC--CCACCAACC--CACC--ACA
*G06a	--DDDBDDDDDDDDDBBDDDBBBDBDDDDDDDDDDDBBDDDDD
*G06b	--CCCCCAAAACACCCCC-CCCCCCACCCCCAACCCACCA
*G06c	--CCACCCCCAAACCCCCC-ACCCACCACCCCACCCCCCA
*R04a	CCCCCACACCCCCCCAACCACCCCACCCCCACCACCCCCC
*R04b	-CACCCCCCCACAACCAACCCCCACAACCAAACCACACC
*V02a	CCCCCACAAAACACCCCCACCCCCCCACCCCCAACCCACCA
*V02b	CCACCCCCCCCACAACCAACCCCCACAACCAAACCACACC
*HIGAT1	CACCCCACACCCCACCCCC-CCCCACCCACCACCCCCCAA
*HIGAT3	BDDDBDDDDDDDDDDDBDBD-DDDDDBBDBBDDBBBDBBBDBD
*HIGAT4	BDDDBDDDDDDDDDDDBDBD-DDDDDBBDBBDDBBBDBBBDBD
*HIGAT5	CAACCCCCCCCCCAACCAA-CCCCACAACCAAAACCACCC
*HIGAT6	DBDBDDDBBBDDDDDDDBDD-DBDBDDDDDDBDDDDDDDDDD
*HIGAT7	DDDBDDDDDBDDDDDDDDD-DDBDDDDDDDBDDDDDDDDDD
*HIGAT9	DDDBDDBDDDBDDDDDBD-DDBBDDDDDDDDDDDDDDDDD
*HIGAT11	DDDBDDBDDDDDDBBDDBB-BDDDDBDBBBDBBBDDDDDDB

oligonucleotide markers) so far could be clearly assigned to linkage groups. Ten linkage groups were constructed, seven of them containing three markers or more (Figure 3.2.2). Two RAPD-markers OPQ14 and OPV08c, were found linked to the Gs marker at 17.6 cM distance. The GS marker is located in chromosome number one in the existing lentil map. The linkage distance ranges from 0.0 cM to 27cM along the 10 groups. Several markers showed a 0.0 cM distance, indicating that these markers may fall in the same locus.

Table 3.2.3. Segregation analysis of the linked RAPD-markers on 40 F_2 individuals. All the markers fit a 3:1 ratio except OPO15c* and OPM18a*.

Marker ID	F_2 Segregation (+/–)	x^2
OPN19	32:7	1.02
OPO6	25:8	0.01
OPO8	26:11	0.44
OPO11	26:11	4.44
OPO13	31:9	0.13
OPO15a	29:10	0.01
OPO15b	28:11	0.19
OPO15c*	12:27	39.6
OPO15d	27:12	0.67
OPO20a	27:11	0.31
OPQ14	28:11	0.19
OPS01b	31:8	0.40
OPS04	7:18:7	0.49
OPSO8a	33:7	1.20
OPSO8b	33:7	1.20
OPS14a	28:9	0.00
OPS16B	33:6	1.92
OPT01	32:7	1.10
OPV08b	29:9	0.04
OPW15	8:20:8	0.09
OPW16a	31:9	0.13
OPW07	31:9	0.13
OPY10	31:9	0.13
OPU01	33:6	1.92
OPU02	31:8	0.43
OPH07a	22:13	3.00
OPM18a*	28:2	6.53

The F_2 population of cross 2 was found to be adequate for mapping because it provides abundant frequencies of polymorphic markers. The constructed linkage groups propose a high potentiality for more markers to be fitted. Therefore screening for more RAPD and RFLP markers is needed. **I. Mahmoud, W. Erskine, B. Bayaa and M. Baum (ICARDA) and F. Muehlbauer (WSU).**

Table 3.2.4. RFLP analyses in lentils.

Clone	Restriction enzyme		Linkage goup(s)
	EcoRI	HindIII	
E5	–	–	3
E8	+	+	–
P119	0	0	4
C95	+	0	–
C25	+	+	–
C45	–	–	–
E1	–	–	4
C32	+	+	–
C33	+	–	5
C52	+	+	1,5
C65	+	+	3,6

= not polymorphic, + = polymorphic, 0 = not tested or blot not readable
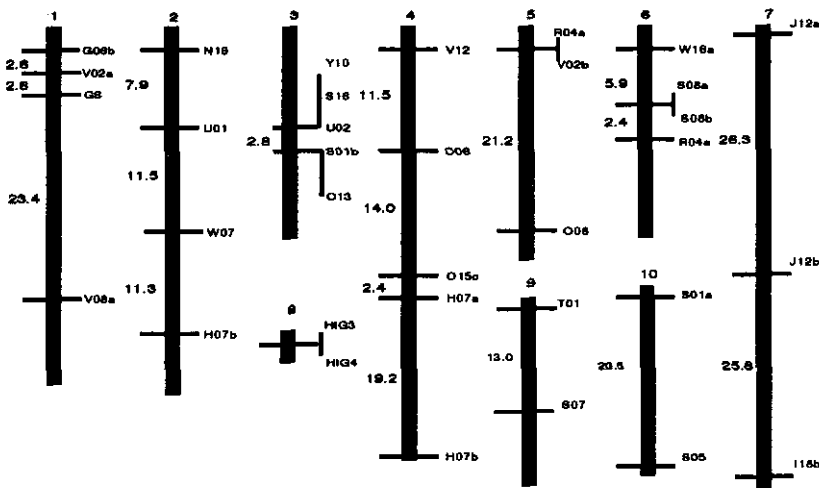
Figure 3.2.3. Possible linkage groups in lentils after F$_2$ segregation analysis of cross 2 using MAPMAKER 1.9 with a LOD score threshold of 3.0. Distances between markers are given in cM. In cases where complete linkages (0.0 cM) occured, markers are connected by horizontal bars (e.g. S08a and S08b in linkage group 6).

3.2.2. RFLP Analysis in Lentil

We started analyse parental and F_2 material with
RFLPs. The RFLP-markers used were already previously
mapped in lentils. Surprisingly, we found a high
degree of polymorphism in genomic degests with only
two restriction enzymes (Table 3.2.4). The use of
already mapped RFLP-markers will help to join the
RAPD-marker map developed at ICARDA with the maps
constructed with the help of isozymes and RFLP-markers
elsewhere. It will also help to link more RAPD
markers into the already established linkage groups.
I. Makmoud, H. Sayed and M. Baum.

3.3. Lentil Mechanization

During the first decade of ICARDA, a major drive was
made to develop economic machine harvest systems for
lentil production (FLIP Annual Reports 1986-1990;
Legume Program Annual Reports 1991-93). Following the
introduction and use by farmers in Syria and Turkey of
such systems, a moratorium has been put on further
research at ICARDA pending the completion of an impact
and adoption survey of producers in Syria.

This survey is being conducted with the Syrian
General Organization of Agricultural Mechanization,
the American University of Beirut and the Farm
Resource Management and Germplasm Programs within
ICARDA.

Eighty lentil producers spread among the major
lentil growing regions of North-West and North-East
Syria have been interviewed using a questionnaire
format. The results are not yet available; but we
expect them to assist greatly in planning future

research and technology transfer of lentil harvest mechanization. **J. Haidar (American University of Beirut), General Organization of Agricultural Mechanization (Syria), A. Salkini, A. Dakermanji & W. Erskine**.

3.4. Lentil Entomology

3.4.1. Effect of *Sitona crinitus* on Lentil Yield

Experiments on *Sitona* damage and control were continued at Tel Hadya and one on-farm location, Alkamiye using Promet insecticide. This season a new formulation of Promet, Promet CS 400 was used as seed dressing at 10 ml/kg seed.

At Tel Hadya the *Sitona* feeding and nodule damage was lower than in previous years. On 23 April 58% nodule damage was recorded in the check as compared to 11% with Promet treatment. Promet treatment significantly reduced nodule damage, but for the first year in these trials seed yield was slightly higher (1724 kg/ha) in the check than in Promet treatment (1633 kg/ha) (Fig. 1). Biological yield, however, was significantly higher with Promet treatment.

At Alkamiye nodule damage was higher and reached 68% and 82% in the check and 21% and 30% with Promet treatment on 11 April and 10 May, respectively. Thus Promet reduced the nodule damage significantly but less effectively than in previous years. Seed and biological yields increased, but not significantly (Fig. 1).

For the second season Promet treatment for *Sitona* control was included in the lentil on-farm verification trials at 5 locations in northern, northeast, and middle Syria. At all locations nodule damage was found, but

severity differed between locations (Table 1). As in Tel Hadya, nodule damage was not high with 50 and 56% in Aleppo and Idleb area. In Hama and Kamishly area high nodule damage between 76% and 84% was recorded (Table 1). At all locations Promet treatment significantly reduced nodule damage. Lentil seed and biological yield, however, did not differ significantly between treatments.

This was the final year of the *Sitona* control experiments. It has been shown, that *Sitona* does occur in all lentil growing areas in Syria. Promet seed treatment reduces nodule damage and increase yield significantly and can be recommended for use in certain situations, like seed production fields. The lower effectiveness in this years experiment is most likely due to the lower dosage of active ingredient used with the new formulation. The new

Table 3.4.1. Effect of Promet (12 ml/kg seed) treatment on lentil seed and total yield and nodule damage by *Sitona* at different locations in Syria, 1993/94.

Location	Treatment	% nodule damage	Lentil yield (kg/ha)	
			Seed	Total
Hama (Souran)	Check	80.8	680	1952
	Promet	5.5	1047	2478
Idleb (Afes)	Check	56.5	1321	2906
	Promet	3.9	1382	2724
Aleppo (Kafr Naseh)	Check	50.0	1685	4042
	Promet	5.2	1929	4412
Hasakeh (Shoudara)	Check	76.0	2191	3123
	Promet	7.5	2116	3378
Kamishly (Hasoud)	Check	84.2	1518	3382
	Promet	4.2	1940	4547

formulation only has 40% a.i. as compared to 66% of the previous Promet formulation, of which 12 ml/kg seed were used. Therefore the dosage of the new formulation has to be increased from 10ml to 15 or 20 ml/kg seed to ensure effective control. **S. Weigand and A. Joubi.**
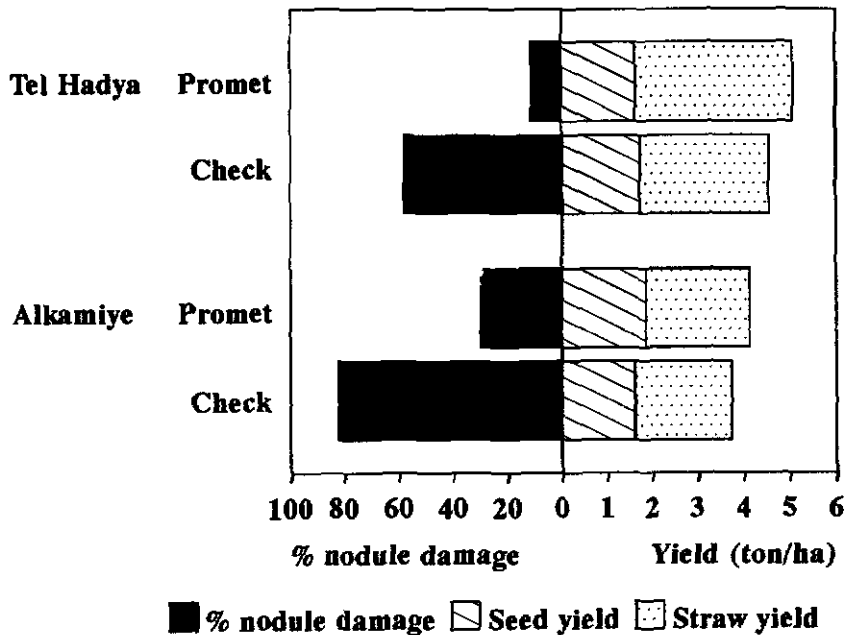
Figure 3.4.1. Effect of application of Promet 400 CS (10 ml/kg seed) on lentil seed and straw yield and nodule damage by *Sitona crinitus* at 2 locations, Syria, 1993/94.

4. FORAGE LEGUMES IMPROVEMENT

Annual forage legume species such as *Vicia* spp and *Lathyrus* spp. are one of the options to replace fallow in the cereal/fallow rotations in dry areas. They are defined as leguminous species sown and harvested in a single year. They can be used for grazing during winter and early spring and harvested either for hay in spring or for grain and straw at full maturity.

These crops may be also sown in mixtures with cereals (oats, barley or triticale) and harvested as hay. They differ from food legume crops only in the end use. They are used mainly to feed livestock, whereas food legumes for human consumption. Therefore, flexibility in forage legume crops to meet different types of utilization in different types agro-ecological zones is always of great importance in producing new adapted cultivars.

Although there is a large diversity of *Vicia* spp. and *Lathyrus* spp. in the Mediterranean region, only few have been used as forage crops and have received little attention of plant breeders and agronomists in the past. Germplasm program pays particular attention to such annual species of these two genera which could be adapted to areas where seasonal rainfall ranges from 250-400 mm.

4.1. Forage Legumes Breeding

The general objective of our breeding program is to develop and produce improved cultivars of *Vicia* spp. and *Lathyrus* spp. to feed livestock in areas receiving less than 400 mm, rainfall. It is also desirable to have widely adapted cultivars that can be recommended for different   locations   with   similar   agro-ecological conditions. While attempting to improve yield potential

and adaptation to environment emphasis is given to ensure
that the palatability, intake and other nutritive values
of herbage, hay, grain and straw are acceptable. This
work is being done in close collaboration with the Pasture
Forage and Livestock Program (PFLP).

In vetches we are dealing with *Vicia sativa* L. (common
vetch), *V. villosa* spp. *dasycarpa* Tan (wooly-pod vetch),
V. ervilia L. (bitter vetch), *V. palaestina* R. (Palaestine
vetch), *V. narbonesis* L. (narbon vetch) and *V. panonica*
GR. (Hungarian vetch). In chicklings we concentrate on
Lathyrus sativus L. (common chickling or grasspea), *L.
cicera* L. (dwarf chickling) and *L. ochrus* (L.) DG (ochrus
vetch). Work is also done on *V. sativa ssp. amphicarpa*
Dorth (underground vetch), and *L. Ciliotatus* L.
(underground chickling) which produce both underground and
aboveground pods.

Two approaches are adopted to develop improved lines
of *Vicia* and *Lathyrus* spp. In one, selection is affected
in the wild accessions to develop improved cultivated
types. In the second, hybridization is used to overcome
specific short comings: shattering pods, prostrate growth,
susceptibility to diseases and nematodes, cold
susceptibility, high content of antinutritional factors
etc. The process begins and ends with on-farm studies to
determine farmers' needs and to see how well our new
improved cultivars meet them. The work is carried out by
a multidisciplinary team involving breeder, physiologist,
pathologist, entomologist and animal nutritionist.

In 1993/94, 1400 accessions of *V. sativa* were
evaluated in collaboration with the Genetic Resources
Unit. Selected genotypes of *L. sativus, L. cicera, L.
ochrus* and *V. narbonesis* were evaluated in nursery
observation rows at Tel Hadya. Promising genotypes
(selections) of *V. sativa* and *V. panonica* were tested in

preliminary microplot field trials at Tel Hadya (seasonal rainfall 337). Promising lines of *V. sativa* were evaluated in advanced yield trials at Tel Hadya, Terbol and Kfardan (seasonal rainfall 337, 475 and 250, respectively). Promising lines of *V. narbonesis* were evaluated in advanced yield trials at Tel Hadya, Breda (seasonal rainfal 298 mm) and Kfardan. Promising lines of *L. sativus, L. cicera, L. ochrus, V. dasycarpa* and *V. palaestina* were tested in advanced yield trials at Tel Hadya and Breda. Promising lines of *V. ervilia* were evaluated in advanced yield trials at Tel Hadya. The F_3 lines of *L. sativus* crosses for low neurotoxine B-N-Oxalylamino-alanin (BOAA) content and other desirable agronomic traits were evaluated at Tel Hadya and Breda. The F_1 crosses of *V. sativa* for nematode resistance and F_3 families of *V. sativa x V. sativa* ssp. *amphicarpa* were tested at Tel Hadya.

A study to investigate the potential of underground vetch under actual grazing conditions and its effect on the subsequent barley crop and its self regeneration after barley was continued. The reaction of promising lines against major foliar and root diseases was monitored in the disease nurseries at Tel Hadya. Table 4.1.1. lists the locations and number of forage legume species studied in 1993/94. All breeding work was done under rainfed conditions without supplementary irrigation.

As international center with major responsibility for WANA we aim to serve the national forage improvement programs through (1) assembling, classifying, evaluating, maintaining and distributing germplasm; (2) developing and supplying breeding populations with adequate diversity to be used in different environments; and (3) co-ordinate international trials to facilitate multiplocation testing and identification of widely adapted genotypes. **Ali M. Abd El Moneim.**

Table 4.1.1.   Trials, location and number of entries of
forage legume species studied in 1993/94.

Trial/species	Location	Entries
Nurseries		
Lathyrus sativus	Tel Hadya	40
L. cicera	Tel Hadya	41
L. ochrus	Tel Hadya	81
Microplot Yield Trials		
Vicia sativa	Tel Hadya	25
V. panonica	Tel Hadya	25
Advanced Yield Trials		
V. sativa	T.H.-Kfardan-Terbol	25
V. narbonensis	T.H.-Breda-Kfardan	36
L. sativus	T.H.-Breda	25
L. cicera	T.H.-Breda	25
L. ochrus	T.H.-Breda	16
V. dasycarpa	T.H.-Breda	25
V. palaestina	T.H.-Breda	16
V. ervilia	Tel Hadya	25
F_3-families *L. sativa* crosses	T.H.-Breda	85
F_4-families of *V. sativa* x *V. amphicarpa* crosses	Tel Hadya	36
Response to cutting	T.H.-Kfardan	10
Total		536

4.1.1. Germplasm Evaluation

4.1.1.1. Agronomic evaluation of common vetch accessions

The appraisal was carried out of 1400 accessions of *V. sativa* in nursery rows in a trial in collaboration with the Genetic Resources Unit (GRU). A total of 144 entries were selected on the basis of visual evaluation of seedling vigour, winter and spring growth, cold effect, leafiness, erect growth habit, and earliness to flowering and maturity. Further evaluation of their herbage and seed yields, reaction against major foliar and root diseases will be done in 1994/95. Results of common vetch germplasm evaluation are reported in 1994 annual report of the GRU. **Ali Abd El Moneim and L.D. Robertson.**

4.1.1.2. Evaluation of three lathyrus species and narbon vetch for adaptation

Two experiments were conducted, in the first 100 accessions of three lathyrus species from different origins, representing 40 *Lathyrus sativus*, 41 *Lathyrus cicera*, and 19 *Lathyrus ochrus* were planted in a triple lattice design with three replicates (5 rows each). In the second, 81 accessions of *V. narbonesis* were planted in the same design. Both experiments were fertilized with 40 kg P_2O_5/ha.

In these trials the accessions were visually scored at 1-5 scale (1-poor; and 5 very good) for establishment, seedling vigour, cold tolerance, winter and spring growth, plot cover, growth habit, plant vigour, leafiness, time to flowering and maturity, and diseases susceptibility.

There was wide variability for the characters studied (Table 4.1.2). Accessions having average score of 3, 4 and 5 were identified as promising genotypes for future breeding program. **Ali M. Abd El Moneim.**

Table 4.1.2. Variation in selection index (1=poor; 5 very good) of three *Lathyrus* spp. and narbon vetch germplasm grown in nursery rows at Tel Hadya, 1993/94.

Species	Selection index (1-5)					Total
	1	2	3	4	5	
Lathyrus sativus	–	–	10	10	20	40
L. cicera	5	5	6	10	15	41
L. ochrus	–	–	4	10	5	19
V. narbonensis	5	10	15	19	37	81

4.1.1.3. Germplasm evaluation of vetches and chicklings for cold tolerance

A total of 327 accessions of six vetch species and 438 accessions of 23 different species of chickling found cold tolerant during 1992/93 and 2659 new accessions belonging to 48 different species of vetches were screened for cold tolerance in 1993/94. The minimum temperature (-4.9°C) experienced on 14 Nov 1993 was not sufficient to kill the susceptible checks (IFLVPa 2727 of vetch and IFLLS 199 of chickling). Hence screening will be repeated in 1994/95. **R.S. Malhotra, Ali M. Abd El Moneim and L.D. Robertson.**

4.1.2. Preliminary Microplot Yield Trials (MYT)

The study of variation in agronomic characters helps the breeders to establish suitable breeding program to develop improved varieties. Selection for desirable traits such as leafiness, leaf-retention, long flowering period (for grazing purpose), rapid winter and spring growth, high herbage yield, high grain yield and harvest index (for grain and straw), non shattering pods, resistance to biotic and abiotic stresses and good nutritive value begins in microplot trials in a year or two after nursery rows evaluation. This leads to more critical tests in advanced yield trials before multilocation testing for selected promising lines.

In 1993/94 season microplots of two *Vicia* species i.e., *V. sativa* and *V. panonica* were tested at Tel Hadya in 3.5^2 plots arranged in triple lattice design. Number of entries for each trial are shown in Table 4.1.1. Seed rate was 100 kg/ha and fertilizers were applied at 40 kg P_2O_5/ha. These microplots were in two sets, one was harvested at 100% flowering to determine the herbage yield (DM) and the other was harvested at maturity to measure

seed and straw yields and other agronomic traits.

4.1.2.1. Common vetch (*Vicia sativa*)

Twenty-five selections were tested at Tel Hadya. Herbage yield (DM) at 100% flowering varied from 800 to 3519 kg/ha, total biological yield at maturity from 2033 to 4200 kg/ha, the grain yield from 400 to 1400 kg/ha; and harvest index from 14 to 39%.

The moderate and high temperatures in winter and spring accompanied with high rainfall at Tel Hadya facilitated development of foliar diseases such as downy mildew (*Peronospora viciae*), powdery mildew (*Erisiphi pisi*), ascochyta blight (*Ascochyta* spp.) and chocolate spot/blight (*Botrytis fabae*). Severe natural infection on leaves and stems caused severe damage and defoliation of leaves which subsequently led to low herbage and grain yields. Great variability was found among the tested selections. Table 4.1.3 shows the performance of the top 5 selections. These genotypes showed a high level of resistance to the above mentioned diseases. These are identified as a source of resistance for future breeding program.

4.1.2.2. Hungarian vetch (*V. panonica*)

Twenty five selection were assessed in microplot field trials at Tel Hadya. Herbage yield varied from 2168 to 4399 kg/ha, and total biological yield from 2742 to 5282 kg/ha whereas grain yield ranged from 699 to 1390 kg/ha.

Hungarian vetch is characterized by cold tolerance and slow winter growth, followed by rapid spring growth. The high herbage yield is due to the mild winter temperatures

that caused rapid winter growth. At the same time
Hungarian vetch was not highly susceptible to the attack
of foliar diseases. It is the first season that Hungarian
vetch out-yielded common vetch. Table 4.1.4 shows the
performance of the top 10 selections for both high herbage
and grain yield. **Ali M. Abd El Moneim.**

Table 4.1.3. Herbage (H), biological (B) and grain (G)
yields, harvest index (HI), and days to
flowering and maturity of the top 5 lines
of common vetch in preliminary yield trials
at Tel Hadya.

IFLVS	Yield (kg/ha)			HI (%)	Days to	
	H	B	G		Flower	Mature
2609	2319	3599	1400	39	115	160
2612	2854	3347	998	29	123	156
2623	2439	4200	1200	28	115	145
2624	3424	3859	1314	33	109	142
2625	3519	3695	1270	34	120	159
Mean+	2281	3417	591	15	115	148
SEM+	245	481	134	3.0	1.2	2.0
LSD (0.05)	700	980	301	8.4	3.4	5.5
CV (%)	22	24	28	30	1.8	2.3

+ Mean for all 25 selections.

4.1.3. Advanced Yield Trials (AYT)

Experiments were carried out to test promising lines
of *Vicia* spp. and *Lathyrus* spp at Tel Hadya (TH),
Breda (Br), Terbol (T) and Kfardan (Kfr) (Table
4.1.1). These lines were selected on the basis of
their performance in microplot yield trials for two
years. The trials were sown and managed as microplots
but with larger plot size ($28m^2$).

Table 4.1.4. Herbage (H), biological (B) and grain (G) yields (kg/ha), harvest index (HI) and days to flower and mature of the top 10 selections of Hungarian vetch in preliminary yield trials at Tel Hadya.

IFLVP	Yield (kg/ha)				Days to	
	H	B	G	HI(%)	Flower	Mature
2653	3277	4980	928	18	119	158
2654	3163	5282	1371	25	118	159
2656	3223	4923	1247	24	117	159
2657	4399	4990	1018	21	117	156
2662	3469	4306	1209	26	118	158
2664	2992	5116	1366	26	121	164
2669	3574	4214	1004	21	121	162
2671	3205	4259	1180	27	120	164
2673	3781	4838	1342	26	120	162
2674	3274	4678	1071	21	122	164
Mean[+]	3202	4090	1057	25	119	161
SEM±	305	487	73	3.1	0.6	1.0
LSD (0.05)	875	1369	220	8.7	1.8	3.0
CV (%)	16	20	15	21	0.9	1.1

+ Mean for all 25 selections.

4.1.3.1. Advanced yield trials of common vetch (*V. sativa*) at Tel Hadya, Terbol and Kfardan

Twenty five promising lines were tested. There were great differences between lines in their winter and spring growth, cold effect, and days to flowering and maturity.

In Tel Hadya, the herbage yield varied from 1271 kg/ha for IFLVS 2483 to 3625 kg/ha for IFLVS 2505, the total biological yield ranged from 2171 kg/ha for IFLVS 2483 to 5800 kg/ha for IFLVS 2487, whereas, grain yield ranged from 507 kg/ha for IFLVS 2483 to 1589 kg/ha for IFLVS2499.

In Terbol, the herbage yield varied from 2793 kg/ha for IFLVS 2497 to 5001 kg/ha for IFLVS 2506, biological yield from 5154 kg/ha for IFLVS 2497 to 8777 kg/ha for IFLVS 2497, and seed yield ranged from 1378 kg/ha for IFLVS 2497 to 2972 kg/ha for IFLVS 2495.

In Kfardan, herbage yield varied from 5039 kg/ha for IFLVS 2505 to 7494 kg/ha, IFLVS 2496, biological yield from 6072 kg/ha for IFLVS 2503 to 8083 kg/ha for IFLVS 2499, whereas, the grain yield varied from 1337 kg/ha for IFLVS 2495 to 2180 kg/ha for IFLVS 2497.

The large variations in both herbage and biological yields (Table 4.1.5) were mainly due to the variation among lines in their winter and spring growth, as indicated by the significant correlation between winter growth and the total biological yield of +0.640, +0.510 and +0.650 at Tel Hadya, Terbol and Kfardan, respectively.

The results in general indicate that there were large differences between the three sites in crop performance. The herbage yields were significantly higher in Kfardan than Terbol and Tel Hadya. In contrast seed yield at Terbol was higher than Kfardan and Tel Hadya. Common vetch responds well to high rainfall during the pod-filling stage. Therefore, seed yield was higher at Terbol.

4.1.3.2. Advanced yield trials of narbon vetch (*V. narbonensis*)

Thirty six promising lines of narbon vetch were evaluated under rainfed conditions at three locations i.e. Tel Hadya, Breda in Syria and Kfardan in Lebanon.

Table 4.1.5. Herbage, biological and grain yields for the 25 lines of common vetch (*Vicia sativa*) in AYT at Tel Hadya (TH), Terbol (T) and Kfardan (Kfr).

Selection IFLVS	Herbage yield (kg/ha)			Biological yield (kg/ha)			Grain yield (kg/ha)		
	TH	T	Kfr	TH	T	Kfr	TH	T	Krf
2483	1271	3861	4507	2171	6301	7276	507	2309	1897
2484	2666	2832	5202	3633	5619	6906	1134	1948	1869
2485	2539	3623	6409	3866	7341	7133	1147	2952	1734
2486	2932	3062	6854	4507	5607	6931	1163	2211	1739
2487	3323	3920	5939	5800	6953	7212	1542	2237	2051
2488	1933	3742	5620	3527	6380	6229	819	2120	1776
2489	2658	4095	6443	4205	8158	7672	1147	2621	1915
2490	3467	3787	5376	5108	6208	7232	1307	2214	2075
2491	3005	3523	7197	5295	6412	7361	1257	2381	1967
2492	2880	4875	5944	3904	6115	7316	1149	1861	1835
2493	3174	4191	6733	4581	5601	6955	1375	1908	1864
2494	2970	3886	7096	4774	5548	7159	1305	2069	1760
2495	2702	3844	6718	4270	8777	6554	1178	2972	1739
2496	2032	4220	7494	2645	6904	7667	774	2286	2096
2497	2494	2793	6934	4156	5154	7984	1051	1378	2180
2498	3370	4236	5810	4738	6968	6259	1180	2093	1337
2499	3424	3899	5636	5614	7556	8038	1584	2835	1947
2500	3068	3889	5948	5777	6231	7513	1443	2071	1902
2501	1900	4172	6760	3155	5689	7630	905	1974	1907
2502	3049	3872	7407	4027	5933	6646	1053	2193	1436
2503	2428	4193	5933	3381	5511	6072	952	1661	1693
2504	2815	3639	5894	4156	6666	6765	924	2429	1508
2505	3625	4074	5039	5038	5743	7448	1468	2255	2123
2566	1565	5001	6377	3144	5459	7028	878	1690	1694
2560	2636	4749	6149	3946	6074	7430	1020	1952	2172
Grand mean	2715	3958	6253	4219	6358	7137	1131	2185	1853
SEM±	189	450	615	490	738	545	152	329	160
LSD (P = 0.05)	1539	1293	1764	1393	2118	1565	433	945	459
CV (%)	12	19	17	20	20	13	23	26	15

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The objective is to determine the relative value to these locations for development of high and stable-yielding narbon vetch cultivars.

The experiments were sown in a triple lattice design with three replicates. Plot size was 5x5.8m^2. The experiments were sown by Oyjord experimental drill soon after the first autumn rains. Seeding rate was 100 kg/ha. Field germination was about 90%. All plots received a basal dressing of 40 kg P$_2$O$_5$/ha.

The results indicate that there were differences between the three locations in biological, straw, grain yields, harvest index and days to flowering and maturity (Table 4.1.6). There were also large differences among lines in their cold effect, winter and spring growth, earliness and reaction against major diseases, especially broomrape (*Orobanche crenata* Forsk), downy mildew (*Peronaspa viciae*) and rust (*Uromyces fabae*). Variations in biological and grain yields were mainly due to the variation in susceptibility to downy mildew and rust, at the three locations, where the natural conditons (rainfall and temperatures) favoured the natural infection and disease development. The high yielding lines at each location are shown in Table 4.1.7. Selections 2377 and 2561 were high yielding at the three locations because of their resistance to downy mildew and rust.

Table 4.1.6. Location means of biological (B) and grain (G) yields, harvest index (HI) and days to flowering and maturity for 36 lines of narbon vetch in AYT.

Location	Yield (kg/ha)			Days to	
	B	G	HI(%)	Flower	Mature
Tel Hadya	5230	1234	23	107	155
	($\pm$615)	($\pm$213)	($\pm$2.0)	($\pm$1.2)	($\pm$2.0)
Breda	4900	1368	28	101	149
	($\pm$453)	($\pm$190)	($\pm$2.8)	($\pm$1.7)	($\pm$2.1)
Kfardan	6114	1530	25	111	160
	($\pm$770)	($\pm$258)	($\pm$2.1)	($\pm$2.0)	($\pm$1.9)

Table 4.1.7. The most promising and adapted lines of narbon vetch at Tel Hadya, Breda and Kfardan.

Location	Promising lines
Tel Hadya	IFLVN, 2367, 2377, 2385, 2379, 2381, 2388, 2561
Breda	IFLVN, 2384, 2377, 2561, 2391, 2463, 2368, 2382
Kfardan	IFLVN, 2377, 2461, 2386, 2367, 2561, 2397, 2465

4.1.3.3. Advanced yield trials of common chickling (*Lathyrus sativus*)

Experiments were carried out to evaluate promising lines of common chickling (grasspea) at Tel Hadya and Breda. Twenty five genotypes were evaluated based on their performance in microplot yield trials in 1992/93. Herbage yield varied from 2580 to 3913 kg/ha at Tel Hadya and from 1967 to 2833 kg/ha at Breda, and seed yield varied from 1032 to 2196 kg/ha at Tel Hadya and from 795 to 1211 kg/ha at Breda. The mean of straw yield was 3569 kg/ha at Tel Hadya and 1834 kg at

Breda. Table 4.1.8 shows the top 9 lines based on their performance at both Tel Hadya and Breda. These lines possessed moderate resistance to downy mildew and ascochyta blight.

4.1.3.4. Dwarf chickling (*Lathyrus cicera*) advanced yield trials at Tel Hadya and Breda

Twenty five lines were evaluated at Tel Hadya and Breda. Mean herbage yield varied from 2953 to 4559 kg/ha at Tel Hadya, and from 1663 to 2640 kg/ha at Breda. Seed yield ranged from 1694 to 2293 kg/ha at Tel Hadya and from 561 to 1106 kg/ha at Breda, whereas, straw yield varied from 3692 to 5630 kg/ha at Tel Hadya and from 955 to 1931 kg/ha at Breda (Table 4.1.9). The mean herbage, seed and straw yields varied widely at each location. Table 4.1.10 shows the most promising lines at each location. No individual line was promising at the two locations. The relatively low herbage and seed yields at Breda was apparently due to the severe infection of downy mildew.

4.1.3.5. Ochrus chickling (*Lathyrus ochrus*) advanced yield trials at Tel Hadya and Breda

Sixteen lines of ochrus chickling were tested at Tel Hadya and Breda. The straw yield varied from 4823 to 7159 kg/ha at Tel Hadya and from 3700 to 5400 kg/ha at Breda. At maturity, the grain yield ranged from 1472 to 2467 kg/ha at Tel Hadya and from 1001 to 1850 kg/ha

Table 4.1.8. Herbage, seed and straw yields and days to flowering and maturity for the top 9 lines of common chickling (*Lathyrus sativus*) in advanced yield trials at Tel Hadya and Breda (B).

Selection IFLS	Herbage yield TH	B	Seed yield TH	B	Straw yield TH	B	Days to flowering TH	B	Days to maturity TH	B
553	3505	2304	1559	1041	3411	2059	120	124	170	166
555	2703	2198	1472	1073	3498	2356	117	128	169	166
556	2909	2694	1767	1048	3811	2166	112	115	169	157
558	3231	2437	1034	1031	3132	2008	120	126	170	166
559	3604	2833	2196	1143	4558	1947	116	126	169	166
560	3443	2402	1754	1001	3855	2190	117	125	169	168
561	3477	2335	2040	111	4192	2041	118	118	170	166
562	3913	2665	1388	1051	3642	1943	115	117	167	159
566	3906	2519	1851	1211	3614	1865	111	117	162	159
Grand mean[+]	3185	2440	1629	1026	3569	1834	115	118	169	162
SEM ±	434	144	216	114	411	212	0.8	0.3	1.5	0.5
LSD (P = 0.05)	1235	413	613	326	1170	607	2.3	0.8	4.5	1.3
CV (%)	24	11	23	20	20	20	2	1.4	1.7	2.5

+ Mean for all 25 selections.

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Table 4.1.9. Mean ±SEM and range of herbage, seed and straw yields (kg/ha) of 25 lines of dwarf chickling in advanced yield trials at Tel Hadya and Breda.

Location	Herbage yield	Seed yield	Straw yield
Tel Hadya			
Yield ±SEM	3776±249	1968±135	4642±309
Range	2953-4559	1694-2293	3692-5630
Breda			
Yield ±SEM	2241±183	844±117	1454±170
Range	1663-2640	561-1106	955-1931

at Breda Table (4.1.11). The most promising lines at Tel Hadya are IFLLO 551, 549, and at Breda IFLLO 185, 549 and 545. The results confirm that ochrus chickling is highly productive only in moderate winter seasons, because of its susceptibility to cold. This season it produced more straw and grain yields than both common chickling and dwarf chickling. The results also confirmed its high resistance to broomrape (*Orobanche crenata* Forsk), whereas both common chickling and dwarf chickling were attacked by broomrape at the pod-filling stage, especially the late maturing selections.

Table 4.1.10. The most promising lines of dwarf chickling (*Lathyrus cicera*) at Tel Hadya and Breda.

Location	Promising lines IFLLC
Tel Hadya	494, 495, 497, 498, 473, 569
Breda	487, 492, 496, 499, 573, 574

Table 4.1.11. Mean± SEM and range of straw and seed yields of 16 lines of ochrus chickling in advanced yield trials at Tel Hadya and Breda.

Location	Straw yield (kg/ha)			Seed yield (kg/ha)		
	Mean	±SEM	Range	Mean	±SEM	Range
Tel Hadya	5811	401	4823-7159	2091	200	1472-2467
Breda	4358	360	3700-5400	1568	160	1001-1850

4.1.3.6. Advanced yield trials of wooly-pod vetch (*Vicia villosa ssp. dasycarpa*)

Twenty five lines were tested at Tel Hadya and Breda. There were differences between lines at both locations in herbage yield (DM), seed and straw yields and days to start flowering, 100% flowering and maturity. The dry matter yield varied from 3647 to 6025 kg/ha, at Tel Hadya and from 3472 to 5001 kg/ha at Breda. Seed yields ranged from 963 to 1410 kg/ha at Tel Hadya and from 534 to 1002 kg/ha at Breda (Table 4.1.12). In contrast to other *Vicia* spp, wooly-pod vetch produced high herbage and straw yields and low seed yield. Seed yield was negatively correlated ($r=-0.590$, $P=0.01$) with days to start flowering. The high herbage yielding lines are characterized by high leaf-retention, which is a good character for high quality hay. The results also confirmed that wooly-pod vetch is resistant to broom-rape (*Orobanche crenata* Forsk.). Lines IFLVD 2562, 2431, 2437, and 2439, were the most promising lines at Tel Hadya and IFLVD 2652, 2433, 2438, 2439, 2457 at Breda, respectively. More emphasis has to be given to reduced pod-shattering, and high leaf-retention and earliness in flowering and maturity to improve productivity of wooly-pod vetch.

Table 4.1.12. Mean +SEM, range of dry matter (DM), seed and straw yields and days to start flower, 100% flowering and maturity of 25 lines of wooly-pod vetch in advanced yield trials at Tel Hadya and Breda.

Location	Yield (kg/ha)			Days to		
	Dry matter	Seed	Straw	Flower	100% flower	Mature
Tel Hadya						
Mean +SE	5184+522	1198+152	5581+541	122+2.0	134+1.2	173+3.0
Range	3674-6025	963-1410	3936-6605	115-130	125-142	161-173
Breda						
Mean +SE	4242+490	799+140	3462+660	124+1.5	130+1.1	162+2.1
Range	3472-5001	534-1002	2235-5005	121-132	127-137	159-167

4.1.3.7. Advanced yield trials of Palaestine vetch (*Vicia palaestina*)

Sixteen promising lines of Palaestine vetch were tested at Tel Hadya and Breda (Table 4.1.13). The herbage yield varied from 2742 to 4320 kg/ha at Tel Hadya and from 2828 to 4324 kg/ha at Breda. At maturity, the grain yield ranged from 1072 to 1965 kg/ha at Tel Hadya and from 856 to 1394 kg/ha at Breda. It produced high straw yield at both locations, due to high leaf-retention, and tall erect plant type.

Table 4.1.13. Herbage (DM), straw and seed yields (kg/ha) of 16 promising lines of Palaestine vetch (*Vicia palaestina*) grown at Tel Hadya and Breda in advanced yield trials.

IFLVP	Tel Hadya			Breda		
	DM	Straw	Seed	DM	Straw	Seed
2523	3335	4052	1407	2852	3052	1126
2524	2742	3244	1409	3290	3030	1112
2525	3481	4247	1367	3462	3422	1093
2526	3851	4048	1978	4136	3384	1582
2527	3952	3815	1642	4072	3222	1313
2528	3839	3602	1409	3002	3080	1127
2529	3647	3978	1532	3424	3674	1225
2530	3129	4010	1242	3582	3900	993
2531	4147	4050	1117	4324	3412	893
2532	3913	3790	1072	3142	3682	857
2533	4257	3853	1536	2848	3296	1228
2534	4320	4370	1176	3326	3136	940
2535	3219	4370	1500	3062	3040	1200
2536	3413	3536	1743	3166	2540	1394
2537	3223	4080	1511	3034	3784	1201
2536	3397	3775	1627	2828	3646	1301
Mean	3616	3926	1454	3346	3270	1161
SEM+	232	255	137	285	210	106
LSD (0.05)	671	737	396	724	439	315
CV (%)	12	13	17	18	16	18

Table 4.1.14. Variation in major attributes of three *Vicia* spp. and three *Lathyrus* spp. at Tel Hadya.

	Days to flower		Yield (kg/ha)			HI
	Start	100%	Herbage	Seed	Straw	(%)
V. narbonensis	107	118	-	1234	3767	24
V.v. ssp. dasy	122	134	5184	1198	5581	17
V. palaestina	104	119	3616	1454	3929	31
L. sativus	115	120	3185	1628	3969	31
L. cicera	110	134	3779	1968	4642	29
L. ochrus	107	120	-	2091	5811	26

The plants are vigorous and compete well with weeds during the vegetative period. No symptoms of downy mildew or ascochyta blight appeared on the plants during this season.

Table 4.1.14 is a summary of the 1993/94 advanced yield trials at Tel Hadya for three *Vicia* and three *Lathyrus* species. Bearing in mind that forage legumes can be used for grazing, hay, straw and grain, we can begin to see how the various species will meet the farmers needs into the prevailing farming systems in the region. The high seed and straw yields and early flowering of narbon vetch and Palaestine vetch will be of value to farmers who want straw and grain. In contrast to narbon vetch, Palaestine vetch can also be used for hay in spring because of its high leaf-retention. For those who require hay or grazing, wooly-pod vetch and perhaps dwarf chickling will be attractive. The results of common chickling are affected by its susceptibility to *Orobanche*, but past experience suggests that in dry areas, it can be used for grain and straw. Because of its resistance to *Orobanch*, ochrus chickling can be used for grazing or for grain and straw in areas heavily infested with *Orobanche*.

4.1.3.8. Advanced yield trials of bitter vetch (*V. ervillia*) at Tel Hadya

Twenty five selections were tested at Tel Hadya. Herbage yield at 100% flowering varied from 3660 to 6062 kg/ha, while the grain yield ranged from 932 to 2532 kg/ha and harvest index from 18 to 37%. Table 4.1.15 shows the performance of the 9 top selections. Bitter vetch showed very rapid winter and spring growth as compared to other vetches. Its yield was, therefore, substantially better than other vetches. No symptoms of downy mildew, powdery mildew or ascochyta blight appeared, but the late maturing selections were affected by *Orobanche*. **Ali M. Abd El Moneim**.

4.1.4. Response of Promising Lines of *Vicia* and *Lathyrus* spp. to Simulated Grazing at Different Growth Stages at Tel Hadya (Syria) and Kfardan (Lebanon)

The objective of this trial was to study the response of *Vicia* and *Lathyrus* spp. to simulated grazing (cutting), the ability for regrowth after cutting under rainfed conditions and to compare some forage quality at various times during the growing season.

One of the questions one can ask in assessing the productivity is as to at what time should herbage production and quality of different *Vicia* and *Lathyrus* spp. be measured. Our practice has been to measure herbage yield at 100% flowering stage, because this is the time normally recommended for hay cuts, and to measure seed yield at maturity. But if farmers are more interested in grazing, this would seem to be unsatisfactory. Furthermore, harvesting at a particular phenological stage can mean big difference in age and hence yield. We need to know whether growth continues

Table 4.1.15. Winter growth, herbage seed and straw yields, harvest index (%), days to 1st flowering, 100% flowering and maturity for the top 9 selections of bitter vetch (*V. ervilia*) in advanced yield trials at Tel Hadya.

IFLVE	Winter[+] growth 1-9	Herbage yield (kg/ha)	Seed yield (kg/ha)	Straw yield (kg/ha)	Harvest index (%)	Days to		
						Start flowering	100% flowering	Maturity
2511	8	5512	2292	5265	30	106	11	149
2513	8	5225	2471	4355	36	103	116	148
2515	8	5951	2376	4431	35	102	115	146
2516	8	5400	2480	4639	33	103	117	144
2517	8	4610	2422	4609	34	103	116	149
2518	8	6252	2342	3997	37	101	115	147
2519	7	5127	2173	5202	29	106	120	151
2522	8	6062	2582	4323	34	100	114	144
2646	6	5245	2421	4391	35	111	123	150
Grand mean[++]	7.9	5348	2029	4456	31	109	121	153
SEM±	0.41	364	166	343	1.8	1.2	1.4	0.9
LSD (P = 0.05)	1.2	1035	474	976	5.1	3.4	3.8	2.5
CV (%)	9	12	15	14	10	1.9	1.9	1.02

[+] On 1 to 9, visual scale basis where 1 = poor and 9 very good.
[++] Mean for all 25 selections.

after cutting at a particular stage and the changes in forage quality. Also, the regrowth rate after cutting at different growth stages may vary among different *Vicia* and *Lathyrus* spp.

Two common vetch lines and one each of bitter vetch, Palaestine vetch, broad podded vetch, wooly-pod vetch, narbon vetch, ochrus chickling, common chickling and dwarf chickling were used in this experiment.

Identical experiments were sown at Tel Hadya on November 26, 1993 and at Kfardan (Lebanon) on November 27, 1993 at a seeding rate of 100 kg/ha except for narbon vetch and ochrus chickling the seed rate was 120 kg/ha, with 40 kg P_2O_5/ha. A randomized block design was used, with three replicates and 5 x 5.8 m plots. Each plot was subdivided to several 1.m^2 subplots, leaving adequate guard areas around the whole plot as well as between the sampling areas. At each cut (harvest), two 1.m^2 subplots were harvested to make up a total of 2.m^2. The first harvest at Tel Hadya was on 20 February, and at Kfardan on 21 February. Harvest 2-5 were taken on March 7, March 22, April 5, April 20 at Tel Hadya and March 9, March 23, April 6, April 21 at Kfardan. The corresponding plant ages were 59, 74, 104, 119 days from 100% germination at Tel Hadya, and 56, 70, 85, 101, 116 days from 100% germination at Kfardan. At maturity subplots were harvested for estimating grain yields.

At each harvest, fresh materials was weighed and subsamples dried at 80-90°C for 24 h to determine dry matter content. The phenological stage of each entry was recorded at each harvest.

Based on dry matter production (dry herbage) of

each entry from the first and second harvest, all
entries were able to survive during the winter at both
locations, however winter at both locations was mild
with less than average number of frost days.

There were clear differences in phenology (Table
4.1.16). Palaestine vetch and narbon vetch were early
reaching start of their flowering at Tel Hadya and
Kfardan at a time when the other entries were still at
vegetative stage. Flowering at Tel Hadya was
generally earlier than Kfardan.

During the vegetative stage of growth differences
in dry matter yield at each cut were large (Table
4.1.17). Up to cut 3, bitter vetch, wooly-pod vetch
and narbon vetch gave the highest herbage yield.
Ochrus chickling produced 0.5 t/ha at the first cut,
but at the fifth cut (100% flowering), its production
increased up to 7.12 t/ha at Tel Hadya and 4.72 t/ha
at Kfardan. Generally, the herbage yield of the first
and second cut of all entries were higher in Kfardan
than Tel Hadya. This is in contrast to the fifth cut
when most of the entries produced higher herbage at
Tel Hadya than at Kfardan (Table 4.1.17).

Regrowth after cutting was recorded two weeks
after each cut. *V. palaestina*, *V. hybrida* and *V.
villosa* ssp. *dasycarpa* showed rapid regrowth after
cutting up to cut 2. All the entries did not make
regrowth after cut 3 at Tel Hadya, but *V. sativa* 713,
V. palaestina 2525 and *V. hybrida* 2548 regrew poorly
after cut 4 at Kfardan (Table 4.1.18). The regrown
materials were left until maturity for seed yields
estimation at Tel Hadya. After cut 1, all entries
produced seeds from the regrown materials which varied
from 174 kg/ha for wooly-pod vetch to 696 kg/ha for
bitter vetch. At harvest 2, seed yield of wooly-pod

Table 4.1.16. Phenological stages at different cutting times and plant ages for *Vicia* spp. and *Lathyrus* spp. grown at Tel Hadya and Kfardan, 1994.

Location/Cut No.	Plant age (days)	Vs713	Vs2560	Ve2517	Vp2525	Vh2548	Vd683	Vn2380	Lo542	Ls587	Lc501
Tel Hadya 1	59	V*	V	V	V	V	V	V	V	V	V
2	74	V	V	V	V	V	V	V	V	V	V
3	89	V	V	V	Start F	V	V	Start F	V	V	V
4	104	Start F	V	Start F	50% F	Start F	Start F	50% F	50% F	V	Start F
5	119	50%	Start F	50% F	100% F	100% F	25% F	100% F	100% F	Start F	50%
Kfardan 1	56	V	V	V	V	V	V	V	V	V	V
2	70	V	V	V	V	V	V	V	V	V	V
3	86	V	V	V	V	V	V	V	V	V	V
4	101	V	V	V	Start F	Start F	V	Start F	Start F	V	V
5	116	Start F	Start F	Start F	50% F	50% F	Start F	50% F	50% F	V	Start F

* V = Vegetative, F = Flowering

Table 4.1.17. Herbage yield (DM) kg/ha of the different vetches and chicklings from cut 1 to 5, at Tel Hadya and Kfardan.

Location/Cut No.	Vs713	Vs2560	Ve2517	Vp2525	Vh2548	Vd683	Vn2380	Lo542	Le587	Lc501	LSD (P=0.05)
Tel Hadya 1	461	529	628	566	256	652	656	561	343	290	191
2	1042	1286	1356	934	930	1873	1682	1534	1004	969	353
3	2506	2346	3082	1945	1984	3346	2950	3319	1886	2111	655
4	3635	2922	5095	3366	3391	4861	3941	4222	2832	2829	738
5	5240	4876	7056	4539	5027	4890	5927	7128	4700	5501	1107
Kfardan 1	590	723	957	849	453	743	953	783	464	375	298
2	1293	1691	1624	1011	910	1838	1779	1392	842	900	361
3	2684	2157	2912	1357	1533	2583	2489	3352	1191	1203	665
4	5194	4006	4596	2289	3014	5545	4922	3950	1893	2630	1173
5	5577	5258	5142	3521	4136	4437	5278	4727	3521	4069	840

Table 4.1.18. Regrowth after cutting, on 1-9 scale (where 1 is poor, 9 excellent) from cuts 1 to 5, for different *Vicia* spp. and *Lathyrus* spp. grown at Tel Hadya and Kfardan.

Location/Cut No.		Vs713	Vs2560	Ve2517	Vp2525	Vn2548	Vd683	Vn2380	Lo542	Ls587	Lc501	LSD (P = 0.05)
Tel Hadya	1	7.3	6.6	6.6	8.6	8.3	8.6	4.3	5.0	7.0	7.0	1.3
	2	5.3	5.6	4.6	7.6	9.0	6.3	5.3	3.6	4.0	5.3	1.4
	3	3.6	2.6	1.0	5.6	3.6	1.0	4.3	1.0	2.3	3.6	0.99
	4	-	-	-	-	-	-	-	-	-	-	-
	5	-	-	-	-	-	-	-	-	-	-	-
Kfardan	1	3.6	5.0	3.0	6.6	5.6	5.6	4.3	3.0	1.6	3.0	2.3
	2	7.0	7.0	3.6	7.6	5.0	5.0	5.0	4.3	3.0	3.6	1.7
	3	4.3	5.6	1.0	5.6	5.0	3.0	3.0	3.0	3.0	2.0	1.3
	4	3.0	-	1.0	4.3	2.0	-	-	-	-	-	1.2
	5	-	-	-	-	-	-	-	-	-	-	-

Table 4.1.19. Seed yield (kg/ha) from the regrowth after cuttings 1, 2 and 3 and at full maturity of different *Vicia* spp. and *Lathyrus* spp. grown at Tel Hadya 1994.

Species/IFLNO	Cut 1	Cut 2	Cut 3	Maturity
Vicia sativa 713	228	320	128	1140
V. sativa 2560	348	355	142	1743
V. ervilia 2517	696	511	-	2323
V. palaestina 2525	386	389	120	1287
V. hybrida 2548	377	308	90	1257
V. v. ssp. dasy. 683	174	70	-	980
V. narbonensis 2380	200	90	-	2753
Lathyrus ochrus 542	395	285	40	1653
L. sativus 587	465	300	110	1550
L. cicera 501	402	420	168	1343
Mean	367	304	114	1602
SEM ±	47.74	43.118	15.45	207
LSD (P = 0.05)	107.9	97.2	35.00	617

vetch, bitter vetch and Palaestine vetch was low and after cut 3 bitter vetch, wooly-pod vetch and narbon vetch did not produce any seeds (Table 4.1.19).

Results of Tables 4.1.17 and 4.1.19 indicate that most of the entries regrew after early cutting (cut 1 and 2) providing more than one ton/ha dry matter yield and enough seed yields for resowing. If farmers desperately need a forage crop for early grazing (late February-beginning of March, common vetch, bitter vetch and wooly-pod vetch are recommended. Grazing at this stage should be moderate to give ability to plants to regrow for seed production.

Changes in quality parameters (CP%, NDF% and ADF%) are shown in Table 4.1.20a and 4.1.20b, at Tel Hadya and Kfardan, respectively. At early growth stage the CP% in herbage was less than 20% in all species in Kfardan except narbon vetch and common chickling, but at Tel Hadya the CP% of all crops was around 20%. The CP % tended to decrease at cut 5. In contrast the NDF% and ADF% increased pregressively with time. This is due to the reduction in leafiness with advance of crop towards maturity.

The crude protein (CP%) for grain and straw was estimated at maturity only at Tel Hadya (Table 4.1.21). Palaestine vetch had the highest protein content in seeds and dwarf chickling had the highest value for the straw.

The results obtained from this trial should be considered tentative as values may vary from year to year and from environment to environment due to variation in weather conditions. However, they can provide good comparison between different species.

It can be concluded that at Tel Hadya and Kfardan conditions and possibly at similar sites *V. sativa* 713 and 2560, *V. ervilia* 2517, *V. villosa* ssp. *dasycarpa* 683 can be grazed at early stages when there is a great need for herbage. *V. palaestina* can be grazed even at a latter stage because of its better ability for regrowth and seed production. *Vicia narbonensis* 67 should not be grazed and should only be grown for seed and straw production. *V. palaestina* 2525, can be used for hay making because of its high leaf-retention and high CP% content making it suitable for winter feeding. **Ali M. Abd El-Moneim and M.C. Saxena.**

Table 4.1.20a. Seasonal changes in crude protein (CP%), neutral detergent fibers (NDF%) and acid detergent fibers (ADF%) of ten feed legume crops grown at Tel Hadya, 1994.

Character	Cut No.	Vs713	Vs2560	Ve2517	Vp2525	Bh2548	Nd683	Vn2380	Lo542	Ls587	Lc501
CP%	1	21.06	22.10	23.80	24.40	24.86	21.14	20.20	20.46	24.46	26.00
	2	21.80	23.30	25.73	26.46	24.40	23.40	23.40	23.20	27.80	27.50
	3	21.50	23.60	21.33	26.50	24.13	25.90	22.26	22.93	27.20	29.66
	4	21.60	23.50	17.46	26.20	24.33	21.33	21.20	22.40	27.33	28.40
	5	16.80	18.80	15.06	16.86	15.90	16.96	15.00	15.50	18.66	17.80
NDF (%)	1	21.60	19.30	15.34	22.49	18.40	19.73	15.60	17.68	18.81	17.84
	2	25.70	25.16	17.85	27.11	22.12	24.38	24.64	20.17	22.38	18.86
	3	29.00	32.16	23.90	33.70	30.34	32.42	34.86	23.84	31.44	28.00
	4	34.40	36.86	31.83	34.50	38.79	41.24	38.10	32.78	36.00	29.00
	5	43.00	40.37	36.87	48.67	49.68	44.88	25.56	35.22	46.27	38.35
ADF (%)	1	13.80	13.14	11.15	13.72	12.00	13.22	11.68	11.17	12.13	12.94
	2	18.00	17.05	14.98	19.00	15.16	18.12	18.12	15.30	16.18	14.36
	3	20.00	19.06	19.14	24.30	18.90	20.80	21.00	17.20	21.50	16.33
	4	22.00	24.23	22.84	24.00	21.00	27.86	20.60	18.38	23.10	18.50
	5	28.00	26.50	27.00	30.70	30.36	27.82	27.10	25.40	27.50	23.94

Table 4.1.20b. Seasonal changes in crude protein (CP%), neutral detergent fibers (NDF%) and acid detergent fibers (ADF%) of ten feed legume crops grown at Kfardan, 1994.

Character	Cut No.	Vs713	Vs2560	Ve2517	Vp2525	Vh2545	Vd683	Nv2380	Lo542	Ls587	Lc501
CP(%)	1	19.46	17.66	20.00	14.90	17.46	16.46	22.00	17.60	20.26	17.26
	2	26.20	25.90	26.40	26.00	26.53	28.26	24.90	22.80	30.33	29.33
	3	26.20	27.53	26.1	28.80	28.53	27.13	26.70	25.00	29.00	30.93
	4	25.13	26.73	22.40	27.00	27.13	26.26	23.50	24.60	28.50	31.20
	5	18.73	19.73	16.26	20.33	19.07	23.33	15.50	17.60	20.35	23.46
NDF (%)	1	26.50	24.25	19.96	24.46	31.80	22.78	26.52	20.42	22.29	18.42
	2	30.00	28.50	24.81	34.10	35.92	27.41	30.00	23.84	27.82	30.74
	3	40.00	38.14	31.11	37.80	42.90	32.60	41.40	34.00	32.86	37.10
	4	38.00	39.36	29.17	39.40	45.28	31.60	41.40	34.00	3286	37.10
	5	43.00	40.00	33.26	34.17	45.12	35.87	42.20	36.70	40.00	38.50
ADF (%)	1	17.74	17.12	14.48	19.25	18.28	17.92	15.00	15.00	15.50	13.50
	2	16.80	18.38	16.26	22.88	21.00	21.30	18.00	16.72	17.00	17.00
	3	20.47	20.78	19.00	26.17	20.70	20.68	19.50	18.50	20.00	19.00
	4	25.88	23.14	23.00	21.20	21.80	23.60	20.78	20.00	21.00	18.50
	5	28.50	25.90	23.60	31.12	29.00	30.30	30.60	23.74	23.50	25.70

Table 4.1.21. Crude protein (CP%) of grains and straw of *Vicia* spp. and *Lathyrus* spp. grown at Tel Hadya, 1994.

Species IFLNO	CP (%)	
	Grain	Straw
Vicia sativa 713	29.4	5.8
V. sativa 2560	28.2	6.7
V. ervilia 2517	25.0	6.0
V. palaestina 2525	32.8	8.4
V. hybrida 2548	29.8	7.3
V. villosa ssp. *dasycarpa* 683	27.0	10.0
V. narbonensis 67	27.4	8.1
Lathyrus ochrus 542	28.0	9.2
L. sativa 587	25.0	9.2
L. cicera 501	28.2	10.6
Mean	28.08	8.03
SEM ±	0.727	0.510
LSD (P=0.05)	1.644	1.153
CV (%)	8.19	20.09

4.1.5. Quality

4.1.5.1. Chemical composition and biological assays of nutritional value of some *Vicia* and *Lathyrus* spp.

Twentyfive straw and 16 herbage samples of *Lathyrus sativus*, *Vicia narbonensis*, and *Vicia ervilia* were analayzed for ash, crude protein, cell constituents (neutral detergent fiber (NDF) and acid detergent fiber (ADF), total phenols (TP), and Tannin, (T) (both as tannic acid equivalent), condensed tannins (CT) (as leucocyanidin equivalent), protein precipitation capacity (PPC), and *in vitro* characteristics predicted from Hohenheim gas test, organic matter digestibility (OMD), and metabolizable energy (ME).

The content of crude protein of the herbage was

significantly higher than that of the straw (152.6 vs 64.5 g/kg) and of ash (82.8 vs 116 g/kg), NDF (332.7 vs, 523.5 g/kg) and ADF (205.2. vs 369.9 g/kg) were significantly lower. For straws of *L. sativus*, *V. narbonensis* and *V. sativa* there was no significant difference in protein and ADF contents, whereas, ash content was significantly lower and NDF% higher in *L. sativus* as compared to both the species of *Vicia*. Predicted from gas volume, the OMD (70 vs 56%) and ME (9.7 vs 7.3 MJ/kg) was significantly higher in the herbage compared to straw. The OMD of *L. sativus* straw was significantly lower compared to the straws of *V. narbonensis* and *V. sativa*. The TP, T and CT for straw were 1.08%, 0.43% and 0.33%, respectively and those for herbage 2.27%, 1.30% and 1.63%, respectively. The PPC was not detected in any herbage or straw sample. The results suggested that the tannin levels of legume straws are negligible and those of the herbage very low, which do not appear to adversely affect the nutritive value of herbage to any appreciable extent (the maximum decrease in OMD by tannins was about 3% units). In addition, the nutritive value of the legume straw is better than cereal straws and is comparable to good quality hay. Details of this study are reported in PFLP annual report. **Harinder Makkar and Kluas Becker, University of Hohenheim, Germany, Antony Goodchild, PFLP and Ali M. Abd El Moneim, GP.**

4.1.5.2. Nutritional quality of chicklings (*Lathyrus* spp.)

Chicklings (*Lathyrus* spp.) are drought tolerant protein rich food and feed legumes of areas with less than 350 mm rainfall. *Lathyrus sativus* (common chickling or grasspea) is particularly adapted to dry conditions and is generally grown on fields with marginal soil fertility and low soil moisture and with poor management practices. One of the major factors limiting the use of *Lathyrus* spp. for human

consumption and animal feed is that the seed contains the neurtoxine B-N-Oxalylamino-alanin (BOAA). The excessive consumption of seeds causes "Lathyrism", a nervous disorder resulting in incurable paralysis of lower limbs of human being and domestic animals. One of our major objectives in breeding chicklings is to develop lines which are early, free from the neurotoxine and adapted to low rainfall areas. We also investigate the association of BOAA content with agronomical and morphological characters of the genotypes, and their reaction against insects and diseases. ICARDA in collaboration with the Grain Research Laboratory of Winnipeg, Manitoba, Canada has developed a rapid method of determing BOAA content using near infrared reflectance (NIR).

4.1.5.3. Evaluation of BOAA content in new lines of Lathyrus spp.

One hundred and ten lines representing 70 *L. sativus*, 24 *L. cicera* and 16 *L. ochrus* were assessed for their BOAA content using NIR (Table 4.1.21). The results indicate that in one line of *L. sativus* the BOAA content was very low (0.07%). This line is characterized by large white seed and white flower and it matured 10 days later than the high BOAA lines. *L. ochrus* lines had the highest BOAA content with a mean of 0.615% and *L. cicera* was low with a mean of 0.159%. The results appear to be highly encouraging as they point to the possibility of developing chickling lines for overcoming the problem of "Lathyrism". **Ali M. Abd El Moneim.**

Table 4.1.22. BOAA content (%) for three *Lathyrus* spp. grown at Tel Hadya, 1994.

Species	Range	Mean	SEM±	No. of lines
Lathyrus sativus	0.070-0.750	0.492	0.12	70
Lathyrus cicera	0.096-0.220	0.159	0.03	24
Lathyrus ochrus	0.49-0.71	0.615	0.06	16

4.1.5.4. Estimation of anti-nutritional or anti-palatability factors in narbon vetch.

It has been reported that narbon vetch contains undesirable substances that decrease its value as feed legume crop. The substances include toxic or anti-nutritional or anti-palatability factors, usually complex organic compounds that are difficult to measure by conventional analysis. If the concentration of these compounds is to be reduced or eliminated, through crop breeding program, rapid techniques of analysis are needed. Dr. Neil Rothine at the Western Australia Chemistry Center developed a new Capillary Zone Electrophoreses (CZE) technique which has produced outstanding results for a compound in narbon vetch, the gamma-glutamyl derivative of S-ethanyl Cystein (GEC), which is responsible for its unpalatability.

Forty-six selections from ICARDA were grown at Muresk Research Station, Western Australia. The seeds were analyzed for GEC%. There were large variations in GEC% in the selections and their crossbreds. The GEC varied from 0.58 to 1.38%. IFLVN 67, 560, 127, 556, 1144 and 568 had the lowest GEC content and IFLVN 582 the highest.

Regarding the crossbreds, a single plant selection from cross IFLVN 2524 x IFLVN 67 had 0.12% GEC and another 1.41%, whereas the GEC content of the parents was 1.83 and

0.60%, respectively. F_3 derived from a cross IFLVN 567 x
IFLVN 575 also gave a wide range of GEC content (from 0.17
to 2.5% GEC). This indicates that there is a good scope
for selection for low GEC content in narbon vetch. **Clive
Francis (CLIMA) and Ali M. Abd El Moneim.**

4.1.5.5. Improving nutritional quality of *Lathyrus sativus* by breeding

This program was initiated after a large number of *L.
sativus* lines maintained in our breeding program were
screened for BOAA content using NIR and conventional
chemical method as described by Briggs et al. 1983. As a
result of screening a few lines were found having low BOAA
content (0.02 to 0.1%) and a large number having high BOAA
content (0.2 to 0.9%). The low neurotoxine lines had
undesirable agronomic traits like late flowering and low
yield. So far, little work has been done to determine the
genetic basis of variation in BOAA and the associated
agronomical and morphological characters. Hence, this
project was started.

The basic material of this study consists of
twenty five genotypes of *L. sativus*, representing the
available variability in BOAA and diversity in origin.
Out of these genotypes 4 testers were crossed with 21
females and 84 hybrids combinations were obtained. Gene
markers such as seed and flower color were used to
eliminate pods that might have developed from selfing and
to identify F_1 plants obtained in 1991/92, and F_2 in
1992/93. Due to transgressive segregation towards
earliness and high BOAA, or due to partial dominance to
high BOAA and early maturity, large proportion of F_2
population matured earlier than the parents. 192 families
descending from F_2 single plant selections, were selected
for early maturity, small seed size, large seed size and

light cream seed color and low BOAA. These characters are considered major selection criteria for breeding low BOAA. In 1993/84, 85 families were evluated at Tel Hadya and Breda for yield potential and the effect of location on BOAA content. Three families (19, 80 and 85) had low BOAA content (0.02, 0.017 and 0.06%, respectively). They had large seed size, white seed color and white flower colour. Seed yield was high at both locations (0.9 to 1.5 t/ha). The effect of location on BOAA was not significant since families with high or low BOAA have retained their levels at both locations. The three low neurotoxine families were 10-15 days late in maturity than lines with high BOAA. The 85 families will be further tested at Tel Hadya and Breda for a second season to select material for international testing trials. **Ali M. Abd El Moneim.**

4.1.6. Studies of Hybrids Between *V. sativa* ssp. *sativa* and *V. sativa* ssp. *amphicarpa*.

To enhance the herbage production of underground vetch (*V. sativa* ssp. *amphicarpa*) and to improve the drought and cold tolerance of common vetch (*V. sativa* ssp. *sativa*) a hybridization program has been underway since 1989/90 as each subspecies possesses some unique agronomic traits. Three promising lines of common vetch with non-shattering-pods i.e. IFLVS 1416, 713 and 1448 were crossed with two lines of underground vetch i.e. IFLVA 2416 and 2614 to make twelve crosses. The F_2 population released enormous variability for selection. Through a multiple trait selection, F_3 families selected had 4-9 underground pods per plant, cold and drought tolerance, more vigorous above ground growth and above ground pods as nonshattering as those of the common vetch parent. Selection for F_4 families was obtained by bulking an equal number of seeds of 20 F_3 plants from the selected F_3 families. The F_4 families were grown in 1993/94 at Tel Hadya. Families

having 6-10 under ground pods and vigorous above ground growth were selected. The above ground biomass of the selected families was three times as much as that of the amphicarpous-type parents whereas the number of underground pods was reduced by 40%. Seed increase will be done for the selected families to study the hardseededness in comparison with their amphicarpous type-parents. **Ali M. Abd El Moneim.**

4.1.7. Mutation Induced Variation in *Lathyrus* spp.

Four accessions each of *Lathyrus sativus, Lathyrus ochrus* and *Lathyrus cicera* were treated with six different mutagenic treatments - 10, 20, 30, 40 and 50 kR of Gama rays and 0.15% of ethyl-methane-sulphate (EMS). The objective of the mutagenic treatments was to assess the possibility of producing neurotoxin-free mutants and to determine inter-and intra-specific differences in responses to mutagens. The seeds of 12 accessions treated with six mutagenic treatments and a control were grown at Tel Hadya during winter season.

The germination in EMS treatment was not effected in *Lathyrus sativus* and *Lathyrus cicera*, however, there was a little reduction in *Lathyrus ochrus* indicating that *L. ochrus* was relatively more sensitive to EMS treatment as compared to the other two species.

L. ochrus was most sensitive to Gama ray treatment. This was followed by *L. cicera* and *L. sativus*. The seed from all the plants from all the treatments were collected and shall be sown next season in M_2 for selection of desirable mutants. **R.S. Malhotra and Ali M. Abd El Moneim.**

4.2. Agronomy/Ecophysiology

4.2.1. Effect of Depth of Burial on Seed of Underground Vetch

The effect of depth of burial on hardseed breakdown for aerial and subterranean seeds was assessed for 4 lines (Selection # 2614, 2647, 2650 and 2660) of underground vetch. For each line, 50 aerial and 50 subterranean seeds were put in mesh bags. The bags were placed on the soil surface, at -5cm and at -10cm in the field at Tel Hadya. Enough bags were placed in the field to take regular monthly samples for germination testing. The experiment was replicated 3 times.

The results show that aerial hardseededness breaks down faster than that of subterranean seeds, with differences between 30 %-40% by day 400 of the trial (Figure 4.2.1).
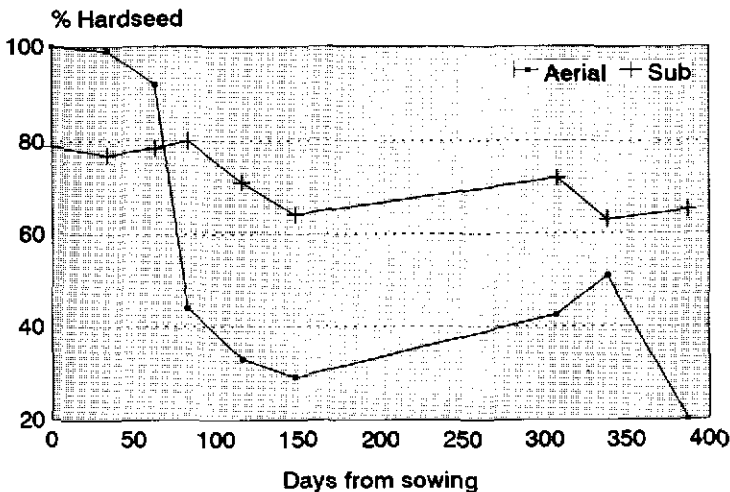
Figure 4.2.1. Comparison of the hardseed breakdown of aerial and subterranean seed buried at -5cm for underground vetch (Selection 2614).

The effect of depth of burial was not evident throughout the trial. However, burying the seed at -5cm resulted in a 5% - 10% lower hardseed level for all accessions for both seed types by day 400. Further, selections did vary in both the rate and final level of hardseed breakdown. For example Figure 4.2.2 shows the hardseed breakdown of underground seed buried at -5cm. Selection 2614 initially broke down more rapidly than the other three selections resulting in a difference of 20% in the final hardseed levels.
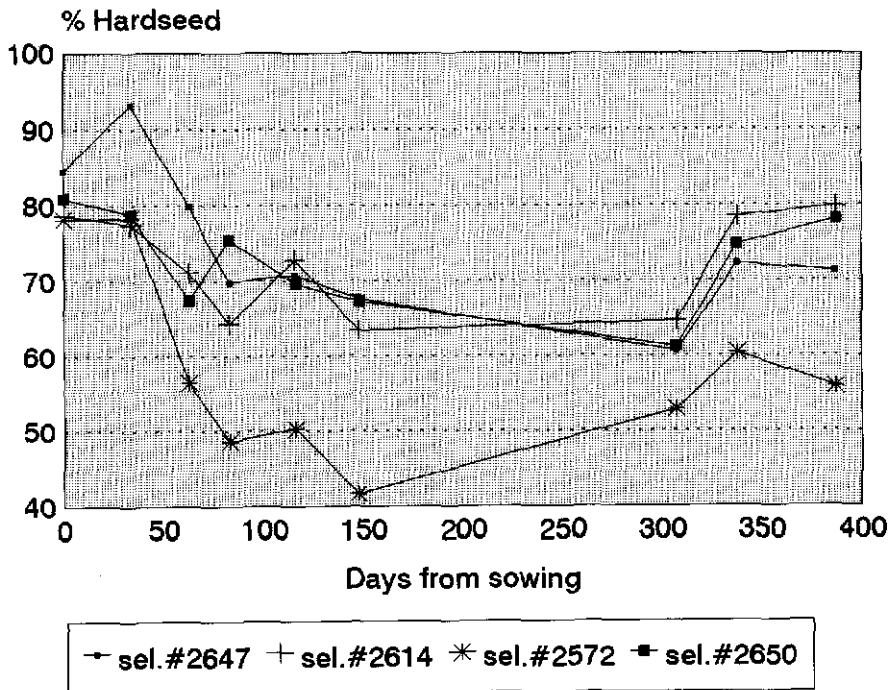
Figure 4.2.2. Hardseed breakdown for four selections of underground vetch buried at -5 cm in the field at Tel Hadya.

4.2.2. Effect of Density on Underground Vetch

Underground vetch (selection 2647) was seeded at 10 rates
(50, 120, 200, 250, 320, 400, 500, 800, 1260 & 2000 seeds
per/m^2) in 0.5m x 6m plots at Tel Hadya. A randomised
complete block design was used with 3 replications. At
maturity a quadrant was harvested and core samples were
taken to assess the above and below ground yields.
Figure 4.2.3 describes the yield response.   The
underground seed yield peaked at a seeding rate of 320
seeds/m^2 ,yielding approximately 250 g/m^2 or 5000 seeds/m^2
(100 seed wt = 5 grams). At higher densities underground
seed yield declined markedly. Likewise, total biomass
(above ground biomass + underground stem weight +
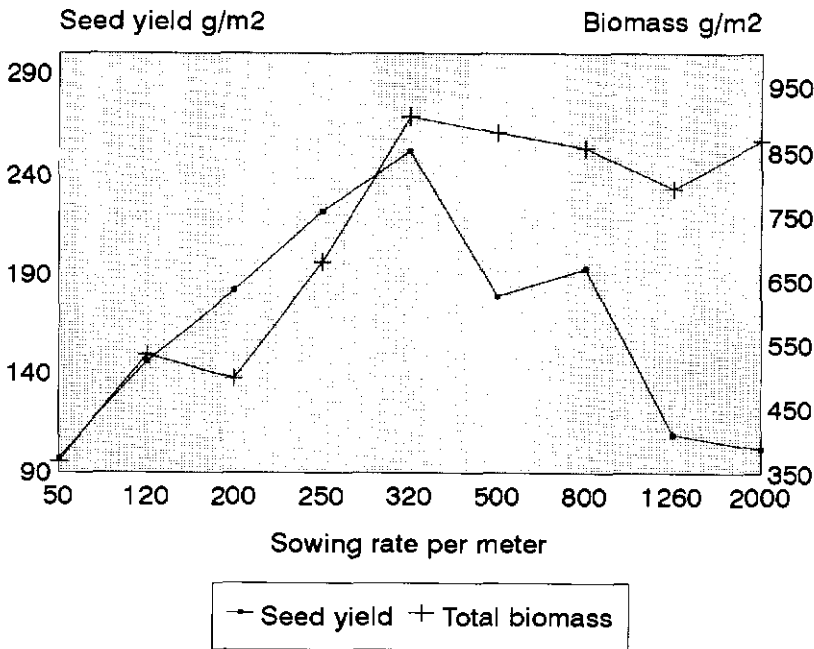
Figure 4.2.3. The effect of density on the underground
seed yield (left y-axis) and total biomass
(right y-axis) of underground vetch
(selection 2647).

underground seed weight + root weight) reached a peak of 900 g/m^2 at a seeding rate of 320 seeds/m^2 and declined to 860 g/m^2 at a seeding rate of 2000 seeds/m^2. Given that the rate of hardseed breakdown ranges from 50%-80% in one year, it is likely that underground vetch in a pure stand will overcrowd itself.

4.2.3. Evaluation of Underground Vetch Germplasm for Morpho-physiological Traits

Twenty-eight accessions of underground vetch were grown out in plots of 40 plants, replicated 3 times in a randomised complete block design. Phenological events and yield parameters were recorded to assess the level of variation within the material on hand at ICARDA.

Figure 4.2.4 shows the time to 50% above ground flowering for each accession. The latest accession flowered 27 days after the earliest. The majority of accessions flowered between 110 and 120 days from sowing.
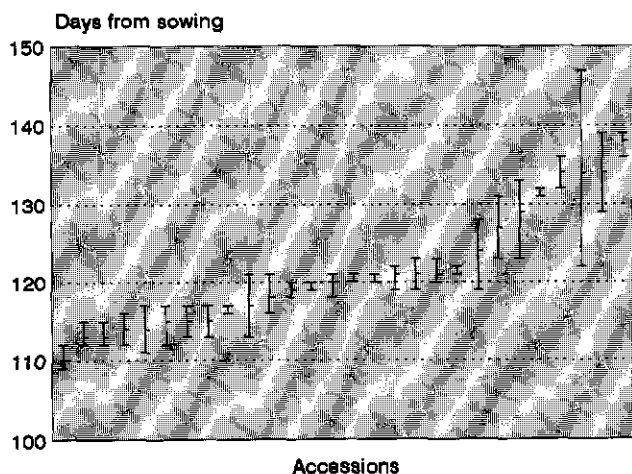
Figure 4.2.4. Time to 50% flowering for 28 accessions of underground vetch. The upper and lower limits of the bars represent within plot standard errors.

Days to 50% above ground pod maturity ranged from 142
to 163 days. In general the late flowering accessions
matured more rapidly.

Underground seed yield ranged from 5 - 35 seeds per
plant. The seed yield was not correlated to flowering or
pod maturity times. However, this could be due to the
mildness of the summer drought.

It is probable that the variation displayed by the
accessions tested could be correlated with the
environmental conditions in the place of origin. A
correlation analysis will be performed to find out whether
this is the case.

4.3. Rotation Trial on Underground Vetch

The background and treatments imposed in this trial are
described in the 1993 Legume Program annual report. The
trial was sown to Barley (var mari.aths*2\\m-ah-73-337-1)
and underground vetch was allowed to volunteer as a weed
under the barley in the 1993/94 season.

The hardseed breakdown measurements undertaken the
previous season were continued. Barley yields were
measured and the size of the underground vetch seed bank
was assessed at the end of this season.

The highest barley grain yields (2.3 ton/ha) were
realised by the grazed in February and no grazing/cut at
maturity treatments. The other subplots yielded between
1.6 and 1.9 tons/ha. By contrast, the continuous barley
control yielded only 0.7 tons/ha.

Except for the grazed in March establishment
treatment (mainplots), there was a 300-400 kg/ha

difference in the barley yields between subplots within their respective main plots. For the February and No graze main plots, yields were superior when cut at flowering. The opposite was true for the grazed in April main plot.

At the end of the 1992/93 season, the underground vetch seedbank contained about 90% hardseed. There was a gradual rate of hardseed breakdown to 70% by September, after which they declined rapidly to below 20% by the end of December.

Table 4.3.1 describes the status of the underground vetch seedbank to June 1994. Seed numbers fell to between 10%-40% of those present in June 1993. There was no significant difference between treatments for seedbank size in June 1993. In June 1994 the 1989 April-grazing and no-grazing / 1992/93 cutting-at-maturity treatments yielded significantly larger seed banks. This was probably due to a higher plant density under the barley rotation in 1993/94.

In summery, the rapid rate of hardseed breakdown displayed by the rotation trial seedbank explains the large reduction in seed numbers over the 1992/93 - 1993/94 seasons. The high rate of germination yields a plant density which is way above the optimal density (320 plants/m^2) indicated by the density trial. In addition many of the plants which did germinate under the barley canopy would have died from shading.

Table 4.3.1. Underground vetch seed yields (seeds/m^2) for the rotation trial. The June 1993 values are averages for the whole plot as there was no statistical difference for within plot treatments.

Treatments		Seeds per meter^2	
1988/89 grazing treatments (Main plots)	1992/93 cutting treatments (Sub plots)	June 1993	June 1994
February	100% flower		526
	Maturity	1429	466
March	100% flower		379
	Maturity	2402	385
April	100% flower		295
	Maturity	2502	1023*
No grazing	100% flower		443
	Maturity	2123	714*

* significantly different for figures in the same column.

At this stage, the results indicate that underground vetch may not be suitable as a self regenerating pasture plant for a ley system. However, the results from both the accession assessment and the depth of burial trial, indicate that some degree of variation exists for both seed yield and rate of hardseed breakdown. Thus it seems worthwhile to explore more fully the genetic variation within the enlarging germplasm collection of underground vetch. **Ken Street, Ali Abd El Moneim and Phil Cocks**.

4.4. Forage Legume Pathology

The program of feed legume improvement of *Vicia* spp. and chicklings (*Lathyrus* spp.) has identified promising genotypes in term of yields and adaptation. The objectives of the pathology section is to evaluate the selected genotypes for resistance to the major diseases and make available information on sources of resistance to

individual and multiple diseases. Since the feed legume genotypes are not yet widely grown in the WANA region, the relative importance of the diseases on the genotypes should be monitored annualy through disease surveys. This would identify the major diseases and their potential economic significance in different agroecological areas where the crops are grown.

4.4.1. Screening for Disease Resistance

Germplasm accessions of *Vicia* and *Lathyrus* species were evaluated for resistance to Ascochyta blight and botrytis stem blight in the field. The weather was particularly favourable for foliar diseases and *Lathyrus* species were severely damaged by ascochyta blight, while *Vicia* species were severely affected by downy mildew. Results are presented in Tables 4.4.1 and 4.4.2 and Figure 4.4.1. Five lines of *V. sativa* (2609, 2624, 2639, 2640, and 1448) were identified as sources of high level of resistance to downy mildew, but all the material in the advanced yield trials (AYT) had intermediate (I) reaction as the highest level of resistance. Six lines of *V. narbonensis* in the AYT were moderately resistant (MR) to downy mildew and *Selection 2561 was MR at two locations*, Breda and Tel Hadya (Figure 4.4.1).

Dual resistance to two diseases is available in most species, but multiple resistance to three of four diseases is not widely available (Table 4.4.2). Dual resistance to downy mildew and ascochyta blight in *L. sativus* is available at I/MR level in only five lines (Selections 553, 555, 563, 529, and 504) effective at two locations. Selection 67/2561 of *V. narbonensis* that originated from Iraq had multiple disease resistance to four foliar diseases (Ascochyta blight, botrytis blight, downy mildew and powdery mildew). Unfortunately, this selection is

highly susceptible to cyst nematode. In addition, Selection 566/2381 of *V. narbonensis* and Selections 2908/2540, 2938/2542, 2988/2543, 2908/2540, 2938/2542, and 2988/2542 of *V. hybrida* had resistance to ascochyta blight, botrytis stem blight and powdery mildew (Table 4.4.2). None of the *L. sativus* and *L. cicera* accessions showed multiple disease resistance (Table 4.4.2).

Basing on observations it is noted that resistance enhancement is required for the breeding lines that will eventually be released to the farmers. Ascochyta blight resistance in *Lathyrus sativus* and resistance to downy mildew in *Vicia sativa* and *V. narbonensis* have to receive priority. **M.T. Mubaga, A.A. El Moneim and M. Bellar.**
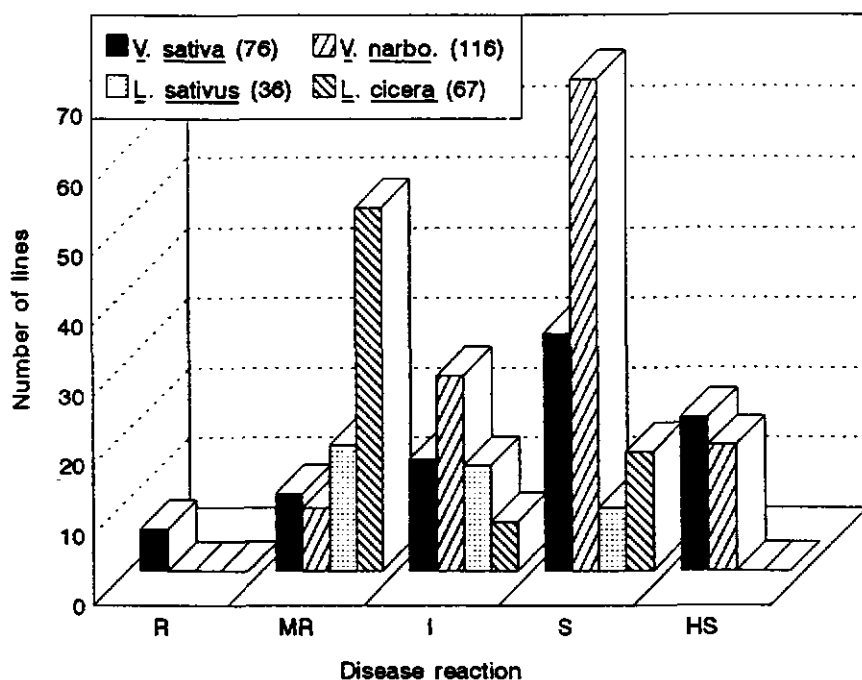
Figure 4.4.1. Disease reaction to downy mildew in selected lines of *Vicia* and *Lathyrus* species (numbers evaluated are in parenthesis).

Table 4.4.1. Disease reaction (on 1-5 scale) of promising *Vicia* spp. and *Lathyrus* spp. lines.

Legume spp. & disease	No. of lines in each disease category				
	1	2	3	4	5
Vicia sativa					
Asc. blight	0	37	50	18	1
Botrytis	2	24	26	11	0
D. mildew	6	23	39	31	18
P. mildew	3	21	38	19	3
V. narbonensis					
Asc. blight	0	7	54	13	0
Botrytis	0	23	44	8	0
D. mildew	2	9	50	76	11
P. mildew	5	46	31	1	0
V. ervilia					
Asc. blight	0	20	9	6	0
Botrytis	0	10	19	11	0
P. mildew	0	35	2	0	0
V. palaestina					
Asc. blight	0	6	14	0	0
Botrytis	0	5	9	5	0
P. mildew	0	0	0	9	7
V. hybrida					
Asc. blight	0	16	0	0	0
Botrytis	0	8	8	0	0
P. mildew	0	7	6	3	0
V. villosa dasycarpa					
Asc. blight	0	36	4	0	0
Botrytis	0	0	30	1	0
D. mildew	0	31	9	0	0
P. mildew	0	3	11	6	0
L. sativa					
Asc. blight	0	3	13	3	1
Botrytis	0	0	4	9	3
D. mildew	5	15	32	1	0
P. mildew	0	2	8	19	20
L. ochrus					
Asc. blight	6	5	12	0	0
Botrytis	6	6	8	0	0
D. mildew	0	18	9	0	0
L. cicera					
Asc. blight	0	10	16	5	0
Botrytis	0	1	12	6	0
D. mildew	1	54	17	9	1
P. mildew	0	3	10	6	0

Table 4.4.2. The availability of sources of multiple disease resistance (rating 1 or 2 on 1-5 scale) in the promising genotypes of *Vicia* and *Lathyrus* species from the disease screening at Tel Hadya, 1993/94.

Legume species	No of lines/selections with resistant to moderately resistant reaction						
	Asc. & Botrytis	Asc. & P.mildew	Asc. & D.mildew	Bot & P. mildew	Asc., Bot & D.mildew	Asc., Bot & P.mildew	Asc., Bot, D.&P.mildew
V. sativa	4	9	13	3	none	none	none
V. narbonensis	3	6	2	13	1	2	1
V. ervilia	8	20	none	9	none	8	none
V. palaestina	none	none	none	none	none	none	none
V. hybrida	16	14	none	6	none	6	none
V. villosa	none	3	31	none	none	none	none
L. sativa	none	none	4	none	none	none	none
L. ochrus	2	none	·4	none	2	none	none
L. cicera	none	none	8	2	none	none	none

4.4.2. Disease Survey for Forage Legumes

Disease incidence and severity in *Vicia* and *Lathyrus* species were evaluated in farmers' fields and on-farm trials in eight locations covering south, central and northern Syria. In addition, farmers fields enroute and around these locations were also included in the survey. The objective was to assess the disease situation in vetches and chicklings and evaluate promising lines for disease reaction.

Table 4.4.3. Disease incidence (on 1-5 scale) in *Vicia* and *Lathyrus* species grown in different locations in Syria, 1993/94 season.

Location	Disease	Disease severity	Pathogen isolated
Himo	Ascochyta blight	2	*Ascochyta* sp.
	Downy mildew	2	NA
	Sclerotinia blight	2	*S. sclerotiorum*
Kamishly	Botrytis blight	3	*Botrytis cinerea*
Tel Hasoud	Sclerotinia blight	4	*S. sclerotiorum*
Hama	Ascochyta blight	2.5	*Ascochyta* sp.
	Downy mildew	3	NA
	Botrytis blight	3	*B. cinerea*
Aphes	Ascochyta blight	3	*Ascochyta* sp.
	Downy mildew	2	NA
	Botrytis blight	2	*B. cinerea*
Soran	Ascochyta blight	2.5	*Ascochyta* sp.
	Downy mildew	2.5	NA
	Botrytis blight	3	*B. cinerea*

Disease incidence and severity was recorded and samples were collected for confirmation of identification. Results are presented in Table 4.4.3. Foliar disease were prevalent in all locations and

wilt/root rot that prevailed during the last season was not a problem. Ascochyta blight, downy mildew, botrytis blight and sclerotinia blight were the major diseases at all locations. **M.T. Mubaga, M. Bellar and Syrian NAR scientists.**

4.5. Forage Entomology

4.5.1. Effect of *Sitona crinitus* on *Vicia* Yield

The experiment on <u>Sitona</u> control in *Vicia villosa* ssp. *dasycarpa* was conducted at an on-farm location in Alkamiye using the same Promet treatment as in lentil (Section 3.4.1). As in previous years, nodule damage started late at Alkamiye but reached 62% and 83% on 11 April and 10 May, respectively. Promet treatment significantly reduced nodule damage and increased seed and straw yield significantly (Figure 4.5.1).
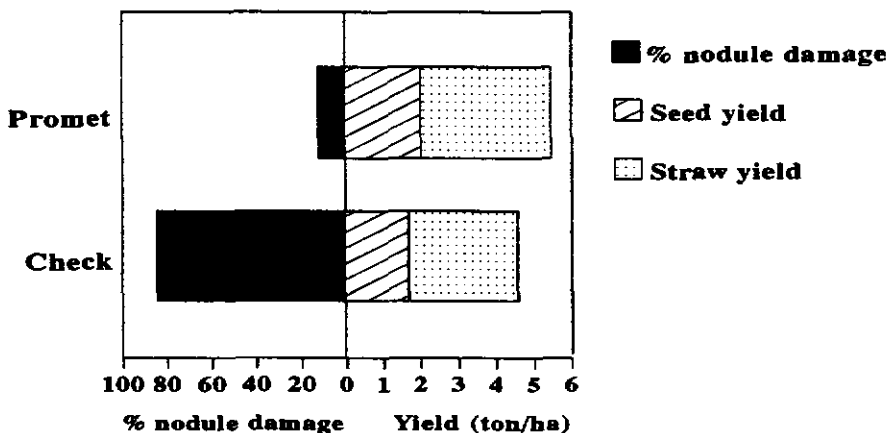
Figure 4.5.1. Effect of Promet seed treatment (400 CS, 10 ml/kg seed) on *Vicia villosa* ssp. *dasycarpa* seed and straw yield and nodule damage by *Sitona crinitus* in northern Syria, 1993/94.

This was the 3rd and final year of the *Sitona* control experiment. It confirmed that *Sitona* does infest this *Vicia* species and control results in significant yield increases. However, Promet treatment may not be economical in subsistence farming, but could be used in commercial seed production fields. **S. Weigand and A. Joubi.**

4.5.2. *Sitona* Damage in other Forage Legumes

The occurrence and importance of *Sitona* in different forage legumes was assessed. Samples were taken from seed increase fields of local varieties of several *Lathyrus* and *Vicia* species and the nodule damage recorded.

The three *Lathyrus* species, *L. ochrus*, *L. sativus*, *L. cicera* had very low nodule damage of 3 to 4% (Table 4.5.1) and no feeding damage. If the adult

Table 4.5.1. *Sitona* nodule damage in local varieties of forage legumes, Tel Hadya, 1993/94.

Forage legume species	% nodule damage
Lathyrus ochrus	4.3
Lathyrus sativus	2.7
Lathyrus cicera	2.8
Vicia pannonica	7.0
Vicia narbonensis	39.0
Vicia sativa	92.8
Vicia ervilia	90.6
Vicia villosa ssp. *dasycarpa*	69.0

Sitona do not feed on the leaves, they do not lay the eggs near these plants and there would be no larvae infesting the nodules. Apparently *Lathyrus* spp. is not a preferred host for *Sitona*.

Differences in *Sitona* damage were also found between the 5 *Vicia* species, *V. pannonica*, *V. narbonensis*, *V. sativa*, *V. ervilia*, and *V. villosa* ssp. *dasycarpa*. In *V. pannonica* the nodule damage was very low (7%), in *V. narbonensis* nodule damage was medium (39%), whereas in *V. sativa*, *V. ervilia* and *V. villosa* ssp. *dasycarpa* high nodule damage of 93%, 91% and 69% was recorded, respectively (Table 4.5.1). These results show that not all forage legume species are attacked to the same extent by *Sitona*. In *Lathyrus* spp., *V. pannonica* and *V. narbonensis* *Sitona* is not of importance. It would be interesting to study the reasons for the preference/non-preference for the different forage species shown by the *Sitona* weevils. **S. Weigand, A. Joubi and A.A. El. Moneim.**

5. DRY PEA IMPROVEMENT

The dry pea research at ICARDA was initiated in 1986/87. As extensive varietal improvement work is being done on dry pea at a number of institutions in the developing and developed countries we capitalise on this research, instead of running our own breeding program, to identify dry pea cultivars adapted to the farming systems of WANA. Our work is concentrated in the following areas:

I. Collecting enhanced germplasm/cultivars from the institutes working on dry pea and testing them at ICARDA sites to identify superior lines for evaluation by the national programs in WANA.

II. Developing suitable production technology and its transfer to the national programs for testing and adaptation.

5.1. Germplasm Collection and Evaluation

5.1.1. Pea Genetic Evaluation Trial

The new accessions obtained from various institutions were evaluated at Tel Hadya in Pea Genetic Evaluation Trial with 49 entries in a 7x7 simple lattice design. The data were analysed for various phenological and morphological characters (Table 5.1.1).

Time taken to flower ranged from 91 days (for Acc No. 572) to 109 days (for Acc No. 225); time taken to mature ranged from 136 days (for Acc Nos. -558, -586, -223, -572, -573, -580, -576, -589, -593, -578) to 147 days (for Acc No. 570); biological yield ranged from 815 kg/ha (for Acc No. 581) to 6661 kg/ha (for Acc No. 225); and the harvest index ranged from 32% (for Acc No. 553) to 58% (for Acc No. 557) (Table 5.1.1). Seed yield varied from 390 to

3044 kg/ha and the five highest seed yielding entries included Acc Nos. 225, 568, 569, 555 and 557 with seed yields of 3044, 2452, 2423, 2227 and 2036 kg/ha, respectively.

5.1.2. Evaluation for Cold Tolerance

Thirty eight accessions of pea screened earlier and 62 new accessions were sown for evaluation for cold tolerance on 6 Oct 1993 with a post-sowing irrigation to ensure adequate moisture supply for germination. During the growing season, below zero temperatures were observed for 20 days and minimum temperature during the season was -4.6^{O}C on 14 Nov 1993. During the season the susceptible check did not get a rating of 9 on a 1-9 scale (where 1 = free from damage, 9 = killed). Thus screening for cold tolerance was not possible. These materials, along with new entries, will be grown next season for their evaluation.

5.2. Yield Trials

5.2.1. Preliminary Yield Trial

Sixty four entries selected from the genetic evaluation trial and preliminary yield trial (PYT) of 1992/93 were evaluated for their performance during the 1993/94 season at six diverse environments. These environments included four locations, Tel Hadya, Jindiress, Kfardan and Terbol; and at Kfardan and Terbol, the trial was sown at two different dates. The stability analysis using Eberhart and Russell (1966) model was done on seed yield. The analysis revealed that only non-linear component of variance was important. Thirty eight entries yielded more than the overall mean across all the environments, and

seven among these exhibited significant deviations and were unstable (Table 5.2.1). Of the 31 entries with above average seed yield and non-significant deviations from regression, 27 entries had regression coefficient equal to one and exhibited general adaptation across environments, and four entries with above average seed yield, regression coefficient greater or less than unity were specifically adaptable to favorable or unfavorable environments.

Seed yield for the entries varied from 1486 to 3420 kg/ha at Tel Hadya; 889 to 2677 kg/ha at Jindiress; and 743 to 4090 kg/ha at Terbol in date 1 (TerD1), and 243 to 3424 kg/ha at Terbol in date 2 (TerD2); 597 kg/ha to 4535 kg/ha at Kfardan in date 1 (KfrD1), and 632 kg/ha to 3132 kg/ha at Kfardan in date 2 (KfrD2). Location means at Tel Hadya, Jindiress, TerD1, TerD2, KfrD1, and KfrD2 were 2589, 1709, 2640, 2374, 2760, and 2049 kg/ha respectively. Based on the mean yield over 6 environments the five best lines included Acc Nos. 225, 403, 21, 526, and 321, which gave seed yields of 3005, 2910, 2866, 2804 and 2772 kg/ha respectively (Table 5.1.2). Among these top yielding accessions, Acc. No. 403 is semi-leafless.

5.2.2. Pea International Adaptation Trial (PIAT)

Twenty three entries selected from Preliminary Yield Trial (PYT) and PIAT conducted during the previous season were tested in this trial at Tel Hadya and Terbol during 1993/94. Six test entries namely PS 210713, Le 23, 305 PS 210572, MG 100726, Umatilla and Syrian Local Aleppo at Tel Hadya, and six entries namely MG 102703, K-129, G 22763-2C, Syrian Local Aleppo, Local Selection 1690, and MG 104325 at Terbol yielded significantly higher than the local check. The location mean yields at Tel Hadya and Terbol were, 1782 and 2667 kg/ha, respectively. **Drs. R.S. Malhotra and M.C. Saxena**

Table 5.1.1. Adjusted seed yield (Yield=kg/ha) and rank (R), biological yield (BYLD=kg/ha), time to flower (DFLR), time to mature (DMAT), and harvest index (HI) of some of the high yielding entries in Pea Genetic Evaluation Trial at Tel Hadya during 1993/94.

Acc No.	Origin	Yield	R	BYLD	DFLR	DMAT	HI
552	AUSTRALIA	1936	6	4090	104	146	48
553	AUSTRALIA	1712	13	3715	102	145	32
554	AUSTRALIA	1677	15	3336	101	146	50
555	AUSTRALIA	2227	4	4085	101	146	54
556	AUSTRALIA	1868	8	3795	103	143	50
557	AUSTRALIA	2036	5	3723	104	146	57
558	AUSTRALIA	1227	33	2400	102	136	50
559	AUSTRALIA	1369	28	2763	102	145	50
560	AUSTRALIA	1700	14	3588	104	144	47
561	AUSTRALIA	1433	24	3336	103	146	42
562	AUSTRALIA	1515	22	3064	97	139	50
563	AUSTRALIA	1586	17	3558	101	146	45
564	AUSTRALIA	1376	26	2670	100	140	52
565	AUSTRALIA	1921	7	3703	104	139	53
566	AUSTRALIA	1853	9	3969	103	143	47
567	AUSTRALIA	1719	12	3404	99	140	50
568	AUSTRALIA	2452	2	4983	105	146	49
569	AUSTRALIA	2423	3	4762	106	146	51
570	AUSTRALIA	1512	23	3263	105	147	46
571	AUSTRALIA	1595	16	3275	100	146	48
572	USA	593	48	1137	91	136	51
573	USA	1373	27	2610	102	136	53
574	USA	881	44	1876	103	139	47
575	USA	1249	32	2450	103	139	51
576	USA	1522	21	2825	104	136	53
577	USA	913	43	1976	104	137	47
578	USA	722	47	1571	102	136	45
579	USA	963	42	1939	96	139	48
580	USA	1054	40	2132	94	136	50
581	USA	390	49	815	104	137	49
582	USA	1387	25	2875	98	139	48
583	USA	1530	19	2951	100	139	52
584	USA	790	46	1762	105	137	44
586	USA	1771	11	3389	94	136	51
587	USA	1101	37	2158	94	139	50
588	USA	1060	39	2164	102	139	48
589	USA	802	45	1657	94	136	48
590	USA	976	41	2132	96	139	46
591	USA	1567	18	3255	108	139	47
592	USA	1260	31	2570	102	139	49
593	USA	1804	10	3308	101	136	55
594	USA	1211	34	2385	102	139	50
596[a]	USA	1064	38	2038	94	139	51
598	USA	1186	35	2438	93	137	50
599	USA	1360	29	2603	95	138	52
600	USA	1165	36	2352	92	137	49
223	SYRIA	1529	20	3577	102	136	42
224	U.K.	1296	30	2428	104	143	55
225	SYRIA	3044	1	6661	109	146	45
Location mean		1443		2929	101	140	49
S.E. of mean		157.1		304.0	0.5	0.9	7.9
L.S.D. (P=0.05)		442.2		855.5	1.5	1.9	8.2
C.V. %		18.9		18.0	0.9	0.8	10.3

a= Semi leafless

Table 5.2.1. Mean seed yield of the entries in Pea Preliminary Yield Trial conducted at Tel Hadya (TH), Jindiress (JIN), Terbol Date 1 (TerD1), Kfardan Date 1 (KfrD1), Terbol Date 2 (TerD2) and Kfardan Date 2 (KfrD2) during 1993/94.

Acc. No.	Origin	Seed yield (kg/ha)						
		TH	JIN	TerD1	KfrD1	TerD2	KfrD2	Mean
8	SYRIA	3019	6771	3243	2931	2431	1986	2552
21	SYRIA	3224	7440	4090	2701	2910	2146	2866
101	ETHIOPIA	2779	6079	3382	2729	2382	2236	2589
172	INDIA	2706	5669	2965	3056	2153	1813	2463
216	AUSTRALIA	2691	5897	3278	2611	2688	1979	2485
267	USA	2574	4547	1861	3389	2507	2826	2511
321	FRANCE	2524	4906	3174	3972	3188	2382	2772
380	INDIA	3420	6589	2285	3292	3069	2236	2743
385	NEW ZEALAND	2816	5149	2972	3132	2806	2181	2586
387	NEW ZEALAND	2805	5372	2729	2813	2611	2181	2419
399	NEW ZEALAND	2502	5104	2653	3500	2701	2014	2541
403	NEW ZEALAND	3225	6022	3514	2722	3125	2639	2910
445	ETHIOPIA	2639	5583	1938	3000	2632	2264	2389
447	ETHIOPIA	3149	6024	2715	2549	2743	2403	2564
448	ETHIOPIA	2515	6142	3410	2528	2840	2146	2658
494	AUSTRALIA	2538	4995	3215	2990	2333	1965	2417
496	AUSTRALIA	2690	6085	3097	2924	2347	1931	2481
497	AUSTRALIA	2556	5475	3785	2806	2201	2090	2486
498	AUSTRALIA	2698	11995	3813	2875	2361	1917	2516
499	AUSTRALIA	2634	5813	3583	3097	2674	2569	2656
501	AUSTRALIA	2644	5509	2972	2833	2854	1868	2472
502	AUSTRALIA	2764	6022	2660	2910	2792	2333	2560
504	ETHIOPIA	2608	5781	3403	3556	1861	2493	2644
517	AUSTRALIA	2757	5701	2736	3549	2667	3132	2721
518	AUSTRALIA	2899	5979	2292	3500	2750	2493	2638
519	AUSTRALIA	2576	5158	2611	2917	2417	2458	2543
520	AUSTRALIA	2340	4443	3125	3417	2424	2368	2532
522	AUSTRALIA	2490	5226	2486	3160	2194	2389	2481
526	AUSTRALIA	2875	5854	2951	3222	2667	3007	2804
533	AUSTRALIA	2453	4833	2694	3347	2403	2201	2364
534	AUSTRALIA	2694	5035	3354	3028	2493	1757	2582
539	AUSTRALIA	2778	6063	2764	3153	2590	2465	2534
542	AUSTRALIA	2788	5678	3111	2806	2688	1778	2502
545	AUSTRALIA	2572	5292	3444	3035	2646	2340	2735
546	AUSTRALIA	2988	6471	1931	3194	2347	2660	2561
548	AUSTRALIA	2810	5940	2438	2521	2931	2882	2463
551	CROATIA	2679	5844	3243	3118	2875	2563	2652
225	SYRIA	2828	6897	3688	3333	3424	2556	3005

Location mean		2589	1709	2640	2760	2385	2050	
S.E. of mean		226.5	256.5	362.8	487.4	211.7	276.1	
L.S.D. (P=0.05)		627.7	710.8	1005.4	1350.9	586.9	765.3	
C.V. %		15.1	26.0	23.8	30.6	15.4	23.3	

6.   OROBANCHE STUDIES

6.1.   Greenhouse Experiment

The purpose of this study on host plant-parasite interaction was to quantify dry matter production and partitioning into different faba bean organs and *Orobanche crenata*, with optimum growing conditions for the host with respect to light, temperature, water, and the supply of mineral nutrients.

The experiment was conducted in the greenhouse with 24°C (day) / 16°C (night) temperatures, and supplemental light for 10 h (high-pressure sodium lamps, 250 W). The soil temperature was measured continuously by using a thermograph (Figure 6.1.1). The characteristics of the soil used for the experiment are described in Table 6.1.1.
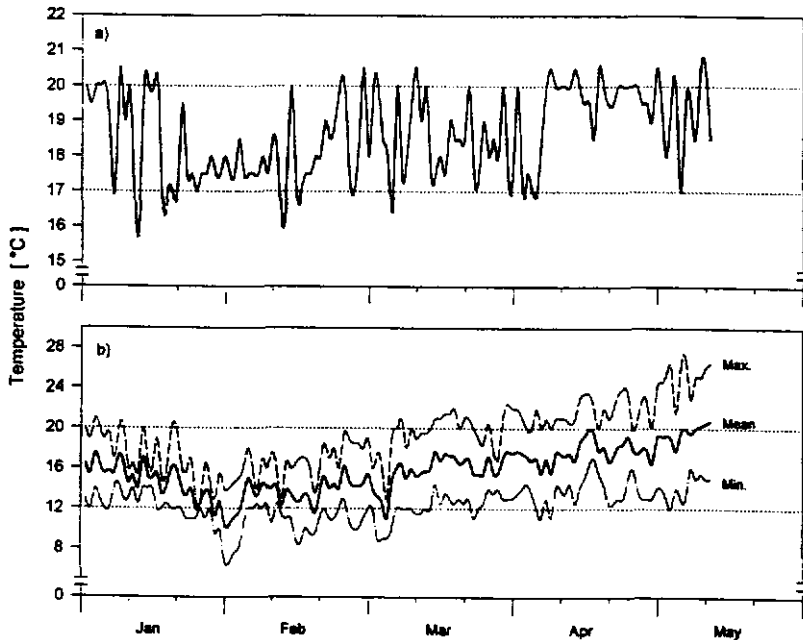
Figure 6.1.1. Mean daily air temperature (a) and daily soil temperature (b) in the greenhouse during the growing period.

Plastic pots (10 l vol.) were filled with 8.5 kg of a 1:1 mixture of soil and sand, which had been steam sterilized to avoid the presence of viable Orobanche seeds. Thereafter, the soil from top 15 cm of each pot was removed and mixed with the required amount of Orobanche seeds to create the densities of $0(O_1)$, $50(O_2)$, $200(O_3)$ and $600(O_4)$ seeds of Orobanche per kg soil.

The number of seeds was calculated from the 1000-seed weight (4 mg) of Orobanche. In addition, nitrogen and phosphorus fertilizer (ammonium sulfate and triplesuperphosphate) were mixed with this soil at the rate of 30 kg N and 100 kg P_2O_5 per ha. The pots were refilled and arranged in a randomized block design with 33 replications. One faba bean seed of the genotype ILB 1814 (Orobanche susceptible) was planted in each pot. Prior to planting the seeds were inoculated with Rhizobium leguminosarum (strain FB: 481). Where the seeds failed to germinate, those pots were transplanted with seedlings grown at the same time in sand in the greenhouse.

During the growing period the water loss through evapotranspiration was estimated every 2-3 days by weighing 10 randomly chosen pots. Whenever the water content of the soil had reached 75% of the field capacity, the pots were irrigated. So, the soil moisture was kept between 75-100% of the field capacity. Every 3 weeks, beginning 4 weeks after emergence, a fluid fertilizer (Hoechst-Complesal, containing 8% N, 8% P_2O_5, 6% K_2O, and trace nutrients) was applied as foliar fertilizer (3 ml/l water).

Table 6.1.1. Characteristics of the soil used for the greenhouse experiment (data from ICARDA's soil laboratory).

NaHCO$_3$-extractable P (Olsen P (Olsen P)	6.7 mg/kg
KCl-extractable NH$_4$-N	5.6 mg/kg
NO$_3$-N (H$_2$O)	20.7 mg/kg
Exchangeable K	7.6 mg/kg
Total CaCO$_3$	18.3 %
DTPA-TEA-extractable Cu	1.4 g
DTPA-TEA-extractable Zn	0.7 mg/kg
DTPA-TEA-extractable Mn	30.4 mg/kg
DTPA-TEA-extractable Fe	7.7 mg/kg
pH (H$_2$O)	8.3
Clay	36%
Silt	17%
Sand	47%
Field capacity (soil water content at a suction of -0.33 bars)	24% by weight
Wilting point (soil water content at a suction of -15 bars)	13% by weight
Available water	11% by weight

At 10-15 day intervals, beginning 3 weeks after faba bean emergence, 12 pots were chosen randomly (3 pots from each *Orobanche* seed density treatment) and the dry weight (after drying at 70°C for 48 h) of different faba bean organs, depending on the stage of growth (roots, leaves, stems, inflorescenses, pod husks, and seeds), and number and dry weight of underground *Orobanche* attachments and emerged *Orobanche* shoots were recorded. Temperature sums were calculated as where T_i is the mean temperature for

$$T_s = \sum_{i=1}^{j} (T_i - T_c)$$

each day i, and T_c is the base temperature, below which the developmental rate equals zero. In this study, the base temperature used was 4°C for air temperature sum and 4°C for soil temperature sum. The total gross dry weight

was calculated as the sum of all host plant parts, including dropped leaves, and *Orobanche* obtained per pot.

6.1.1. Dry Matter Production and Partitioning

Different *Orobanche* infestation levels did not show any significant effect on the total gross dry matter produced per pot until 101 days after emergence (Figure 6.1.2). At this stage the host plants with 200 and 600 *Orobanche* seeds/kg soil (O3 and O4) were almost dead, whereas the ones without parasite seeds (O1) and with 50 seeds/kg soil (O2) were still growing. They reached the physiological maturity at 115 days after emergence. At this time the total dry weight in O1 was significantly higher than in O3 and O4. The total dry weight in the treatment with low *Orobanche* seed density (O2) was lower than control and higher than O3 and O4. These differences, however, were not significant (TUKEY test at $\alpha=0.05$).

In O3 and O4 the first visible *Orobanche* attachments (tubercles >1 mm) on faba bean roots were observed 55 days after emergence of the host plant, and in O2 at the commencement of flowering (69 days after emergence). Figure 6.1.3 shows the relative proportions of total weights of faba bean organs and *Orobanche* after the occurrence of visible *Orobanche* attachments. In O4 the relative proportion of *Orobanche* increased rapidly and reached its maximum of 66.5% about 92 days after emergence, and there was no increase in total dry matter thereafter. The period of flowering was very short and the host plants did not set any pods. In O3 only few pods were observed, which became brownish-black with the time. No seeds could be harvested in this treatment. The maximum value for the relative proportion of *Orobanche* was obtained 101 days after emergence (52%).
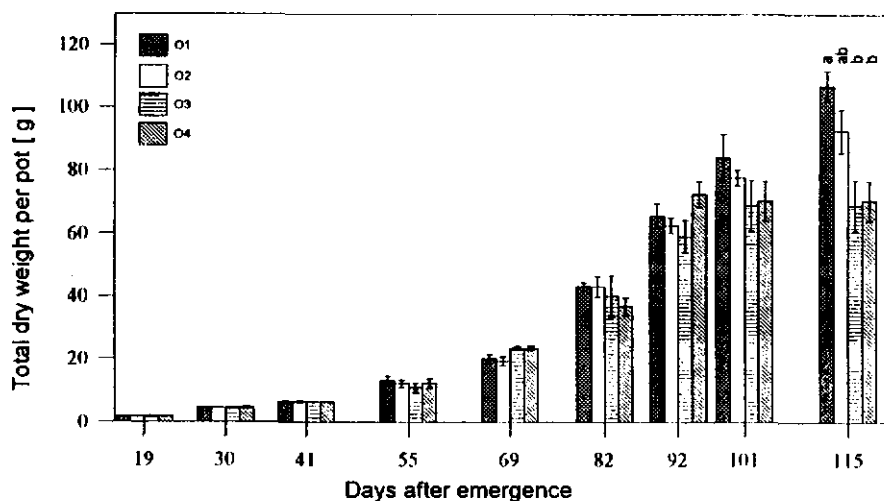
Figure 6.1.2. Total gross dry weights per plot (faba bean organs + *Orobanche crenata*) during the faba bean development. Means with the same letter are not significantly different (α=0.05). Vertical bars: SE.
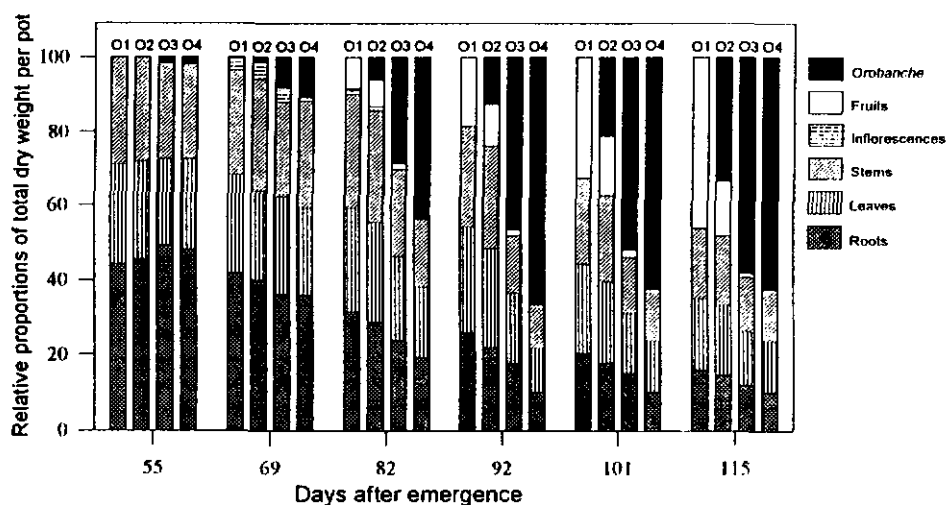
Figure 6.1.3. Relative proportions of dry weight of faba bean roots, leaves, stems, inflorescences, fruits (pods+seeds) and *Orobanche crenata* during various stages of crop development.

As for the treatment with low *Orobanche* seed density (O2), the amount of the dry matter partitioned into parasites and faba bean fruits was almost the same as the one partitioned only into fruits in the control pots (O1). Concerning the pattern of dry matter partitioning into *Orobanche* and fruits, two periods could be distinguished. During the first period, from faba bean flowering to the beginning of rapid *Orobanche* growth (69-92 days after emergence), the sink strength of both organs seems to have been equal. Therefore, the faba bean pods and the parasites had almost the same growth rate. In the second period, from 92 days after emergence up to physiological maturity of the host, *Orobanche* should have had a higher sink capacity, as there was a rapid increase of the parasites' dry weight. The pods that did not contain seeds at the end of the first period, became brownish-black and died without setting any seeds.

6.1.2. Dry Matter Partitioning into *Orobanche crenata*

To quantify as to on the expense of which of the host-plant organs did *Orobanche* grow, the total dry matter produced between two successive sampling dates, through the growing period, were calculated. The relative portions of these that were partitioned into the faba bean organs and the parasites are presented in Figure 6.1.4.

In the treatment with low *Orobanche* seed density (O2), the pattern of dry matter partitioning into roots and leaves+stems was similar to the control plants (Figure 6.1.4a and 6.1.4b). The *Orobanche* infestation thus did not affect the vegetative growth of host plants. In the reproductive stage of faba bean, there was a competition between parasites and faba bean fruits for the photosynthates being produced. The relationship between the relative portions of the total dry matter produced

between two successive sampling dates and partitioned into
Orobanche, and the accumulated daily mean temperature was
quadratic (Figure 6.1.4b).

With moderate and high *Orobanche* seed densities (O3
and O4), where the first visible *Orobanche* attachments
were already observed before commencement of flowering,
the growth of both vegetative and generative organs of
faba bean was affected by *Orobanche crenata* (Figure 6.1.4c
and 6.1.4d). The portions of the total newly produced dry
matter partitioned into leaves+stems, up to 1000°C d after
emergence, were almost equal to the control plants.
Thereafter, however, the vegetative shoot growth rate was
strongly reduced in O3 and O4, whereas in O1 the
partitioning rates were almost constant (50-60%) up to the
beginning of pod filling (1300 °C d) and decreased with
the increasing of the fruit growth rate. Furthermore, the
sink strength of the parasites was so high, that no faba
bean seeds were produced in both above mentioned *Orobanche*
treatments. Concerning the dry matter partitioning into
Orobanche as shown in Figure 4c and 4d (O3 and O4) and
fruits as shown in Figure 6.1.4a (O1) a logistic equation
of the form provided good fit to the data, where PDM is

$$PDM = \frac{PDM_{max}}{1 + \exp^{(a_1 + a_2 T_s)}}$$

the portion of the total dry matter produced between two
successive sampling dates per pot and partitioned into
Orobanche or faba bean fruits, and T_s is the accumulated
mean daily air temperature (°C d). The coefficients a_1
and a_2 of these equations are given in Table 6.1.2.
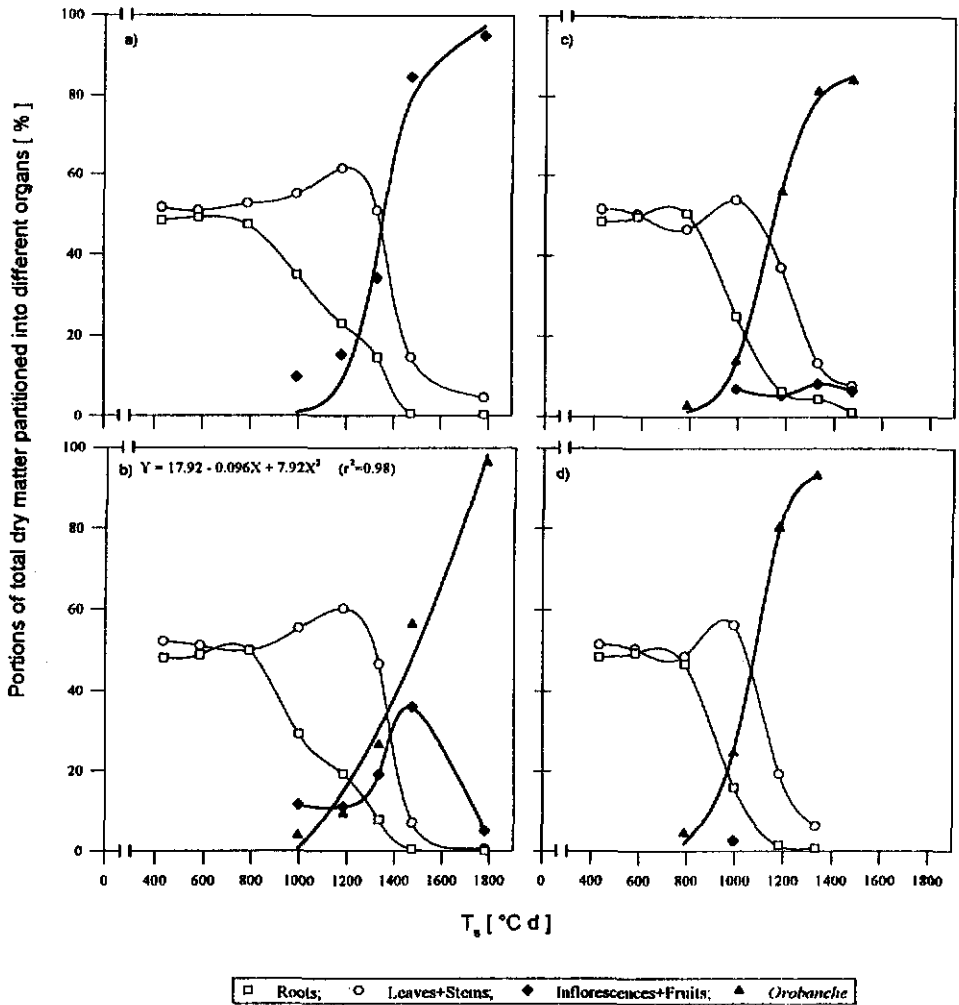
Figure 6.1.4. Partitioning of the total dry matter produced between 2 successive sampling dates into faba bean organs and *Orobanche crenata* during various stages of crop development (T_s; air temperature sum °C d). (a) Control without *Orobanche* seeds; (b) 50 *Orobanche* seeds/kg soil; (c) 200 *Orobanche* seeds/kg soil; 9d) 500 *Orobanche* seeds/kg soil.

Table 6.1.2. Coefficients a_1 and a_2 (SE in parentheses) of the logistic equation $PDM=PDM_{max}/(1+EXP(a_1+a_2T_s))$*

Orobanche treatment	PDM_{max}	a_1	a_2
O1 (control)	Fruits:97.85 (10.07)	17.55(6.23)	-0.013 (0.005)
O3 (200 Orobanche seeds/kg soil)	Orob.:86.59 (1.85)	13.88(1.08)	-0.012 (0.001)
O4 (600 Orobanche seeds/kg soil)	Orob.:95.54 (3.77)	15.05(1.85)	-0.014 (0.002)

* PDM: portion of the total produced dry matter between two successive sampling dates partitioned into Orobanche.
T_s: accumulated mean daily air temperature (°C d).

In addition, close linear relationships were found between *Orobanche* dry weight and total dry weight per pot in O3 and O4 (Figure 6.1.5, b and c). With low *Orobanche* seed density (Figure 6.1.5a), this relationship was exponential.
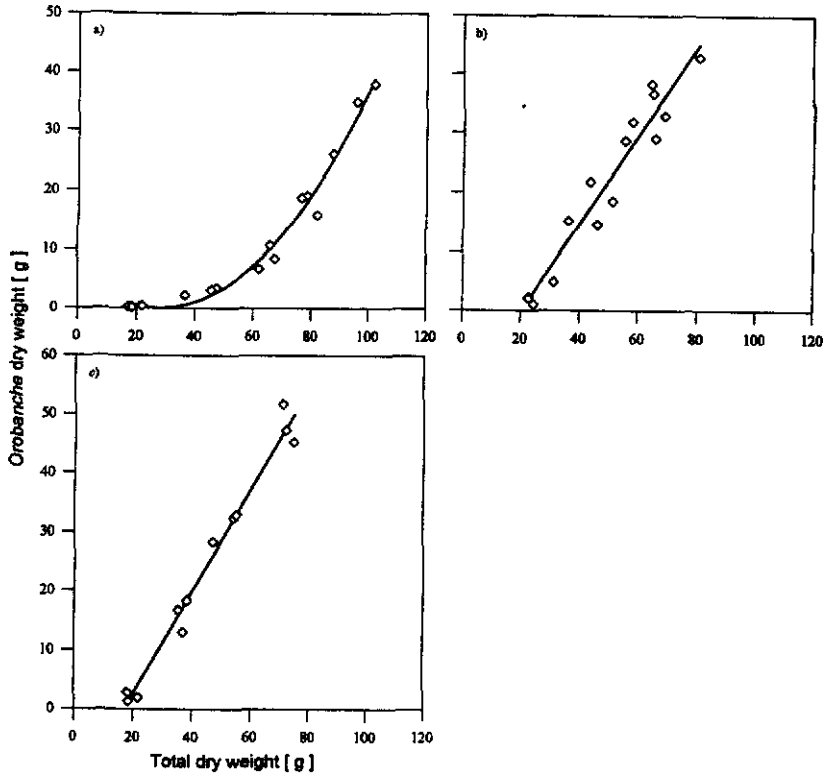
Figure 6.1.5. Relationship between dry weight of *Orobanche crenata)* (ODW) and total dry weight produced per pot (TDM) with 50 orobanche seeds/kg soil (a); 200 *Orobanche* seeds/kg soil (b); and 600 *Orobanche* seeds/kg soil (c). Regression equations are:

(a) ODW = 5.76 - 0.40 TDM + 7.13 TDM^2 r^2=0.98

(b) ODW = 0.75 TDM - 15.19 r^2=0.93

(c) ODW = 0.87 TDM - 14.89 r^2=0.98

* ODW: Orobanche dry weight per pot; TDM: total dry weight per pot

6.1.3. Development of *Orobanche crenata* on Faba bean during the Growing Period

With moderate and high *Orobanche* seed densities (O3 and O4), the durations of the development of both host plant and *Orobanche* were shorter than with low *Orobanche* seed density (O2). Different *Orobanche* seed density treatments led to a significant difference in numbers of parasite attachments observed on faba bean roots at the end of the growing period (Figure 6.1.6). The development of parasite in pots with high *Orobanche* seed densities (O4) was very fast. Maximum of both the number (80) and the dry weight (48.1 g) of *Orobanche* were reached around 900°C d after emergence of faba bean. In O3, the maximum number of *Orobanche* (44) was observed at the same time as O4, but the dry matter peak was reached at different times, around 1040°C in O_3 as against around 950°C in O_4. The value in O_3 was lower than in O_4. With low *Orobanche* seeds in the soil (O2), the maximum number of *Orobanche* (15.7) was significantly lower than the one in O3, but there was no significant difference in parasite dry weight between the two treatments.

The relationship between the *Orobanche* number (ORO_N) and accumulated soil temperature (T_S) could be described by following quadratic equations:

For O_2: $ORO_N = -28.84 + 0.07T_S + 0.97\ T_S^2$ ($r^2 = 0.97$)
O_3: $ORO_N = -118.37 + 0.33T_S - 1.68\ T_S^2$ ($r^2 = 0.98$)
O_4: $ORO_N = -119.30 + 0.53T_S - 2.48\ T_S^2$ ($r^2 = 0.98$)

Similarly, the relationship between the *Orobanche* dry weight (g/pot) (ORO_w) with accumulated soil temperature (T_S) could be described by the following functions:

For O_2: $ORO_W = -0.43 - 0.024T_s + 3.7\ T^2_s$ ($\sqrt{}^2 = 0.98$)

O_3: $ORO_W = 35.80/(1+e^{(13.75-0.016T_s)})$

O_4: $ORO_W = 49.60/(1+e^{(28.28-0.034T_s)})$

Ahmed M.-Manschadi, M.C. Saxena (ICARDA), Jürgen Kroschel and Joachim Sauerborn (University of Hohenheim).
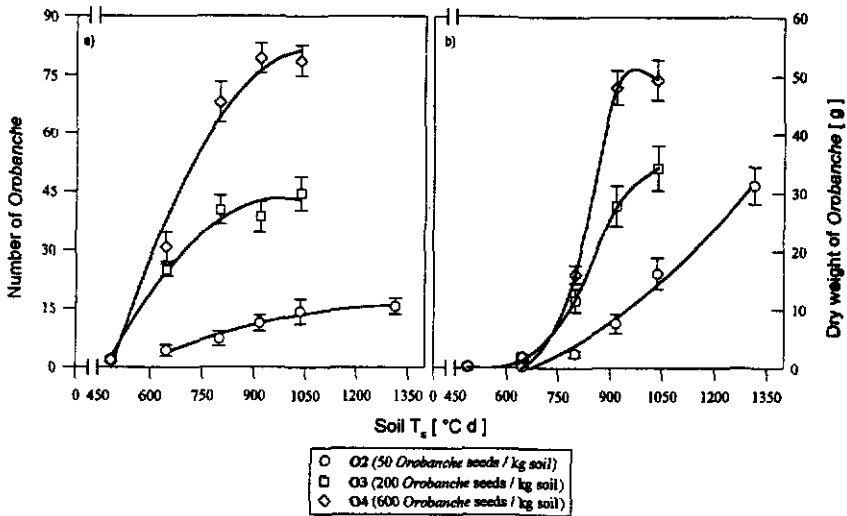
Figure 6.1.6. Relationship between number (a) and dry weight (b) of *Orobanche crenata* on faba bean and accumulated soil temperature, T_s (°C d), for host plants grown in pots with different *Orobanche* seed densities (02, 03, 04) in the soil.

6.2. Field Experiment for Modelling

The objectives of this study were to: a) validate a faba bean growth model developed by Stützel (1990[*]); b) study the faba bean root growth and distribution within the upper 30 cm soil layer; c) quantify the relationship between faba bean root-length density (cm/cm3) and the number of *Orobanche crenata* attachments; d) study the dry matter partitioning between different faba bean organs and *Orobanche* according to the abiotic factors soil moisture and temperature; and e) determine the extent of damage caused by *Orobanche* on faba bean according to different *Orobanche* seed banks in the soil. The study was conducted at Tel Hadya during the 1993/94 season. The soil of the study site is a chromic luvisol, poor in organic matter and more than two meters deep (Table 6.2.1). Total precipitation during the crop season amounted to 373 mm and therefore was higher than the long-term mean annual rainfall (331 mm). Its distribution, however, was poor, most of the rain falling between January and February. The total rainfall received during March and April was 12 mm, only. During the winter months, the temperatures were above the long-term averages and no severe frost spells occurred (Figure 6.2.1).

The trial was designed as split-split-plot with moisture supply as main plot, sowing date as subplot, and *Orobanche* seed load as sub-subplot (Table 6.2.2). There were four replications.

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* Stützel, H. 1990. Modellierung des Ertragsbildungssystems Ackerbohnenstand. Habilitaionsschrift, Fakultät III-Agrarwissenschaften I (Pflanzenproduktion und Landschaftsökologie) der Universität Hohenheim, Germany, pp 131.

Table 6.2.1. Characteristics of the soil at the experimental site in Tel Hadya (data from ICARDA's soil laboratory).

$NaHCO_3$-extractable P (Olsen P)	1.8 - 2.3 mg/kg
KCl-extractable NH_4-N	2.6 - 4.7 mg/kg
NO_3-N (H_2O)	10.1 - 19.2 mg/kg
Exchangeable K	8.4 - 9.4 mg/kg
Total $CaCO_3$	23.6%
DTPA-TEA-extractable Cu	1.5 mg/kg
DTPA-TEA-extractable Zn	0.7 mg/kg
DTPA-TEA-extractable Mn	7.1 mg/kg
DTPA-TEA-extractable Fe	5.5 mg/kg
pH (H_2O)	7.9
Organic matter	0.85%
Clay	60%
Silt	30%
Sand	10%
Field capacity (soil water content at a suction of -0.33 bars)	40% by weight
Wilting point (soil water content at a suction of -15 bars)	23% by weight
Available water	17% by weight
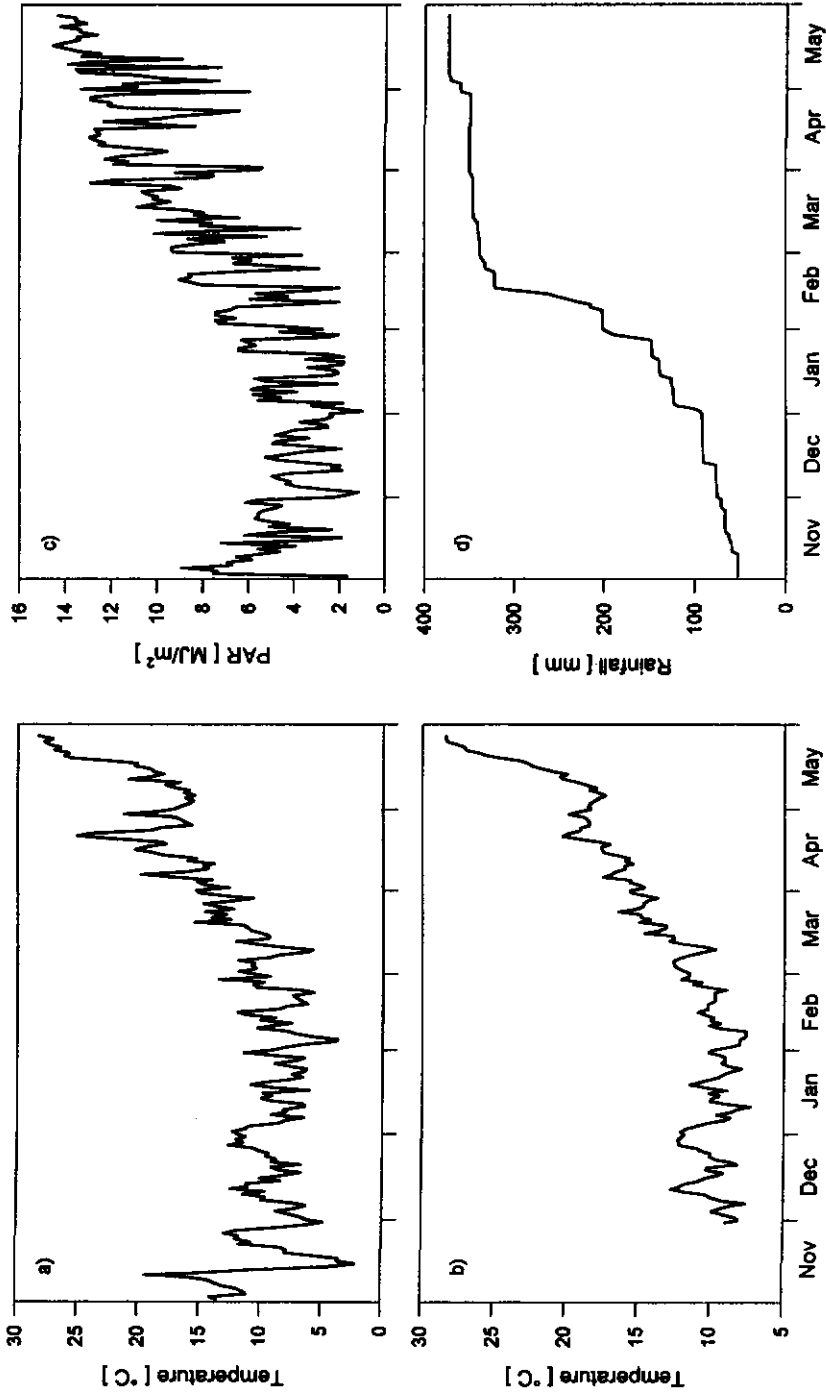
Figure 6.2.1. Daily mean air temperature (a), daily mean soil temperature in 10cm depth (b), photosynthetically active radiation, PAR (c), and cumulative precipitation (d) during the growing period.

Table 6.2.2. Summary of the experimental factors.

Factor	Level	Description
Moisture supply	MS1	no water stress
	MS2	with water stress
Sowing date	SD1	10 Nov. 1893
	SD2	17 Dec. 1993
Orobanche seed load	O1	without *Orobanche* seeds
	O2	50 *Orobanche* seeds/kg soil
	O3	200 *Orobanche* seeds/kg soil
	O4	600 *Orobanche* seeds/kg soil

In order to avoid the presence of viable *Orobanche* seeds naturally occurring in the soil, the area of this trial was solarized in the summer of 1993 (July-August). At the beginning of November, different *Orobanche* seed densities (O2, O3, and O4) were created by mixing the *Orobanche* seeds with the soil. The number of seeds was calculated from the 1000-seed weight (4 mg) of *Orobanche*. Nitrogen and phosphorus fertilizers (ammonium sulfate and triplesuperphosphate) were incorporated into the soil at the rates of 20 kg N and 50 kg P_2O_5 per hectare.

At both sowing dates, the seeds of faba bean genotype ILB 1814 (*Orobanche* susceptible) were sown by hand in rows with 45 cm spacing (22 seeds/m^2). Plots were 3m wide and 3m long. A fluid culture of *Rhizobium leguminosarum* (strain FB: 481) was applied directly on the seeds to ensure nodulation. In both moisture supply treatments (MS1 and MS2), the average volumetric water content of the two soil layers, 0 - 15 and 16 - 30 cm, was determined gravimetrically at 7-10 day intervals, and water was applied by surface irrigation method. In order to avoid the movement of *Orobanche* seeds between the treatments, plots were

separated from each other by constructing ridges around them. In MS1, water was applied, when the soil moisture in the active rooting zone had been reduced to 50% of the available water. The crops in the treatment MS2 were grown mainly under rainfed conditions.

All data were collected on a per plot basis. Sequential samples were taken during plant growth, beginning 4 weeks after emergence and ending at physiological maturity. Plants from 5 adjacent positions were collected at each sampling date and separated into: stems, leaves, inflorescences, pod walls, and seeds. Number and dry weight (after drying at 70°C for 48 h) of these organs were measured and the number of missing leaves was calculated according to the number of bare nodes. Leaf areas were determined with a calibrated Licor Leaf Area Meter. For two adjacent plants, out of 5 collected, the root length density RLD (cm roots/cm^3 soil) was measured. Three soil samples were taken from each plant with an auger (diameter 11 cm). The first sample was taken as a core centering around the plant (C_M), and the two others as adjacent left/right cores ($C_{L/R}$). The soil samples were collected from the soil depths, 0-15 and 16-30 cm, respectively. In total, 12 samples per plot were obtained (Figure 6.2.2).

The soil samples were soaked over night in water with sodium bicarbonate (about 50 g per 15-L bucket) to disperse the bulky clay soil. The roots were washed
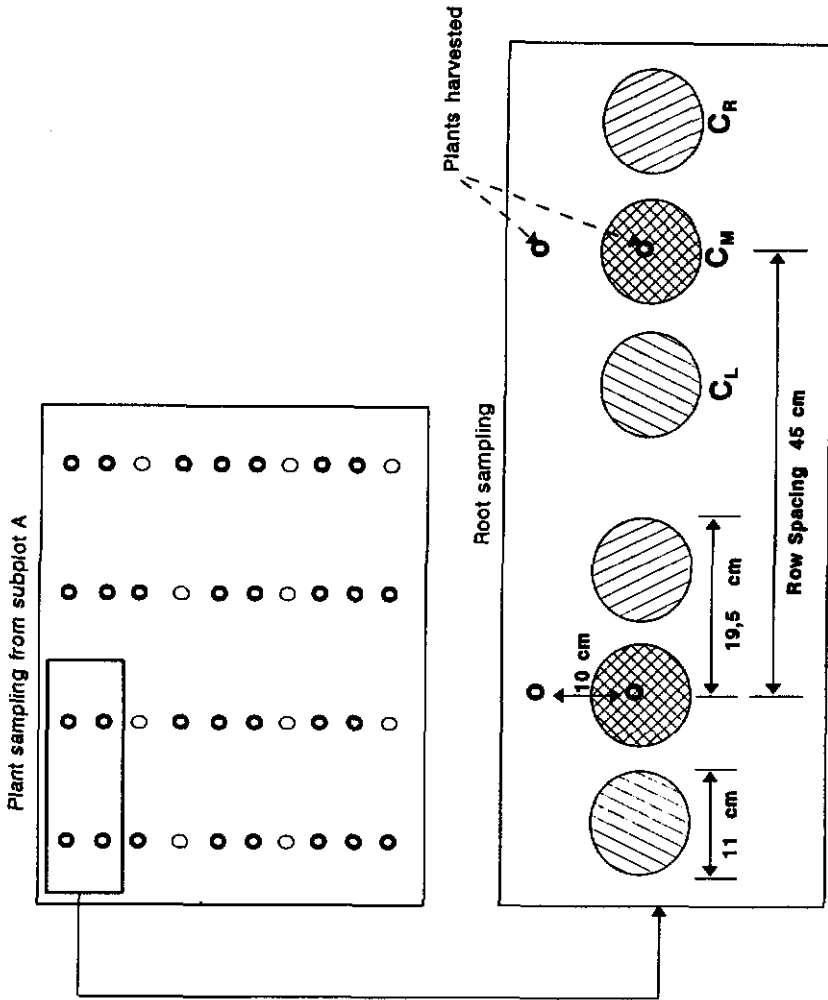
Figure 6.2.2. Pattern of plant and root sampling; (O) plants not harvested; (0) plants harvested; (C_M) core centring around the plant; ($C_{L/R}$) adjacent left and right cores.

free from soil on a 1-mm sieve. Living roots were
separated from dead roots and debris by hand. The
obtained roots from each sample were cut into small
pieces, approximately 2-3 cm long. The root length was
measured by using the Comair Root Length Scanner. For
each soil sample *Orobanche* tubercles (>1 mm), buds,
underground and emerged shoots were counted. After
separating the parasites from the faba bean roots,
their dry weight (after drying at 70°C for 48 h) was
measured per host plant for the two soil depths,
respectively.

At each sampling date, total incident and total
intercepted solar radiation were measured with two
tube solarimeters, model TSL (Delta-T devices,
Cambridge, UK, see Szeicz 1965*). One solarimeter (97
cm long) was mounted above the crop canopy. The second
(identical) solarimeter was inserted into the base of
the crop at right angles to the rows. Solarimeter
output was integrated for 60 sec using a millivollt
integrator (Delta-T devices). All the measurements
were made within 2 h of solar noon. The fraction of
total incident radiation transmitted through the
canopy (T) was calculated according to the equation

$$T = C_b / C_a$$

where C_a and C_b are the integrated counts above and
below the canopy, respectively. Using the technique
of Gallagher & Biscoe (1978*), transmissivity readings
were recalculated into the fraction (Q) of absorbed
photosynthetically active radiation (PAR): To obtain
$$Q = (1 . 0 - T^{1.2}) / 1 . 1$$

total PAR between successive sampling dates,
arithmetic means of Q, as measured at successive
sampling dates, were multiplied by 50% of the global

radiation recorded from the Tel Hadya weather station. PAR has been reported to comprise 50% of the total incident shortwave radiation (Szeicz, 1974[*]).

With a datalogger, soil temperature was recorded continuously at 5, 15, and 30 cm depth in both moisture supply treatments. Temperature sums were calculated as where T_i is the mean temperature for

$$T_s = \sum_{i=1}^{j} (T_i - T_c)$$

each day i, and T_c is the base temperature, below which the developmental rate equals zero. In this study, the base temperature used was 0 °C for air temperature sum and 4 °C for soil temperature sum.

The plots in the moisture supply treatment MS1 were irrigated 5 times (29 Dec, 15 & 23 Mar, 5 & 13 Apr) during the growing season (Figure 6.2.3). Under this soil moisture condition, the faba bean crops did not show any water stress. In MS2, irrigation was applied only 2 times during the growing period. First time on 29 Dec to ensure the germination and emergence of faba bean plants, and second time on 13 Apr at pod-filling stage due to the long drought period during March and April. Plants were suffering from water stress mainly during the reproductive phase.

* Sceicz, G. 1965. A muniature tube solarimeter. Journal of Applied Ecology 2:145-147.

Sziecz, G. 1974. Solar radiation for plant growth. Journal of Applied Ecology. 11:617-636.

Gallagher, J.N. and Biscoe, P.V. 1978. Radiation absorption, growth and yield of cereals. Journal of Agricultural Science, Cambridge 91:47-60.
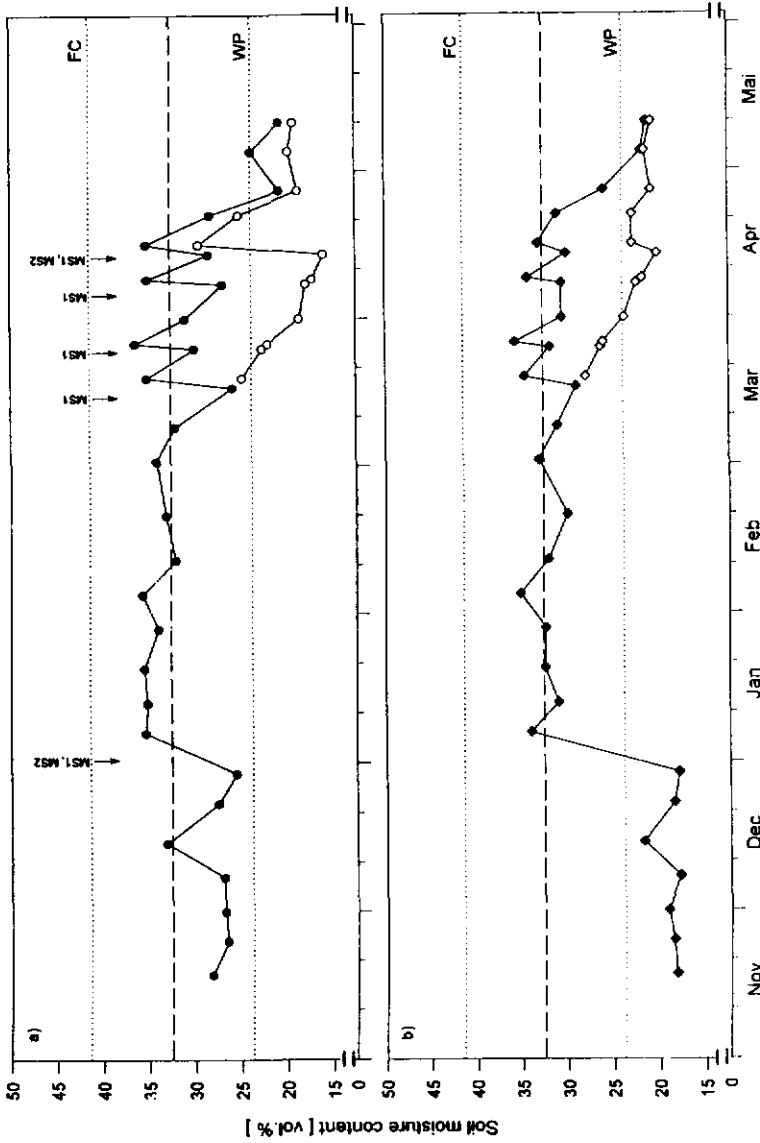
Figure 6.2.3. Soil moisture content in 0-15cm(a), and 16-30 cm(b) soil depth during the growing period. Closed symbols represent data from the first moisture supply treatment (MS1) and open symbols from the second one (MS2). Dashed lines show the 50% available water, FC is field capacity, and WP is wilting point. Arrows are for irrigation dates.

6.2.1. Simulation of Growth and Development in Faba bean (ILB 1814)

The first step in modelling the faba bean-*Orobanche* interaction, is to build a model for the simulation of host-plant growth and development under stress-free environment. Since drought stress is the main abiotic yield-limiting factor in faba bean production in semi-arid zones, in the next step, a water stress module should be developed to consider the effect of water shortage on faba bean growth. Having such a model developed, the final step would be to integrate an *Orobanche* module simulating the effect of *Orobanche* infestation on faba bean growth and development. One of the objectives of this study was to validate a faba bean growth model developed by Stützel (1990). The model was built mainly based on data and relationships obtained from field experiments with indeterminate and determinate faba bean cultivars grown at Hohenheim, Southern Germany. In the following, a brief description of this model and the comparisons between simulated and measured data on faba bean growth are presented. The data on faba bean growth and development were collected from the control plots (without *Orobanche* infestation) of the moisture supply treatment MS1.

6.2.1.1. Model description

In the model, dry matter is produced as a function of light interception and utilization. After reading basic inputs (genotypic, locational, and agronomic data; Table 6.2.3), and calculating derived basic data, simulation starts in daily intervals, beginning with the day of emergence. Every day, mean temperature, global radiation, and relative humidity

are read from a weather data file. Provided that the crop is still growing, i.e. not mature or killed, phenological parameters such as flowering, leaf production, branching, and pod formation are calculated. The end of the natural growth period (physiological maturity) is reached when the leaf area index reaches zero. The model estimates the faba bean yield under ample supply of water and nutrients in a pest-, disease-, and weed-free environment (Figure 6.2.4).
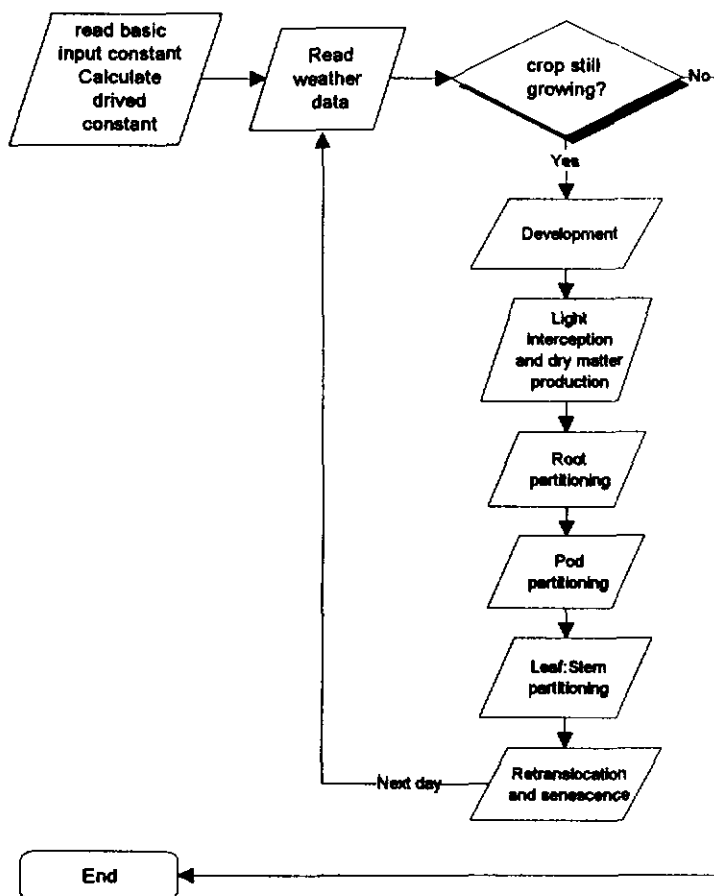

Figure 6.2.4. Flow diagram of faba bean model.

Table 6.2.3. Description of basic input data.

Parameter	Abbr	Unit	Used in simulation*		
			H. Freya	ILB-1	ILB-2
Latitude	LAT	degrees	48.72	36.01	36.01
Soil water holding capacity	WHC	mm	100.0	100.0	100.0
Cultivar	CVR	–	H. Freya	ILB 1814	ILB 1814
Thousand seed weight	TSW	g	400.0	1700.0	1700.0
Number of seeds per pod	NSP	–	4.0	2.7	2.7
Optimum temperature for flowering	TOF	$°C$	14.8	14.8	14.8
Constant A for flowering	AFL	d^{-1}	0.0	0.0	0.0
Constant B for flowering	BFL	$h^{-1}d^{-1}·c^{-1}$	0.000106	0.000106	0.000106
Constant C for flowering	CFL	d^{-1}	0.05326	0.05326	0.05326
Constant D for flowering	DFL	$h^{-1}d^{-1}·c^{-1}$	-0.000118	-0.000118	-0.000118
Maximum node number per stem	MXN	–	40	30	30
Branching? 1=No, 2=Yes BRA-122	BRA	–	1	2	2
Assimilate flux per branch	ANT	$g\ d^{-1}$	0.353	0.353	0.353
Assimilate flux per pod	ANP	$g\ d^{-1}$	0.034	0.034	0.034
Minimum light extinction coefficient	K	–	0.093	0.093	0.093
Specific leaf area	SLA	$cm^2\ g^{-1}$	270	270	270
Temperature sum for pod filling	TPF	$°C\ d$	1000.0	1000.0	800.0
Seedling density	SED	m^{-2}	18.5	20.0	20.0
Date of emergence (Julian date)	DEM	-126	98	130	
Date of initial LAI (Julian date)	JD1	–	138	118	150
Sowing density (germinated seeds per m^2)	SOD	m^{-2}	18.5	20	20
Sowing technique (score: 0=excellent, 9=poor)	SOT	–	1.0	1.0	1.0
Date of sowing (Julian date)	DSO	–	105	71	108

* Herz Freya: an indeterminate German faba bean cultivar;
ILB-1 and ILB-2: an indeterminant faba bean genotype (ILB 1814, Syrien Local Large) planted on Nov. 10 and Dec. 17, respectively.

6.2.1.2. Initial simulation

For the initial simulation, a weather data file of the study site (Tel Hadya, 1993/94) and the input constants for the faba bean genotype ILB 1814 (Table 6.2.3), planted at two sowing dates, were used. For the weather data file and the agronomic input parameters, in this study, the Julian calendar starts with the 1st of September since the ILB 1814 genotype, in contrast to H. Freya, was planted as a winter crop. The model starts the simulation of dry matter production and partitioning when the crop has reached a certain leaf area per plant (JD1, initial LAI). At this time, it is assumed, that most of the cotyledon reserves have been depleted and that the further growth of plants is related to newly produced assimilates. In cultivar H. Freya, with a thousand-seed weight of 400 g, the initial LAI was calculated to be 0.0925 and was reached 12 days after emergence. For a large-seeded faba bean genotype like ILB 1814 with a thousand-seed weight of 1700 g, the same calculation resulted in an initial LAI of 0.21. This stage of leaf area was observed in ILB 1814 around 20 days after emergence (Table 6.2.3).

The comparison between simulated and measured data showed that, the model overestimated the LAI as well as the total gross dry matter, WTO, for both sowing dates (Figure 6.2.5). The maximum LAI simulated was higher than the one observed in the field, and it was reached earlier. This indicates that, the model overestimated the faba bean growth during the winter months. Consequently, the predicted fruit and seed dry weights were higher than the measured ones (Figure 6.2.6). The dry matter partitioning into leaves was simulated realistically, whereas the predicted stem dry weight exceeded the experimental data

considerably. The model underestimated slightly the root dry weight for the first sowing date until 100 days after emergence and overestimated thereafter. For the second sowing data the simulated root dry weight corresponded to the measured data until 80 days after emergence, and as for the first sowing date, it exceeded the observed data thereafter (Figure 6.2.6). This means, with higher temperature and lower rainfall in the mid spring, the model overestimates the root dry weight considerably.

The reasons for these discrepancies between simulated and measured data could be: **a)** for initial simulations some genotypic input parameters such as specific leaf area, SLA, assimilate flux per branch (ANT) and assimilate flux per pod (ANP) were chosen from H. Freya. These parameters might differ in the case of ILB 1814 and they had to be determined. **b)** the faba bean model is based mainly on analytical results from field experiments with small-seeded cultivars grown under temperate climatic conditions. This means that some of the physiological relationships and assumptions on which this model is based might be genotypic or location specific, and thus should be modified for the genotype ILB 1814 grown in a mediterranean environment.

6.2.1.3. Determination and modification of some basic input parameters

a) Specific leaf area, SLA (cm^2/g): The faba bean model calculates the leaf area index, LAI, according

$$LAI = WLG * SLA / 10000$$

to the equation where WLG is the green leaf dry matter (g), and SLA the specific leaf area. Higher SLAs thus
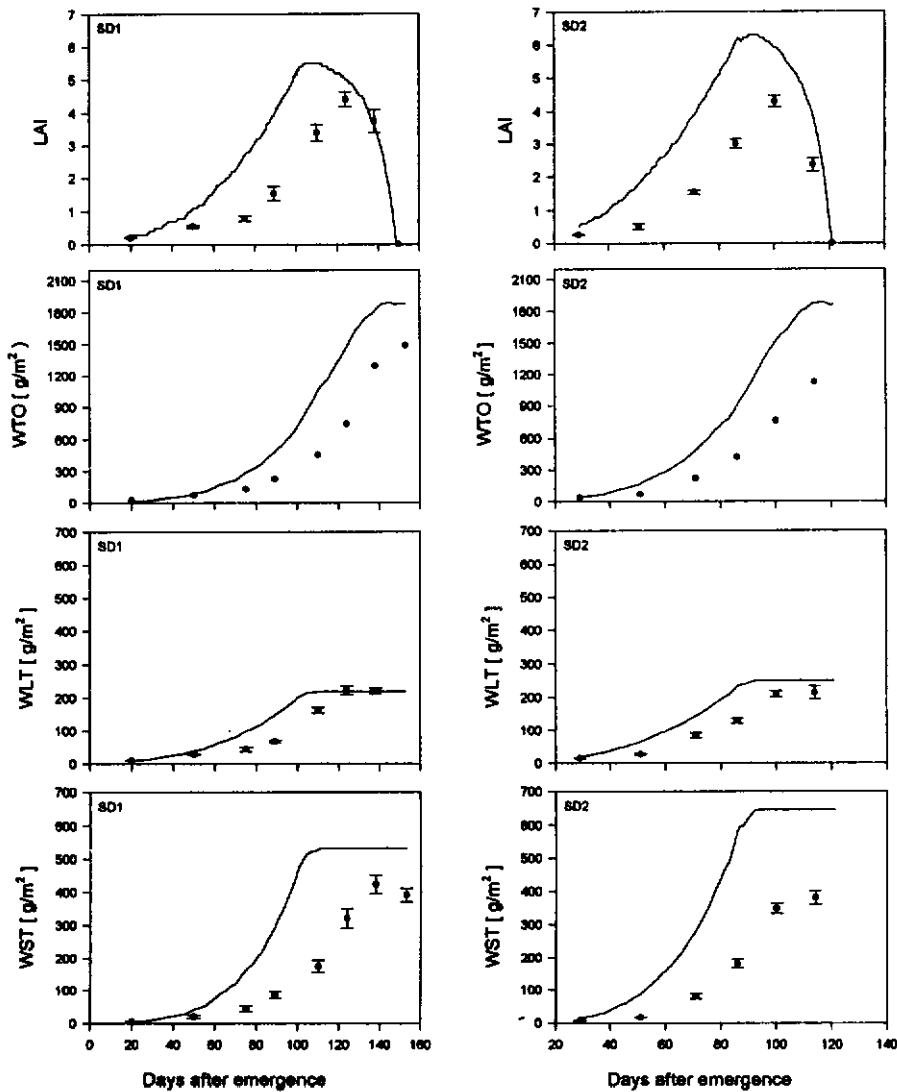
Figure 6.2.5. Simulated (curves) and measured (points) leaf area index, LAI, gross total dry matter (WTO), leaf dry matter (WLT) and stem dry matter (WST) in faba bean genotype ILB 1814 planted on Nov. 10 (SD1) and Dec. 17 (SD2). Bars indicate standard errors.
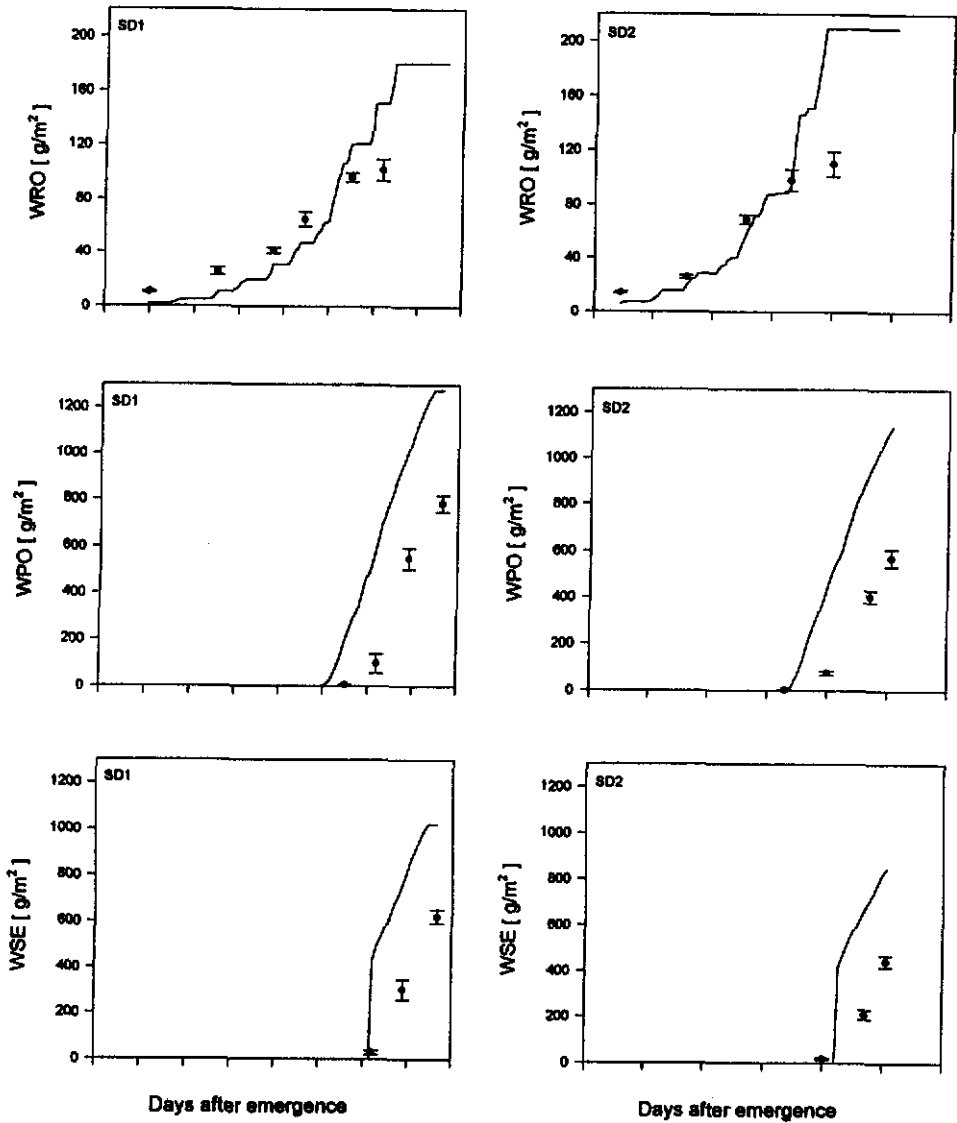
Figure 6.2.6. Simulated (curves) and measured (points) root dry matter (WRO), fruit dry matter (WPO), and seed dry matter (WSE) in faba bean genotype ILB 1814 planted on Nov. 10 (SD1) and Dec. 17 (SD2). Bars indicate standard errors.

lead to higher LAIs. In ILB 1814, the SLA was found to be 217 for the first sowing date, and 225 for the second one (Figure 6.2.7). Thus SLA is lower in ILB 1814 than in H. Freya (270).
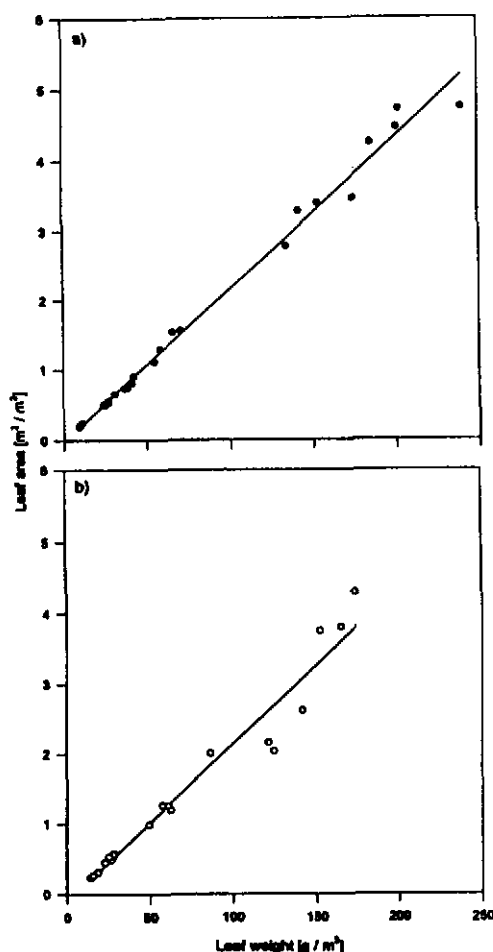
Figure 6.2.7. Relationships between leaf weights, W_L, and leaf area, A_L, for the faba bean genotype ILB 1814 planted on Nov. 10(a) and Dec. 17(b). Regression equations are:

(a) $A_L = -0.08 + 0.0217\ W_L$, $r^2 = 0.99$
(b) $A_L = -0109 + 0.0225\ W_L$, $r^2 = 0.96$

b) Stem:leaf partitioning: The overestimation of stem dry matter in the initial simulations (Figure 6.2.5) led to the assumption, that the coefficient h and g of the linear regression between the natural logarithm of stem and leaf weights used in the model

$$lnW_s = h + g\,lnW_l$$

might be different in the case of ILB 1814. Throughout the period of stem and leaf growth in ILB 1814, the above mentioned relationship was also linear, as graphically shown for the both sowing dates in Figure 6.2.8. The coefficients h and g, however, differed from those assumed in the model. Having found the relationship between h and g in ILB 1814, the original equation used in the model was substituted by the one presented in Figure 6.2.9

c) Dry matter partitioning into roots: In the model, dry matter partitioning into roots depends on total gross dry weight, (W_T), crop growth rate, (G05), and vapour-pressure deficit of the air, (V05), as shown in the equation:

$$W_R = (0.046 * W_T) + (0.33 * G05 * V05)$$

This means that the root growth accelerates when the vapour-pressure deficit of the air increases. Using this equation, the model overestimated the root dry weight during the warmer and dryer period in mid-spring although the plants were irrigated (Figure 6.2.6). When the crops are grown under ample supply of water, one can assume, that the root growth is related only to the plant total gross weight, and to the soil temperature. In ILB 1814, a strict linear relationship was found between the root fraction and the accumulated soil temperature throughout the root growth period (Figure 6.2.10). For the time being, the linear regression model shown in Figure 6.2.10.

will be used for the simulation studies, when faba
bean is grown under irrigation conditions. This root
model, however, will be developed further to account
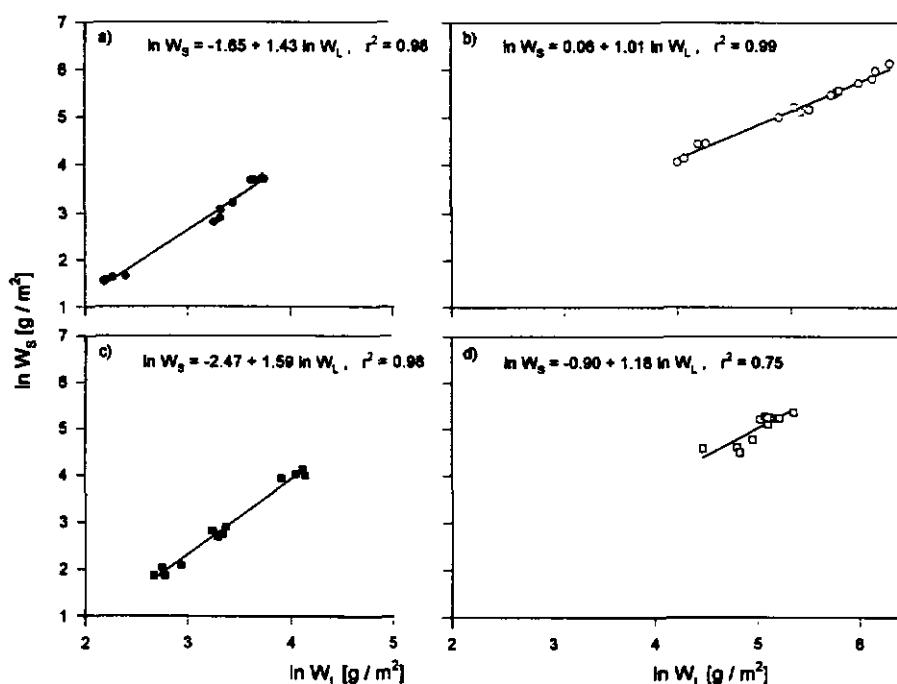for the effect of water stress on the root growth.

Figure 6.2.8. Relationships between the natural
logarithm of stem weight (In W_s) and
the natural logarithm of leaf weight,
(In W_L) for faba bean genotype ILB 1814
planted on Nov. 10 (a, b) and Dec. 17
(c, d). Closed symbols represent data
from first sampling until commencement
of flowering and open symbols for data
obtained after flowering.
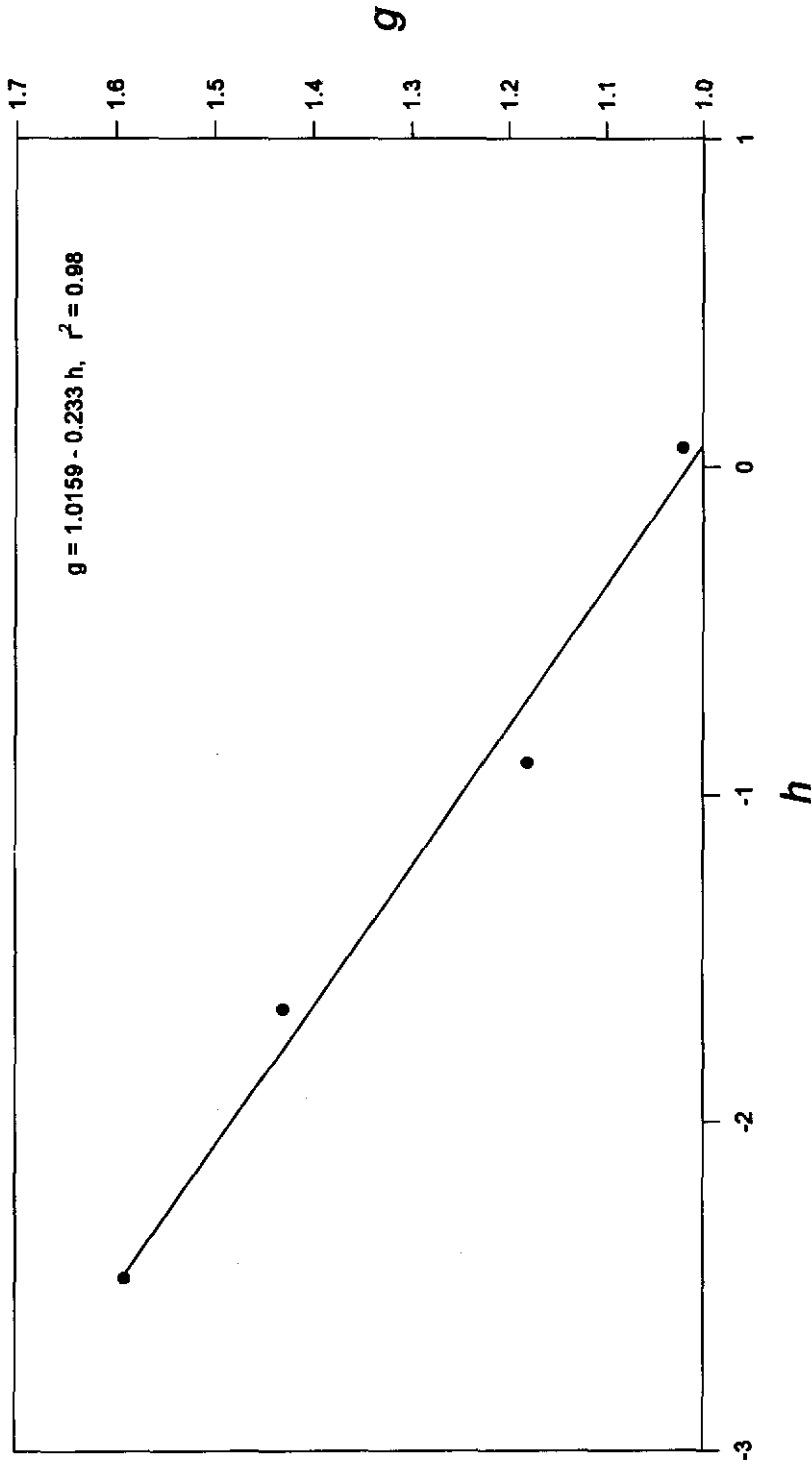
Figure 6.2.9. Relationship between regression coeffients h and g of the equation $\ln W_S = h + g$ $\ln W_L$. From this equation we obtain: $W_S = e^h W_L^{1.0159 - 0.233h}$ in contrast to the values in Stützel's Model: $W_S = e^h W_L^{1.159 - 0.188h}$

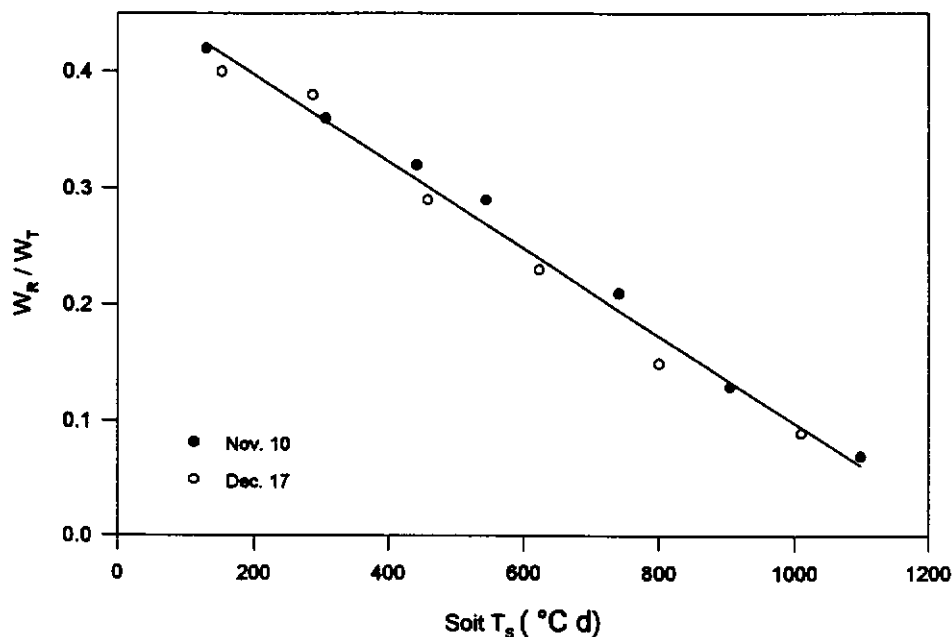Within the figure:

$g = 1.0159 - 0.233\,h, \quad r^2 = 0.98$

Figure 6.2.10. Relationship between the root fraction, W_R/W_T, and the accumulated daily mean soil temperature in 10 cm depth in faba bean genotype ILB 1814 planted on Nov. 10 and Dec. 17 (W_R:root dry weight; W_T:plant total dry weight). Regression equation is: $W_R = (0.47*W_T) - (0.00037*T_S *W_T)$ $r^2=0.98$ in contrast to the values in Stützel's Model: $W_R = (0.046*W_T) + (0.33*G05*V05)$.

d) **Dry matter partitioning within the pod:** At the physiological maturity of faba bean, the model overestimated the proportion of pod husk on total fruit dry weight (Figure 6.2.6). The model simulates the dry matter partitioning within the pod based on the assumption that the proportion of pod husk on the total fruit mass is related linearly to the single fruit weight. Although, a similar relationship was found in ILB 1814, the intercept of the regression equation was higher than the one assumed in the model (Figure 6.2.11).
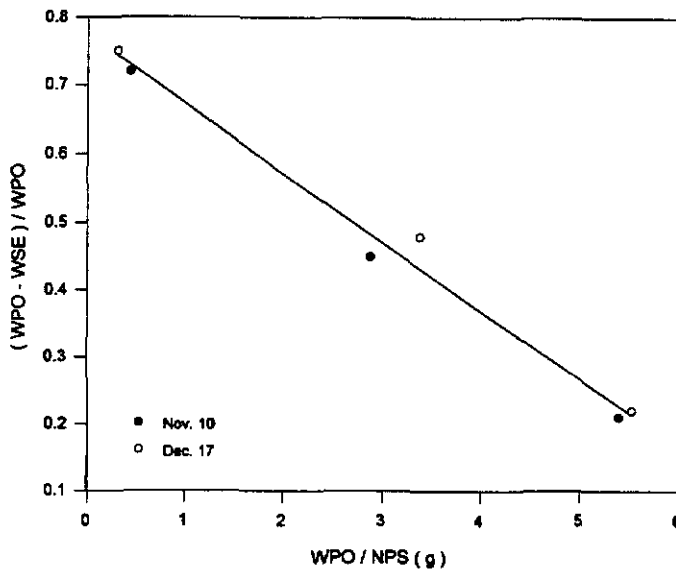
Figure 6.2.11. Proportion of the pod husk, WPO-WSE, on whole fruit mass, WPO, as dependent on single fruit mass, WPO/NPS, in faba bean genotype ILB 1814 planted on Nov. 10 and Dec. 17, respectively, (WSE: Seed dry matter $[g/m^2]$; NPS:Number of seed bearing pods/m^2). Regression equation is: (WPO-WSE)/WPO=0.78-0.10*(WPO/NPS), r^2=0.98

WSE=WPO*(1-(0.78+0.10*(WPO/NPS)))

Stützel's Model:WES=WPO*(1-(0.42+0.10*(WPO/NPS)))

e) Dry matter production: In the model, daily rates of dry matter production (GTO) are the product of photosynthetically active radiation incident (PAR) the proportion of radiation intercepted (Q) and the light use efficiency (LUE) (g dry matter per MJ intercepted PAR:

$$GTO = LUE * Q * PAR$$

LUE depends only on light extinction coefficient, K, (given input constant, Table 6.2.3):

$$LUE = 1 / (-0.078 + 0.733 * K)$$

At the study site (Tel Hadya, Northern Syria), temperature, and not radiation, is the main abiotic growth-limiting factor during the winter period. As shown above, the model, however, does not consider its effect on dry matter production, and this might be the reason for the overestimation of dry matter production observed during the winter period (Figure 6.2.5). Therefore, the equation for the calculation of LUE has been modified according to the assumption that, with daily mean temperatures up to 12 °C, the LUE is reduced linearly in relation to temperature:

$$LUE = (1 / (-0.078 + 0.733 * K) * (-0.17 + (0.1 * T))$$

where T is the daily mean temperature.

6.2.1.4. Simulation with the modified growth model

After having modified the above-mentioned relationships, the model was run again to see whether the prediction of growth and development in ILB 1814 faba bean agreed with the experimental data. The basic input parameters for ILB 1814, as shown in Table 6.2.3, were used again, except that the input value of SLA, for ILB 1814 was 220 cm^2/g (average of both sowing dates, Figure 6.2.7), and due to the shortage of experimental data, the values for the assimilate

flux per branch (ANT) and the assimilate flux per pod, (ANP) were assumed to be 0.1 g/day, respectively.

As shown in Figure 6.2.12 and Figure 6.2.13 for both sowing dates LAI and the total WTO predicted by the modified model, agreed well with the measured data. The dry matter partitioning into different plant organs was also simulated realistically. This indicates that the modified model is able to take into consideration the effects of delayed sowing on vegetative and generative growth of faba bean genotype ILB 1814.

6.2.3. Future Development of the Model

As presented above model is now able to simulate the growth and development in faba bean genotype ILB 1814 grown under water-stress free conditions. For other faba bean cultivars, the genotypic input parameters should be determined. The model was tested with the experimental data from one growing season (1993/94) only. Further validation of the model will be undertaken with the data from the same field trials to be conducted in the 1994/95 season. In addition, the effect of water shortage on faba bean growth will be considered in the model by developing a water-stress subroutine. For this, the data already collected from the control plots in moisture supply treatment MS2 (with water stress) will be used. Root-length density, as one of the most important factors for germination and attachment Orobanche, can not yet be simulated by the model. A subroutine for the estimation of root length from root dry weight will be integrated in the model. After having analyzed the data already collected on Orobanche infestation in faba bean, the effect of Orobanche infestation on dry

matter production by faba bean and its partitioning within the faba bean-*Orobanche* system will be simulated. **Ahmed M. Manscahdi, M.C. Saxena, W. Goebel (ICARDA), J. Kroschel and J. Sauerborn (Univ. of Hohenheim).**
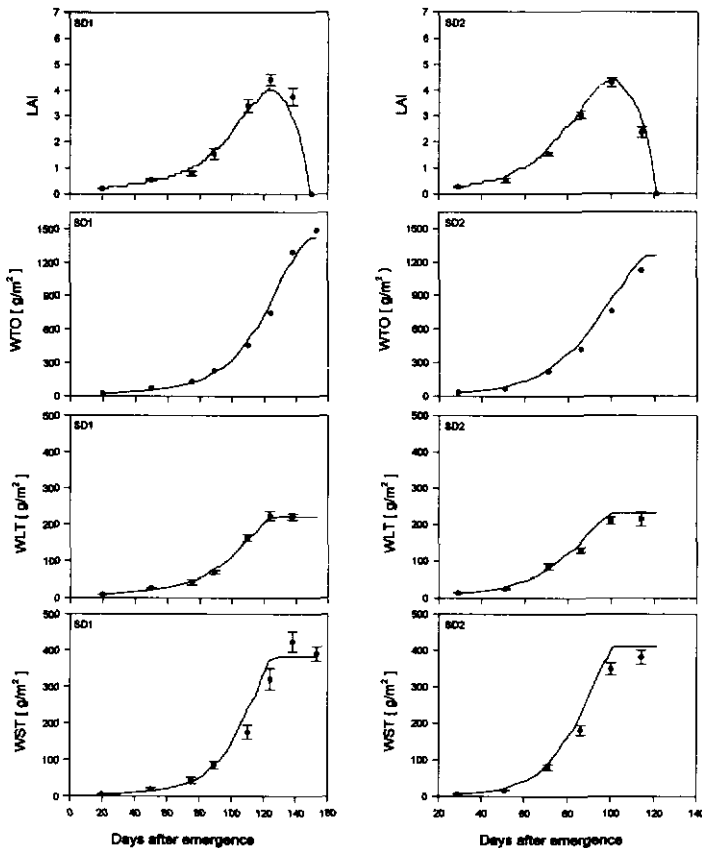

Figure 6.2.12. Simulated (curves) and measured (points) leaf area index (LAI), gross total dry matter (WTO), leaf dry matter (WLT), and stem dry matter (WST), in faba bean genotype ILB 1814 planted on Nov. 10 (SD1) and Dec. 17 (SD2). Bars indicate standard errors.
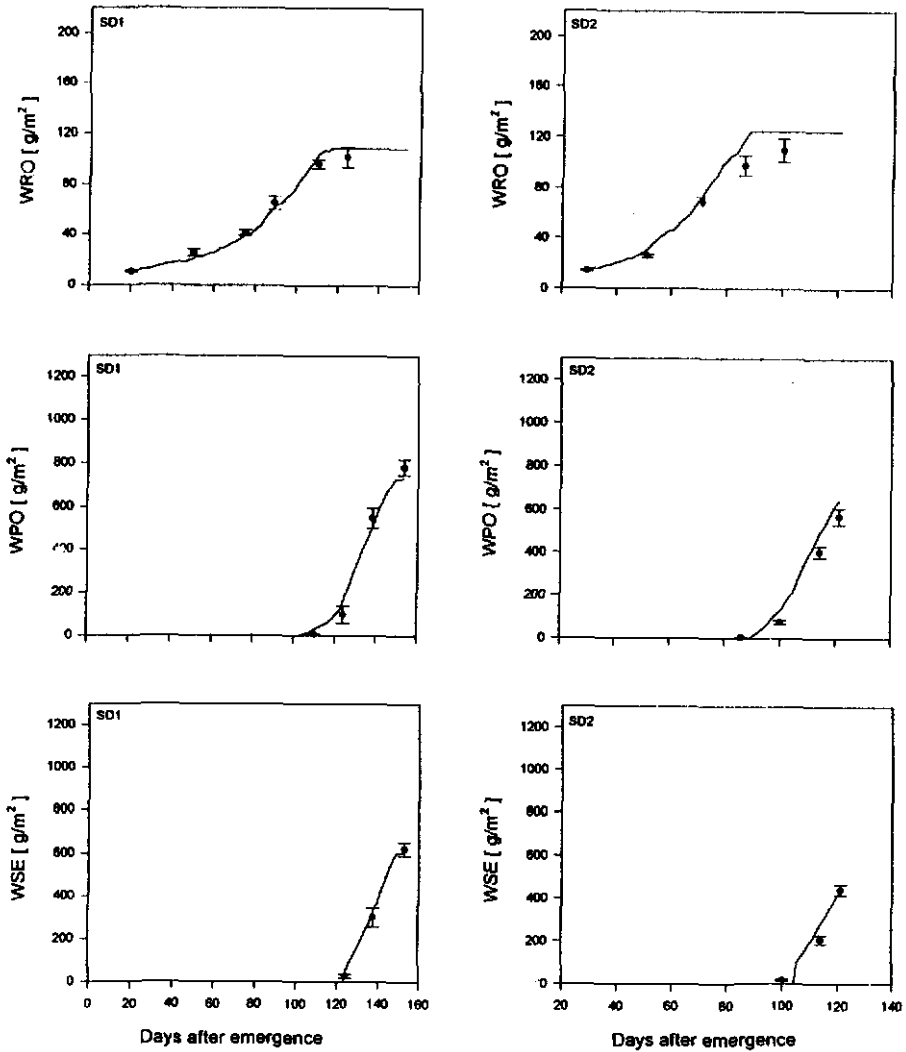
Figure 6.2.13. Simulated (curves) and measured (points) root dry matter (WRO), fruit dry matter (WPO), and seed dry matter (WSE), in faba bean genotype ILB 1814 planted on Nov. 10 (SD1) and Dec. 17 (SD2). Bars indicate standard errors.

7. INTERNATIONAL TESTING PROGRAM

The international testing program on lentil, kabuli chickpea, dry pea, lathyrus and vetches is a vehicle for the dissemination of genetic materials in the form of international nurseries and trials, to the national programs in and outside the WANA region. The genetic materials comprise early segregating populations in F_3 and F_4 generations, and elite lines with wide or specific adaptation, special morphological or quality traits, and resistance to common biotic and abiotic stresses. Nurseries are only sent on request and often include germplasm specifically developed for a particular region or a national program.

The testing program helps in identification of genotypes with specific and wide adaptation. The performance data permit assessment of genotype x environment interaction and help in targeting breeding efforts for specific agroecological conditions.

In 1994 two separate drought tolerance nurseries, one each in chickpea and lentil, were developed for 1994/95 season. The Chickpea International Leaf Miner Nursery (CILMN) was dropped as there was no demand from the cooperators. We supplied 1027 sets of 40 different nurseries (Table 7.1.1) to cooperating scientists in 48 countries for the 1994/1995 season. Several cooperators requested large quantities of seed of elite lines, identified by them from the earlier international nurseries/trials for multilocation yield testing and on-farm verification.

The salient results of the 1992/93 international nurseries, received from cooperators until 1 October 1994, are presented here. The stability analyses of some of the trials were done using Eberhart and Russell (1966)[*] model.

7.1. Lentil

For Lentil International Yield Trial-Large Seed (LIYT-L) data were analyzed for seed yield for 31 locations. At Guelma in Algeria, Larissa in Greece; Gazvin, Nishabour and Sanandaz in Iran; Caltagirone in Italy; Rabba in Jordan; El Saf Saf in Libya, Badajoz in Spain; Gelline, Idleb Izra'a and Breda in Syria; El Kef in Tunisia; and Hymana in Turkey, 1, 3, 2, 5, 1, 14, 1, 1, 2, 1, 3, 1, 1, 5 and 14 of the test entries exceeded the respective local check in seed yield by a significant margin ($P \leq 0.05$). On the basis of mean across locations the five heaviest yielding lines were FLIP 91-9L, FLIP 87-16L, FLIP 88-7L, FLIP 90-11L and FLIP 89-5L.

Stability analysis for seed yield of LIYT-L entries, based over 30 locations, revealed that linear and non-linear portion of entry x environment (GxE) interaction were significant (Table 7.1.2). The stability parameters exhibited that 2 out of 23 entries, namely FLIP 87-16L and FLIP 87-17L, with above average mean yield, regression coefficient (b) greater than 1, and non-significant deviations from regression, exhibited specific adaptation to the high yielding environments. Another entry FLIP 88-7L with above average yield, non-significant deviations from regression and regression equal to unity exhibited general adaptation over all the environments.

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[*] Eberhart, S.A. and Russel, W.A. 1996. Stability parameters for comparing varieties. Crop Science. 6:36-40.

Table 7.1.1. Distribution of Legume International Nurseries for the 1994/95 season to cooperators.

International Trial/Nursery	No. of sets
Lentil	
Yield Trial, Tall (LIYT-L)	45
Yield Trial, Small (LIYT-S)	30
Yield Trial, Early (LIYT-E-95)	37
Screening Nursery, Large-Seed (LISN-L-95)	33
Screening Nursery, Small-Seed (LISN-S-95)	21
Screening Nursery, Early (LISN-E-95)	30
Screening Nursery, Drought Tolerance (LISN-DT-95)	30
F_5 Nursery, Large Seed (LIF$_5$N-L-95)	23
F_5 Nursery, Small Seed (LIF$_5$N-S-95)	15
F_5 Nursery, Early (LIF$_5$N-E-95)	15
F_5 Nursery, Cold Tolerance (LIF$_5$N-CT-95)	6
Cold Tolerance Nursery (LICTN-95)	19
Ascochyta Blight Nursery (LIABN-95)	11
Fusarium Wilt Nursery (LIFWN-95)	26
Rust Nursery (LIRN-95)	14
Chickpea	
Yield Trial Spring (CIYT-Sp-95)	45
Yield Trial Winter, Mediterranean Region (CIYT-W-MR-95)	45
Yield Trial Southerly Latitudes-1 (CIYT-SL1-95)	12
Yield Trial Southerly Latitudes-2 (CIYT-SL2-95)	16
Yield Trial Latin American (CIYT-LA-95)	12
Screening Nursery Winter (CISN-W-95)	28
Screening Nursery Spring (CISN-Sp-95)	26
Screening Nursery, Southerly Latitudes-1 (CISN-SL1-95)	11
Screening Nursery, Southerly Latitudes-2 (CISN-SL2-95)	11
Screening Nursery, Latin American (CISN-LA-95)	13
F_4 Nursery, Mediterranean Region (CIF$_4$N-MR-95)	13
F_4 Nursery, Southerly Latitudes (LIF$_4$N-SL-95)	10
Ascochyta Blight Nursery: Kabuli (CIABN-A-95)	20
Ascochyta Blight Nursery: Kabuli & Desi (CIABN-B-95)	21
Fusarium Wilt Nursery (CIFWN-95)	28
Cold Tolerance Nursery (CICTN-95)	22
Drought Tolerance Nursery (CIDTN-95)	30
Forage Legumes	
Lathyrus Adaptation Trial (ILAT)	
- *Lathyrus sativus* (ILAT-LS-95)	49
- *Lathyrus cicera* (ILAT-LC-95)	29
- *Lathyrus ochrus* (ILAT-LO-95)	27
Vetch Adaptation Trial (IVAT)	
- *Vicia sativa* (IVAT-VS-95)	48
- *Vicia narbonensis* (IVAT-VN-95)	37
- *Vicia ervilia* (IVAT-VE-95)	31
- *Vicia villosa* ssp *dasycarpa* (IVAT-VD-95)	37
Peas	
Adaptation Trial (PIAT-95)	51
TOTAL	1027

Table 7.1.2. ANOVA for stability analysis of seed yield for the entries in LIYT-L, LIYT-S, and LIYT-E conducted during 1992/93.

Source of variation	LIYT-L		LIYT-S		LIYT-E	
	df	MS	df	MS	df	MS
Entry	22	208105.59**	22	368870.20**	22	114543.30
Entry x Location (Linear)	22	191665.01**	22	242026.91**	22	177686.66
Pooled deviation	644	57130.80**	322	129478.33**	184	174319.44**
Poled error	1320	27864.68	704	58909.65	440	58076.04

* Significance at $P \leq 0.05$, ** Significance at $P \leq 0.01$.

The results of Lentil International Yield Trial-Small Seed (LIYT-S) received from 17 locations revealed that at 11 locations, Gemmiza in Egypt; Tolentino in Italy, El Safsaf in Libya, Lincoln in New Zealand; Badajoz in Spain, Gelline, Hama, Heimo, Idleb, Izra'a and Tel Hadya in Syria, 4, 1, 1, 1, 6, 7, 2, 9, 2, 1, and 1 of the test entries exceeded the respective local check in seed yield by a significant ($P \leq 0.05$) margin. The five heaviest yielders in this trial included 81 S 15, FLIP 90-41L, FLIP 89-37L, FLIP 90-36L, and FLIP 90-30L. Stability analysis for seed yield based over 16 locations revealed that both entry x location (linear) and deviations from regression (nonlinear) were significant (Table 7.1.2). Three entries namely, FLIP 90-41L, FLIP 90-30L, and FLIP 90-40L exhibited above-average yield and nonsignificant deviations from regression. Among these entries FLIP 90-30L and FLIP 90-41L had regression coefficient (b) equal to 1, and exhibited general adaptation and the entry FLIP 90-41L with b>1.0 exhibited adaptation to favorable environments.

The results of Lentil International Yield Trial-Early (LIYT-E) from 13 locations revealed that at 3 locations namely Gemmiza in Egypt, Gassim in Saudi

Arabia, and Hudeiba in Sudan 1, 7, and 2 of the test entries exceeded the local check in seed yield by a significant margin. The five heaviest yielders across locations included FLIP 89-71L, ILL 7165, FLIP 86-39L, ILL 7164, and FLIP 87-72L. The stability analysis done across 10 locations revealed that only non-linear portion of G x E interaction was significant (Table 7.1.2). Five entries namely ILL 7165, FLIP 87-72L, L1282, ILL 7164, and ILL 4403, with above average seed yield, non-significant deviation from regression and regression b=1.00, were adaptable across environments.

For Lentil International Screening Nursery - Large (LISN-L), Small (LISN-S), and Early (LISN-E), the data for seed yield were reported from 17, 9, and 11 locations, respectively. The analyses of data revealed that at 5 locations in LISN-L (Caltagirone in Italy; Lincoln in New Zealand; Tel Hadya and Heimo in Syria; and Erzurum in Turkey), two locations in LISN-S (Oristano in Italy and Izra'a in Syria), and 2 locations in LISN-E (Sri Nagar in India and Tel Hadya in Syria) some of the test entries exceeded the respective local check by a significant margin ($P \leq$ 0.05). The five heaviest yielding lines across locations in these nurseries are given in Table 7.1.3.

Table 7.1.3. The five heaviest yielding lines across locations in different lentil screening nurseries, 1992/93.

Rank	LISN-L	LISN-S	LISN-E
1	FLIP 93-28L	FLIP 93-20L	FLIP 93-44L
2	FLIP 92-11L	FLIP 93-19L	FLIP 92-50L
3	FLIP 93-21L	FLIP 89-39L	FLIP 93-46L
4	FLIP 92- 4L	FLIP 89-40L	FLIP 93-47L
5	FLIP 93-33L	FLIP 91-21L	FLIP 91-27L

The results of Lentil International F_5-Nursery Large (LIF$_5$N-L), F_5-Nursery Small (LIF$_5$N-S), F_5-Nursery Early (LIF$_5$N-E), and F_5-Nursery Cold Tolerance (LIF$_5$N-CT), were received from 9, 7, 6, and 2 locations, respectively. At 4, 6, 3 and 2 locations, respectively, some individual plant selections were made by the cooperators.

The results of Lentil International Cold Tolerance Nursery were received from 6 locations. None of the lines at Sanadaz in Iran and Hymana in Turkey; one line ILL1878 at Toshevo in Bulgaria; all the lines except ILL1878 at Elvas in Portugal; and three lines namely ILL 323, ILL 780 and ILL 7155 at Diyarbakir in Turkey exhibited tolerance reaction (rating ≤4). At Tolentino in Italy all the entries exhibited a rating of 1.

The results of Lentil International Ascochyta Blight Nursery were received from Akaki and Ghinchi in Ethiopia. All the entries including the susceptible check rated ≤ 5 at Ghinchi. Al Akaki, however, three entries namely Lenka, 78S 26013, and FLIP 93-13L showed resistant reaction (rating ≤ 3).

The results of Lentil International Rust Nursery were reported from Ishurdi in Bangladesh, Chilan in Chile and Kanpur in India. At Chillan all the entries including susceptible check showed tolerant reaction. All the entries except ILL 358 at Ishurdi and ILL 857, FLIP 87-19L, FLIP 87-60L and local check at Kanpur were tolerant. The tolerant lines across two locations included, Lenka, FLIP 84-112L, 81S15, FLIP 86-16L, FLIP 86-38L, FLIP 87-17L, FLIP 87-74L, FLIP 88-32L, FLIP 92-52L, FLIP 93-2L and FLIP 93-3L.

The results of Lentil International Fusarium Wilt Nursery were reported from 6 locations. At Aleppo and Hama in Syria, there was no disease infestation. At Lumle in Nepal all the entries were rated at 9. At Toshevo in Bulgaria only one entry Naslada, and at Izra'a in Syria all the entries including susceptible check showed tolerant reaction.

7.2. Chickpea

The results of Chickpea International Yield Trial-Winter-Mediterranean Region (CIYT-W-MR) were reported from 33 locations in 11 countries. A number of test entries exceeded the respective local check by a significant margin ($P \leq 0.05$) at six locations, namely Khroub and Oued Smar in Algeria; Hashamabad in Iraq; Caltagirone and Papiano in Italy; Mushagar in Jordan; Terbol in Lebanon; El Safsaf and Seba in Libya; Elvas in Portugal; Gelline, Hama, Idleb, Izra, Jindiress and Tel Hadya in Syria; Adana I, Adana II, Diyarbakir and Menemen in Turkey. The five best entries across locations were FLIP 90-96C, FLIP 88-85C, FLIP 89-29C, FLIP 90-132, and FLIP 90-13C. The stability analysis (Table 7.2.1) revealed that only entry x location (non-linear) component was significant. The entries FLIP 90-109C and FLIP 86-6C, with above average seed yield, regression equal to 1.0, and deviations approaching to zero, exhibited average stability.

For Chickpea International Yield Trial-Spring (CIYT-SP) data were reported from 29 locations in 13 countries. At 10 locations namely Thessaloniki in Greece, Mashad in Jordan; Papiano and Tolentino in Italy; Jimmah in Oman; Heimo in Syria; Geni Roral in Tunisia; and Amasya, Erzurum and Menemen in Turkey, some of the entries exceeded the respective local

check by a significant margin ($P \leq 0.05$). The five best entries across locations included FLIP 90-64C, FLIP 89-67C, FLIP 90-37C, FLIP 90-79C and FLIP 89-127C. The ANOVA for stability for seed yield indicated that mean squares due to pooled deviations and entry x location (linear) were significant (Table 7.2.1). The entries FLIP 90-64C, FLIP 89-67C, FLIP 89-127C, FLIP 82-150C and FLIP 90-136C had regression coefficient equal to 1, deviations approaching to zero and seed yield more than the general mean, and were thus widely adaptable.

The results of Chickpea International Yield Trial Southerly Latitudes-1 (CIYT-SL1) were reported from 10 locations in 8 countries. At some locations namely, Sids in Egypt, Durgapura in India, Al Kharaj in Saudi Arabia and Tel Hadya in Syria some of the test entries exceeded the local check by a significant margin. The five best entries across locations included, FLIP 88-87C, FLIP 87-60C, FLIP 90-78C, FLIP 82-150C and FLIP 88-86C. The ANOVA for stability for seed yield revealed the significance of non-linear portion and non-significance of linear portion (Table 7.2.1.). FLIP 90-65C, with regression coefficient equal to unity, deviations approaching zero, and seed yield above the overall mean over locations, was stable across environments.

The results for Chickpea International Yield Trial Southerly Latitudes-2 (CIYT-SL2) were reported from 6 locations in 4 countries. None of the test entries exceeded the local check in seed yield by a significant margin at any of the locations. The five heaviest yielding entries across locations included FLIP 88-47C, FLIP 88-46C, FLIP 88-34C, FLIP 89-39C and FLIP 88-36C. The ANOVA for stability revealed the significance of both linear and non-linear portion of

G x E interaction variance (Table 7.2.1). The adaptable lines across environments included FLIP 88-34C, FLIP 88-30C, and FLIP 90-180C.

The results for Chickpea International Yield Trial Latin America (CIYT-LA) were reported from 5 locations in 4 countries. The ANOVA for seed yield revealed that only at Graneros in Chile 11 test entries exceeded the local check in seed yield by a significant margin ($P \leq 0.05$). The five heaviest yielders across locations included FLIP 90-15C, FLIP 88-6C, FLIP 87-90C, FLIP 85-5C and ILC 4184. The ANOVA for stability revealed the significance of non-linear portion of G x E interaction only. A large number of lines namely, FLIP 88-6C, FLIP 87-90C, ILC 4184, FLIP 86-110C, ILC 136, ILC 4178, ILC 99, ILC 613, and ILC 3356 were adaptable across environments.

The data on seed yield of Chickpea International Screening Nurseries -Winter (CISN-W), -Spring (CISN-SP), -Southerly Latitudes-1 (CISN-SL1), -Southerly Latitudes-2 (CISN-SL2) and -Latin America (CISN-LA) were reported from 22, 13, 4, 5, and 4 locations, respectively. Some of the test entries exceeded the local check by significant margins at 14, 2, 1, 2 and 1 locations, in CISN-W, CISN-SP, CISN-SL1, CISN-SL2 and CISN-LA, respectively. The five best entries across locations are given in Table 7.2.2.

Chickpea International F_4 Nurseries for Mediterranean (CIF_4N-MR) and for Southerly Latitudes (CIF_4N-SL) were supplied to cooperators at 28 and 15 locations, respectively, and only 7 and 3 locations made the plant selections for use in their own breeding programs.

Table 7.2.1. ANOVA for stability analysis of seed yield for the entries in Chickpea International Yield Trials-Spring (CIYT-SP), Winter (CIYT-MR), Southerly Latitudes-1 (CIYT-SL1), Southerly Latitudes-2 (CIYT-SL2), and Latin America (CIYT-IA) conducted during 1992/93.

Source of variation	CIYT-SP		CIYT-MR		CIYT-SL1		CIYT-SL2		CIYT-IA	
	df	MS(x10)^3	df	MS(x10)^3	df	MS(x10)^3	df	MS(x10)^3	df	MS(x10)^3
Entry	22	472.341**	22	293.678**	22	484.539**	22	167.003*	22	224.592
Entry x Location (Linear)	22	269.040**	22	163.649	22	119.887	22	94.799	22	85.994
Pooled deviation	575	138.697**	621	151.684**	161	216.197**	69	79.750**	46	145.440**
Pooled error	1188	69.974	1276	68.563	396	56.110	220	39.431	176	71.346

* Significant at $P \leq 0.05$.

Table 7.2.2. The five heaviest yielding lines across locations in different chickpea screening nurseries, 1992/93.

Rank	CISN-W	CISN-SP	CISN-SL1	CISN-SL2	CISN-IA
1	FLIP 90- 12C	FLIP 91- 34C	FLIP 91-203C	FLIP 91-102C	FLIP 90- 18C
2	FLIP 90- 8C	FLIP 90-173C	FLIP 90- 94C	FLIP 90-126C	FLIP 91- 85C
3	FLIP 90-182C	FLIP 90-172C	FLIP 91-163C	FLIP 91-129C	FLIP 89-121C
4	FLIP 91- 52C	FLIP 91- 31C	FLIP 91-178C	FLIP 82-150C	FLIP 91- 86C
5	FLIP 91-131C	FLIP 82-150C	FLIP 91- 26C	FLIP 91-127C	FLIP 90- 2C

The Chickpea International Ascochyta Blight Nursery results for kabuli type (CIABN-A) were reported from 11 locations and for desi+kabuli type (CIABN-B) from 9 locations. None of the entries (kabuli or desi) was tolerant to Ascochyta blight infestation across all locations. Considering the frequency of occurrence of an entry among the tolerant group (with rating $\leq$ 4 on 1-9 scale) in Kabuli types, entry FLIP 84-182C, showed tolerance at 17 out of 21 locations and appeared best, and was followed by ILC 200, and FLIP 84-92C (which occurred 16 times), and ILC 72, FLIP 85-84C, and FLIP 90-56C (which each occurred 15 times). Similarly, among desi lines ICC 4475, ICC 13269, ICC 13508, ICC 13555 and FLIP 87-505C were tolerant at 4 out of 10 locations. These entries thus exhibited relatively broad-based resistance to Ascochyta blight as compared to others in this nursery. The differential reaction of lines at various places further revealed the presence of variability in the pathogen.

The results of Chickpea International Fusarium Wilt Nursery (CIFWN) were received from 12 locations in 8 countries. At four locations namely, Dholi, New Delhi and Badnapur in India, and Parwanipur in Nepal all the entries exhibited 9 rating. All the entries at Oued Smar in Algeria and Graneros in Chile were tolerant with rating ≤ 3, 8 entries at Guelma in Algeria; 10 entries at Debre Zeit in Ethiopia; 28 entries at Behrampore in India; 2 entries at Oroumieh in Iran; one entry at Hudeiba in Sudan; and 22 entries at Gemmiza in Egypt took rating of 4 or less and were tolerant. Five lines namely FLIP 85-29C, FLIP 85-30C, UC27, Be Sel 81-48 and Be Sel 81-103 were tolerant at 6 out of 12 locations.

The results of Chickpea International Leaf miner Nursery (CILMN) were reported from four locations (Tarquinia in Italy, Terbol in Lebanon, and Bornova and Izmir in Turkey). The susceptible check took a score of 3 at Tarquinia in Italy, 5 at Terbol in Lebanon, and 9 at other two places (on 1-9 scale, 1=free, 9=highly susceptible). Out of 30 test entries, none of the test entries was tolerant to leaf miner at Bornova and Izmir in Turkey where the susceptible check took 9 rating.

For Chickpea International Cold Tolerance Nursery (CICTN) the cold tolerance score was reported from six locations (Durgapura in India, Tolentino in Itlay, Terbol in Lebanon, Elvas in Portugal, Tel Hadya and Breda in Syria). Only at three sites the susceptible check took rating of 9 on 1-9 scale (where 1=free, 9-killed). On the basis of reaction across locations four entries namely ILC 3857, ILC 5667, ILC 5947 and FLIP 85-4C showed tolerant reaction to cold at all the locations and were the best.

7.3. Forage Legumes

The lathyrus and vetch adaptation trials were supplied to cooperators for the third year in 1992/93. The International Lathyrus Adaptation Trial (ILAT) included 13 entries of *L. sativus* and 10 entries of *L. cicera*. The results for ILAT were received from 20 locations. The ANOVA for stability was done using *L. sativus* entries as one group and *L. cicera* as another group. The ANOVA for *L. sativus* entries (Table 7.3.1) revealed the importance of both linear and non-linear portion of GxE interaction in expression of seed yield in *L. sativus* however, ANOVA for *L. cicera* entries exhibited the significance of only entry x location

(linear) interaction. The L. sativus accessions with above average seed yield across locations included Acc No. 188 sel 38, Acc. No. 178 sel 29, Acc No. 199 sel 452, Acc No. 232 sel 471, Acc No. 206 sel 463, Acc No. 277 sel 476, Acc No. 200 sel 453, and Acc No. 205 Sel 459. Among these, the later five accessions exhibited non-significant deviations and exhibited average stability across a range of environments. Among *Lathyrus cicera* six accessions, namely Acc No. 142 sel 496, Acc No. 119 sel 489, Acc No. 136 sel 495, Acc No. 536 local selection, and Acc No. 121 sel 491 had above average yield, non-significant deviations from regression, and regression equal to unity. These five *L. cicera* accessions thus possessed general adaptation across locations.

The International Vetch Adaptation Trial (IVAT) included 17 entries of *Vicia sativa* and 6 entries of *Vicia narbonensis*. The results for IVAT were reported from 17 locations. The ANOVA for stability of yield performance were done separately for *V. sativa* and *V. narbonensis* (Table 7.3.1). The seed yields of *Vicia narbonensis* accessions were, in general, more as compared to *Vicia sativa* accessions. The five high yielding *V. narbonensis* entries across the locations included Acc No. 568 sel VN 2383, Acc No. 565 sel – 2380, Acc No. 577 sel -2391, Acc No. 574 sel -2388, and Acc No. 573 sel -2387. Among these, three accessions, Acc No. 565, -577 and -574 with above average yield and regression equal to one were adaptable to a wide range of environments, and the Acc No. 568 Sel 2583 was adaptable to favorable environments. Among *V. sativa*, 8 accessions gave above average performance and included, Acc Nos. 384 sel 2062, Acc No. 705 sel 2556, and Acc No. 4 sel 2057, Acc. No. 482 sel 2096, and Acc no. 709, Acc No. 7 sel 1135, Acc No. 2 sel 1134 and Acc No. 507 sel

2019. Among these Acc No. 4 sel 2057 was unadaptable; Acc No. 384 sel 2060, Acc No. 705 sel 2556, Acc No. 482 sel 2096, Acc No. 7 sel 1135 and Acc No. 1331 sel 1437 were specifically adaptable to favorable environments; and Acc No. 709, and Acc No. 2 sel 1134 and Acc No. 507 sel 2019 were adaptable across a wide range of environments.

7.4. Dry Pea

The results of Pea International Adaptation Trial (PIAT) were reported from 28 locations. At eighteen locations, namely, Toshevo in Bulgaria; Gisozi in Burundi; Athalassa in Cyprus; Holetta in Ethiopia; Makedonia in Greece; Caltagirone in Italy; Mushagar and Maru in Jordan; Cochabama in Bolivia; New Delhi and Kanpur in India; Terbol in Lebanon; Zahra in Libya; Kedainiai in Lithuania; Rawdat Harma in Qatar; Gassim in Saudi Arabia; Badajoz and Valladolid in Spain; and Tel Hadya in Syria; some of the test entries exceeded the local check in seed yield by a significant ($P \leq 0.05$) margin. The five heaviest yielding entries across locations included, MG 104325, Local Selection 1690, MG 102029, Collegian, and Early Dun. The ANOVA for seed yield for stability parameters based on 24 environments revealed that both linear and non-linear components of GxE interaction mean squares were significant (Table 7.3.1). Three entries namely MG 104325, Local Selection 1690, and MG 102256 with above average yield, and deviation approaching zero, were relatively adaptable across a range of environments.

Table 7.3.1. ANOVA for stability parameters for seed yield for the entries in International Vetch Adaptation Trial (IVAT) - *Vicia narbonensis* (VN), and *Vicia sativa* (VS), International Lathyrus Adaptation Trial (ILAT) - *Lathyrus cicera* (LC) and *Lathyrus sativus* (LS) and Pea International Adaptation Trial (PIAT), during 1992/93.

| Source of variation | IVAT | | | | ILAT | | | | PIAT | |
| | VN | | VS | | LC | | LS | | | |
	df	$MS(x10)^3$	df	$MS(x10)^3$	df	$MS(x10)^3$	df	$MS(x10)^3$	df	$MS(x10)^3$
Entry	6	822.464**	15	761.492**	9	107.382ns	12	459.429**	22	1159.370**
Entry x Location (Linear)	6	398.886**	15	546.631**	9	114.726ns	12	468.978**	22	1103.297**
Pooled deviation	91	99.862**	208	160.860**	150	72.377ns	195	154.400**	506	276.490**
Poled error	180	102.254	450	119.019	306	65.657	408	82.942	1056	117.448

*, ** Signifiance at $P \leq 0.05$, and at $P \leq 0.01$ respectively.

7.5. Identification of Superior Genotypes by the NARS

The national program scientists participating in the
Legume International Testing Program identified
several lines for multilocation testing, on-farm
trials and pre-release multiplication and release for
general cultivation. The releases for general
cultivation are listed in Table 7.5.1. Also a large
number of lines resistant to various stresses were
identified and they are being used for direct or
indirect exploitation. **National Program Scientists,
R.S. Malhotra, K.B. Singh, W. Erskine, A.E. Moneim,
M.C. Saxena and S. Weigand**

Table 7.5.1. Legume cultivars reported in 1994 as released by the
national programs.

Country	Cultivars released	Year of release	Specific features
Chickpea			
Ethiopia	DZ 10-16-2	1994	For mid-altitude areas, high yield, tolerance to wilt/rust
Turkey	Aziziya (FLIP 84-15C)	1994	For cultivation in Erzurum region
Lentil			
Ethiopia	NEL 2704	1994	For lowland moisture-stressed conditions; early maturity, high yield, tolerance to rust
Sudan	Aribo 1 (ILL 818)	1993	High yield, for Jebel Mara area
Peas			
Cyprus	Kontemenos (PS 210713)	1994	High yield
Ethiopia	061K-2P-2192	1994	For high altitude areas, high yield, tolerance to powdery mildew and ascochyta blight.

8. LEGUME VIRUSES

Work on legume viruses during the growing season 1993/94
included (i) survey for faba bean viruses in Egypt,
Morocco and Sudan, and (ii) evaluation of lentil genotypes
for faba bean necrotic yellows virus (FBNYV) and lentil
luteovirus resistance. Activities also included regular
testing for seed-borne viruses in seeds dispatched for
international nurseries and testing gene bank accessions
to free them from seed-borne infections. The virology lab
continued to provide ELISA kits for virus testing to a
number of NARSs laboratories upon request.

8.1. Survey for Faba Bean Viruses in Sudan

Virology Laboratory personnel, in collaboration with
Sudanese colleagues, conducted a survey on legume viruses
during the period 1-9 February, 1994. A total of 33 faba
bean fields were selected to represent three different
production areas (i) Khartoum (ii) Gezira and Rahad (new
introduction areas) and (iii) the northern region
(traditional production area).

Virus symptoms observed were classified for
convenience into two categories (i) mosaic/mottle, and
(ii) leaf yellowing/rolling/stunting/necrosis. The number
of samples collected of each category depended on its
frequency in the field. A total of 339 samples showing
mosaic/mottle and 426 samples with leaf
roll/yellowing/necrosis/stunting were collected.

Field observation indicated that 50% of the fields
surveyed had, at the time of the survey, a virus disease
incidence of 5% or less, and the majority of these fields
were in the non-traditional faba bean production areas.
In the faba bean traditional areas, virus diseases more

commonly encountered were those that induce mosaic or mottle symptoms. Around 20% of the fields surveyed had a virus disease incidence of 6-20%, 18% of the fields had an incidence of 21-50% and 12% of the fields had an incidence of 51-100%.

A total of 765 faba bean diseased samples were collected and tested by the tissue-blot immunoassay immediately after collecting them for the presence of any of the following nine viruses: faba bean necrotic yellows virus (FBNYV), bean leaf roll luteovirus (BLRV), bean yellow mosaic potyvirus (BYMV), broad bean wilt fabavirus (BBWV), broad bean mottle bromovirus (BBMV), alfalfa mosaic alfamovirus (AMV), broad bean stain comovirus (BBSV), pea seed-borne mosaic potyvirus (PSbMV) and broad bean true mosaic comovirus (BBTMV). Laboratory tests showed that out of the 339 samples showing mosaic/mottle symptoms, 296 were infected with BYMV, 67 with BBMV and one samples each with AMV and PSbMV. However, when the 426 samples showing yellowing/rolling/stunting were tested for the presence of BLRV and FBNYV none of them reacted positively with FBNYV and only two reacted with BLRV antiserum. Selected faba bean (15) and chickpea (15) samples, which reacted negatively with BLRV and FBNYV, were tested against chickpea chlorotic dwarf geminivirus (CCDV) antiserum by DAS-ELISA, all chickpea and 6 faba bean samples reacted positively with CCDV antiserum. This study suggest that the yellowing of chickpea and faba bean in the Sudan is caused by CCDV which is transmitted by leafhoppers. The vector of CCDV in the Sudan, however, still need to be identified. **K.M. Makkouk and S.G. Kumari.**

8.2.   Survey for Faba Bean Viruses in Egypt

In response to the planning meeting of the Nile Valley
Regional Program, a survey for faba bean viruses in Egypt
was planned for February 1994.   This is a continuation of
the survey conducted in 1993.   In both years the main
objective of the survey was to monitor the viruses and the
extent of their spread in faba bean fields in the
different production areas in Egypt.

Virology Laboratory personnel, in collaboration with
Egyptian colleagues, conducted a survey during 14-22
February, 1994.   A total of 64 faba bean fields were
selected by a predetermined criteria and covered the Nile
Valley from Assiut to Mansoura including the Fayoum
Governorate.

Virus symptoms observed in the field were classified
for convenience into two categories (i) mosaic/mottle, and
(ii) leaf yellowing/ rolling/necrosis with or without
stunting.   The number of samples collected from each
category depended on its frequency in the field.   A total
of 408 samples showing mosaic/mottle and 758 samples with
leaf roll/yellowing/necrosis/stunting were collected.

Symptoms observed in the fields indicated that around
53% of the fields surveyed had, at the time of the survey,
a virus disease incidence level of 5% or less.   In 17% of
the fields virus disease incidence was in the range of 6-
20%.   Around 30% of the fields surveyed had a virus
disease incidence more than 20%. The field situation on a
regional basis showed that in Middle Egypt (Beni Suef,
Minya, Assiut) the estimate of virus disease incidence was
in the range of 10-20%, whereas it was 0.5-1% in the Delta
region.   In Fayoum, virus disease incidence was
surprisingly high (60-80%), and the dominant type of
symptoms observed was mosaic or mottle.

A total of 1166 samples with symptoms suggestive of virus infection were collected and tested by DAS-ELISA immediately after collecting them for the presence or absence of any of the following nine viruses: faba bean necrotic yellows virus (FBNYV), bean leaf roll luteovirus (BLRV), bean yellow mosaic potyvirus (BYMV), broad bean wilt fabavirus (BBWV), broad bean mottle bromovirus (BBMV), alfalfa mosaic alfamovirus (AMV), broad bean stain comovirus (BBSV), pea seed-borne mosaic potyvirus (PSbMV) and broad bean true mosaic comovirus (BBTMV). Results obtained from laboratory tests showed that only seven viruses were detected, namely FBNYV (62%), BYMV (31.2%), AMV (2.6%), BBWV (2.0%), BLRV (1.7%), PSbMV (1.1%) and BBMV (0.08%). These tests clearly showed that, for a second year in a row, FBNYV was the virus with the highest incidence in both Middle Egypt and Delta. It is also the most prevalent in fields surveyed regardless of its incidence in each field. In addition, symptoms observed in the field corresponded very well with the laboratory results obtained; out of the 758 plant samples collected from the fields with leaf roll/yellowing/stunting/necrosis symptoms 724 were found positive for FBNYV in laboratory tests. **K.M. Makkouk and S.G. Kumari**.

8.3. Survey for Faba Bean Viruses in Morocco

This survey was conducted during the period of 19-21 April, 1994. The main objective of the survey was to study the spectrum of viruses that are causing yellowing/stunting/necrosis and samples were collected accordingly. A total of 167 faba bean, 12 lentil and 13 chickpea samples were collected from 17 fields surveyed in the Meknes/Fes area, and tested by the tissue-blot immunoassay in Morocco immediately after collecting them for the presence of faba bean necrotic yellows virus (FBNYV) and luteovirus (e.g. bean leaf roll). Laboratory

tests showed that among 192 samples tested 179 were found infected with a luteovirus (158 faba bean, 11 chickpea and 10 lentil) and only one faba bean sample was infected with faba bean necrotic yellows virus. This is the first report of FBNYV in Morocco. It is interesting to note the difference between Egypt and Morocco. In Egypt, FBNYV is the virus most commonly associated with yellowing of faba bean and luteoviruses being rare, whereas in Morocco it is the other way around. **K.M. Makkouk.**

8.4. Screening for Faba Bean Necrotic Yellows Virus (FBNYV) Resistance in Lentil Genotypes

One hundred and sixteen lentil genotypes were evaluated for resistance to a local FBNYV isolate (SV292-88) using artificial inoculation by aphids. Genotypes tested were planted in the field in two replicates, composed each of two one meter rows, with 10 plants per meter in a randomized complete block design for both the inoculated and non-inoculated treatments. Disease incidence and yield loss was determined for all the genotypes tested and are presented in Tables 8.1.1 and 8.1.2. Based on these results it was possible to divide the genotypes tested into four categories: (1) Highly resistant genotypes: These genotypes did not show disease symptoms, no virus was detected in the leaves by ELISA, and no loss in grain yield was determined (e.g. ILL 6245, ILL 6198); (2) Resistant genotypes: Disease incidence in these genotypes did not exceed 10% and grain yield loss was between 0-10% (e.g. ILL 75, 291, 5816, 6193); (3) Tolerant genotypes: Disease incidence was high (80%) but the grain yield loss was about 10% (e.g. ILL 204, 212, 214, 324); and (4) Susceptible and sensitive genotypes: Disease incidence in these genotypes was high (reached 100%) and the grain yield loss was also high (reached 100%) (e.g. ILL 4803 and ILL 4774). This is a preliminary screening and results

need to be confirmed by second and a third year trials. Results obtained clearly indicated the presence of useful genetic diversity in *Lens culinaris* with respect to FBNYV resistance. **K.M. Makkouk and A. Muhanna**.

Table 8.1.1. Yield loss categories of lentil genotypes inoculated with faba bean necrotic yellows virus (FBNYV) in inoculated as compared to protected field plots.

Genotype	% Grain yield loss
ILL 75, 86, 204, 291, 292, 5816, 6198, 6245	0
ILL 212, 214, 324, 6193	0.1-5
ILL 203, 4400	5.1-10
ILL 74, 85, 478	10.1-20
ILL 112, 213, 259, 344	20.1-30
ILL 221, 271	30.1-40
ILL 64, 67, 70, 76, 84, 105, 107, 202, 227, 236, 623, 705.	40.1-50
ILL 71, 205, 211, 219, 222, 342, 757, Chilean 78	50.1-60
ILL 3, 65, 81, 97, 265, 341, 345, 346, 3485	60.1-70
ILL 2, 50, 96, 98, 226, 258, 260, 336, 347, 707, 921, 3433, 4401, 6229.	70.1-80
ILL 1, 24, 25, 31, 49, 54, 57, 68, 79, 104, 269, 339, 711, 716, 855, 920, 1646, 1647, 1855, 1856, 1857, 1922, 3484, 3500, 4408, 4409, 4410, 4411, 4412, 4550, 4605, 4667, 4735, 4736, 4737, 4774, 4803, 5582, 5699, 5700, 5873, 5588, 5994, 6015, 6220, 6226, Palouse, Crimson, Red chief.	80.1-100

Table 8.1.2. Incidence (%) of FBNYV infection based on visual symptoms after artificial virus inoculation.

Genotypes	FBNYV Infection (%)
ILL 6198, 6193, 6245	0
ILL 213, 291	1-5
ILL 75, 5816	5.1-10
ILL 86, 226, 227	10.1-20
ILL 85, 292	20.1-30
ILL 74, 204, 214, 344	30.1-40
ILL 70, 203, 478, 757, 4400	40.1-50
ILL 112, 84, 205, 4401	50.1-60
ILL 67, 65, 76, 81, 105, 107, 211, 219, 236, 324, 342, 623, 711, 706	60.1-70
Chilean 78, Red chief, ILL 3, 31, 54, 64, 71, 79, 96, 97, 104, 202, 222, 245, 271, 336, 341, 212, 346, 705, 707, 855, 921, 3433, 5700, 5582, 5994, 6220, 6229	70.1-80
ILL 1, 2, 24, 25, 49, 50, 57, 68, 98, 221, 258, 259, 265, 269, 339, 347, 260, 920, 1857, 1646, 1647, 1855, 1856, 1922, 3485, 3484, 3500, 4408, 4409, 4410, 4411, 4412, 4413, 4550, 4605, 4667, 4735, 4737, 4736, 4774, 4803, 5588, 5699, 5700, 5873, 6015, 6226, Palouse, Crimson	80.1-100

8.5. Screening for Lentil Luteovirus Resistance in Lentil Genotypes

Forty five genotypes were evaluated for resistance to a local isolate of lentil luteovirus using artificial inoculation by aphids. Genotypes tested were planted in the field in three replicates each composed of three one meter rows, with 15 plants per meter in a randomized complete block design for both the inoculated and non-inoculated treatments. Disease incidence and yield loss was determined for all the genotypes tested and are summarized in Table 8.1.3. **K.M. Makkouk and S.G. Kumari.**

Table 8.1.3. Variability in disease incidence and yield
loss among lentil genotypes in response to
infection with lentil luteovirus evaluated
during spring of 1993.

Lentil genotypes	Disease incidence (%)
ILL 75, 76, 204, 212, 213, 214, 222, 3433	0.0
Palouse, Crimson, ILL 71, 74, 85, 86, 259, 291, 344	0.1-5
ILL 81, 112, 757	5.1-10
ILL 2, 24, 202, 265, 271, 292, 6229	10.1-20
Chilean 78, ILL 3, 478, 4401	20.1-30
ILL 203, 211, 221, 346, 920, 6245	30.1-40
ILL 6193, 6198	40.1-60
Red Chief, ILL 105, 324, 707, 4400, 5816	60.1-100

	Yield loss (%)
Palouse, ILL 76, 85, 212, 214, 259	0.0
ILL 75, 222, 3433	0.1-5
ILL 71, 86	5.1-10
ILL 74, 204, 213, 265, 344, 757, 6229	10.1-20
Chilean 78, ILL 112, 291, 346, 4401	20.1-30
Crimson, ILL 24, 478, 920, 6198	30.1-40
ILL 2, 81, 202, 203, 221, 271, 292, 6245	40.1-50
ILL 3, 211, 4400, 5816	50.1-60
Red chief, ILL 105, 324, 707, 6193	60.1-100

8.6. Testing for Seed-borne Viruses

8.6.1. Testing Lentil Seed Lots for International Nurseries

A total of 457 lentil seed lots from the international nurseries were tested serologically for the presence of seed-borne virus (BYMV, BBSV or PSbMV) infection. Eighty two lentil lots were eliminated due to seed-borne infection. Rejection was based on detection of at least one seed infection in a 400 seeds sample per seed lot.

8.6.2. Cleaning Germplasm in the Gene Bank from Seed-borne Infection

A total of 1227 faba bean accessions increases for the gene bank were continuously inspected during the growing season 1993/94. Roguing was excersised two times during the growing season (April-May, 1994). Any plant with symptoms suggestive of virus infection was eliminated.

Two hundred and forty eight barley accessions stored in the Gene Bank were tested for the presence of barely stripe mosaic hordeivirus and 528 lentil accessions were tested for the presence of BBSV, BYMV or PSbMV infections. Thirty eight barley and 279 lentil accessions were found to contain a seed-borne infections. Accessions identified with seed-borne viruses will be cleaned up when multiplied in the field in the future. **K.M. Makkouk and N. Attar.**

8.7. Production of ELISA Kits

During 1994, antiserum to wheat streak mosaic potyvirus (WSMV) was produced, ELISA kits were prepared and made available for laboratories of the national programs in

WANA. In each kit there is enough immunoglobulins and enzyme conjugate to test 2000 samples. Accordingly, ELISA kits for the following viruses are now available:

Legume viruses
- Alfalfa mosaic alfamovirus
- Bean yellow mosaic potyvirus
- Broad bean mottle bromovirus
- Broad bean stain comovirus
- Broad ben wilt fabavirus
- Chickpea luteovirus
- Cucumber mosaic cucumovirus
- Pea seed-borne mosaic potyvirus

Cereal viruses
- Barley yellow dwarf luteovirus
- Barley stripe mosaic hordeivirus
- Wheat streak mosaic potyvirus
- Wheat soil-borne mosaic virus from Turkey
  (virus not fully characterized yet). **K.M. Makkouk and S.G. Kumari.**

9. TRAINING

The training activities were continued to strengthen the technical capabilities of researchers involved in the germplasm improvement activities in the national programs. Table 9.1.1 summarizes the activities undertaken during 1994. Much of these activities were done jointly with the cereals improvement scientists. A total of 90 participants received training in the improvement of lentil, kabuli chickpea and annual forage legumes at Aleppo (Table 9.1.1). In addition, several participated in training activities organized in the regional programs or with other ICARDA programs.

Table 9.1.1. Summary of training activities in legume improvement in 1994.

Type of training	Number of participants
1. Group courses at Aleppo	
1.1. Legume diseases and their control	
10 to 21 April 1994	9
1.2. Insect control in legumes and cereals	
18-28 April 1994	14
1.3. Breeding methodology in legumes	
2-12 May 1994	10
1.4. Diagnosis of plant viruses	
28 Aug to 8 Sept 1994	13
1.5. DNA molecular marker techniques	
19 to 29 Sept 1994	9
2. Group course in the region	
2.1. Mechanical harvest of legumes,	
in lebanon	10

Cont'd ...

Cont'd ...

2.2. Field research techniques, in Lebanon	15
3. Individual non-degree	16
4. Graduate students including those doing B.Sc. and diploma work	27

Details of some of the group courses are given in the 1994 annual report for cereals. The individual non-degree training, which ranged in duration from a few days to 4 months, was provided based on the specific needs of the national programs. The curriculum for this was tailored to meet the specific needs of the individuals.

The Program hosted for 2 days (23-24 November 1994) the participants of a regional training course on "mutation techniques for improvement of stress tolerance in basic food crops" organized by the International Atomic Energy Agency (IAEA) and the Syrian Atomic Energy Commission (AEC). There were 15 participants from 9 countries (Bulgaria, Hungary, Jordan, Lithuania, Poland, Romania, Syria, Turkey and UAE). Presentations were made by ICARDA scientists on breeding wheat, barley, chickpea, and lentil for stress environments, and on use of tissue culture and wide hybridization for cereal improvement. The participants visited the biotechnology lab facilities and observed demonstrations of some aspects of anther culture and the use of DNA-marker techniques.

The list of participants in graduate research training program, registered for M.Sc. or Ph.D. degree is given in Table 9.1.2. Eight candidates completed their M.Sc./Ph.D. degree in 1994.

Table 9.1.2. Participants in graduate research training in 1994.

Name	Degree	University	Quality
Registration in 1994			
1. Mr. Adel Omar A.L.	M.Sc.	AUB	Sudan
2. Mr. Abdel Majid Adlan	M.Sc.	AUB	Sudan
3. Mr. El-Tahir Ahmed A.A.	M.Sc.	Jordan	Sudan
4. Mr. Ken Street	Ph.D.	W. Australia	Australia
5. Ms. Huda Nassan	M.Sc.	Aleppo	Syria
6. Mr. Said Hassan	M.Sc.	Aleppo	Syria
7. Mr. George Ghandour	M.Sc.	Aleppo	Syria
8. Ms. Jouana Haidar	M.Sc.	AUB	Lebanon
9. Mr. Alexander Franz	Ph.D.	Brawnscheig	Germany
10. Mr. Mohamed Ibrahim Ismail	M.Sc.	Jordan	Sudan
Registration continuing from previous years			
1. Ms. Widad Shehadeh	M.Sc.	Damascus	Syria
2. Mr. Abbas Abbas	Ph.D.	Aleppo	Syria
3. Mr. Ahmed M. Manschadi	Ph.D.	Hohenheim	Austria/Iran
4. Mr. Hassan Ahmed Ali Tambal	M.Sc.	AUB	Sudan
5. Mr. Hassan Khalid Ali	M.Sc.	AUB	Sudan
6. Mr. Mohamed A.M. Adlan	M.Sc.	AUB	Sudan
7. Mr. Ismail Kusmenoglu	Ph.D.	Selcuk, Konya	Turkey
8. Ms. Suhaila Arslan	M.Sc.	Aleppo	Syria
9. Ms. Sara Nour	Ph.D.	INRA, Leon	Sudan
10. Mr. Mohamed Labdi	Ph.D.	INRA	Algeria
11. Mr. Bruno Ocampo	Ph.D.	Cordoba	Italy
12. Mr. Imad Mahmoud	Ph.D.	Helsinki	Sudan
13. Ms. Bianca van Dorrestein	M.Sc.	Wageningen	Holland
14. Ms. Kholoud Sultan	M.Sc.	Aleppo	Syria
15. Mr. Mohamed Lammouni	D.E.S.	Marrakesh	Morocco
16. Mr. Ali Chettou	M.Sc.	ENA, Meknes	Morocco
17. Mr. Mohamed Noor Al-Hamad	M.Sc.	Jordan	Jordan

Research completed and degree awarded in 1994

1.	Ms. Aziza Dibo Ajouri	Ph.D.	Aleppo	Syria
2.	Mr. Hossam El Sayed Ibrahim	Ph.D.	Alexandria	Egypt
3.	Ms. Marja van Hezewijk	Ph.D.	Amsterdam	Holland
4.	Ms. Safaa M. Ghassan Kumari	M.Sc.	Tishreen	Syria
5.	Ms. Susanne Pecher	M.Sc.	Hohenheim	Germany
6.	Mr. Elias El-Hajj Mousa	M.Sc.	CIHEAM	Lebanon
7.	Mr. Habib Halila	Ph.D.	London	Tunisia
8.	Mr. Ahmed Mohanna	M.Sc.	Tishreen	Syria

GP hosted a large number of visitors for varying periods of time during 1994. Scientists from Germany, Italy, the Netherlands and ICRISAT visited the Program to discuss ongoing and proposed joint projects for research on legume crops. Other scientists from Australia, Bangladesh, Egypt, FAO, Germany, Lebanon, Pakistan, Sudan, Syria, Tunisia and UK came to get information about GP's work or to discuss joint research or training in legume improvement.

Students from Syrian Universities came with some teachers for a one-day visit as part of a tour of ICARDA's facilities and on-going research and training activities.

GP scientists also visited NARS in WANA and other countries to discuss collaborative work and evaluate legume germplasm grown in these countries. **S. Weigand, H. Ketata and other legume scientists.**

10. PUBLICATIONS

10.1. Journal Articles

Aletor, V.A., Abd El Moneim, A.M. and Goodchild, T. Evaluation of the seeds of selected lines of three *Lathyrus* spp for B-N-Oxalylamino-L-alanin (BOAA), tannins, tribsin inhibitor activity and certain in-vitro characteristics. J. Sci. Food Agric. 1994. 65, 143-151.

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van Hezewijk, M. 1994. Germination ecology of *Orobanche crenata* - implications for cultural control measures. Doctoral Thesis Vrije Univeriteit, Amsterdam, Holland.
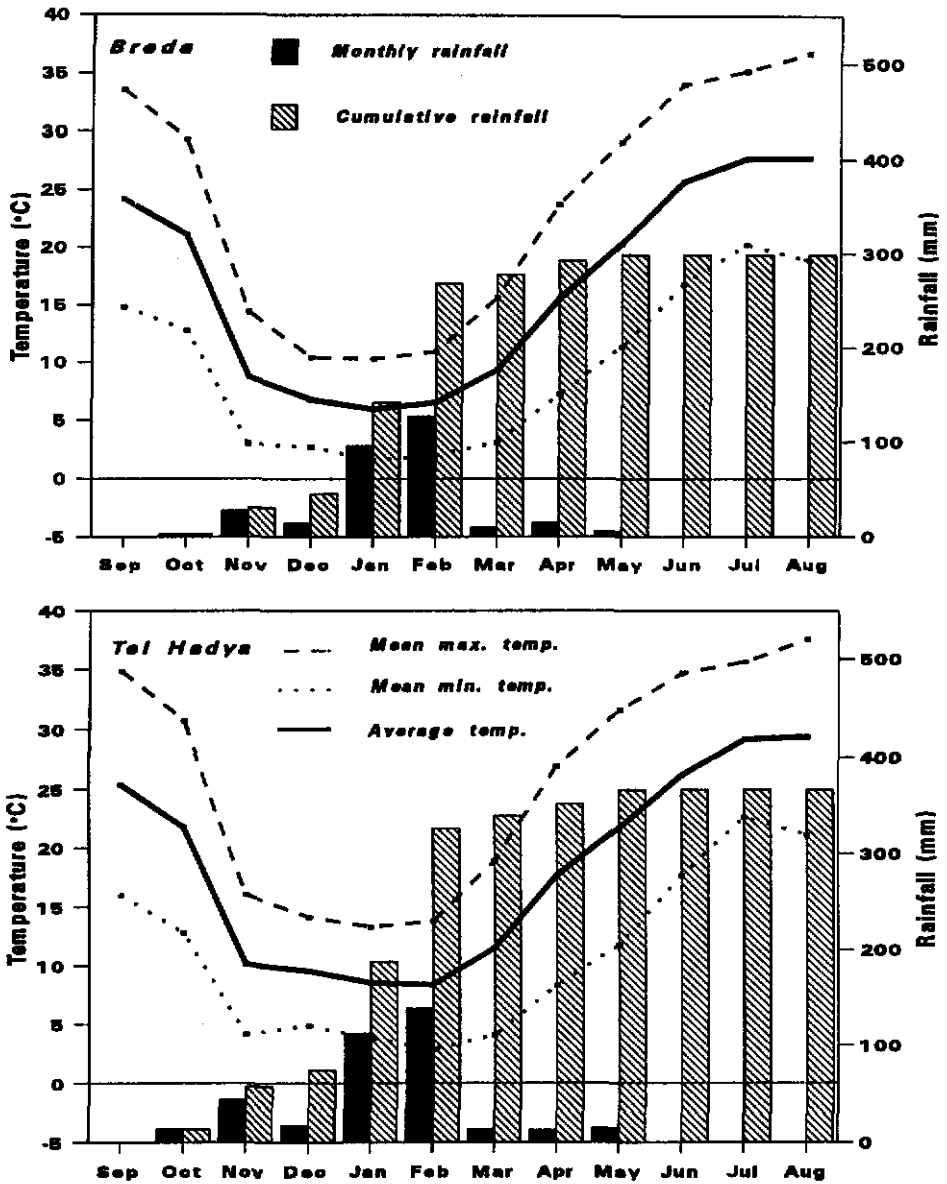
11. WEATHER DATA 1993/94

Figure 11.1. Mean monthly average, maximum and minimum temperatures, and monthly total and cumulative rainfall at Breda and Tel Hadya during 1993/94.
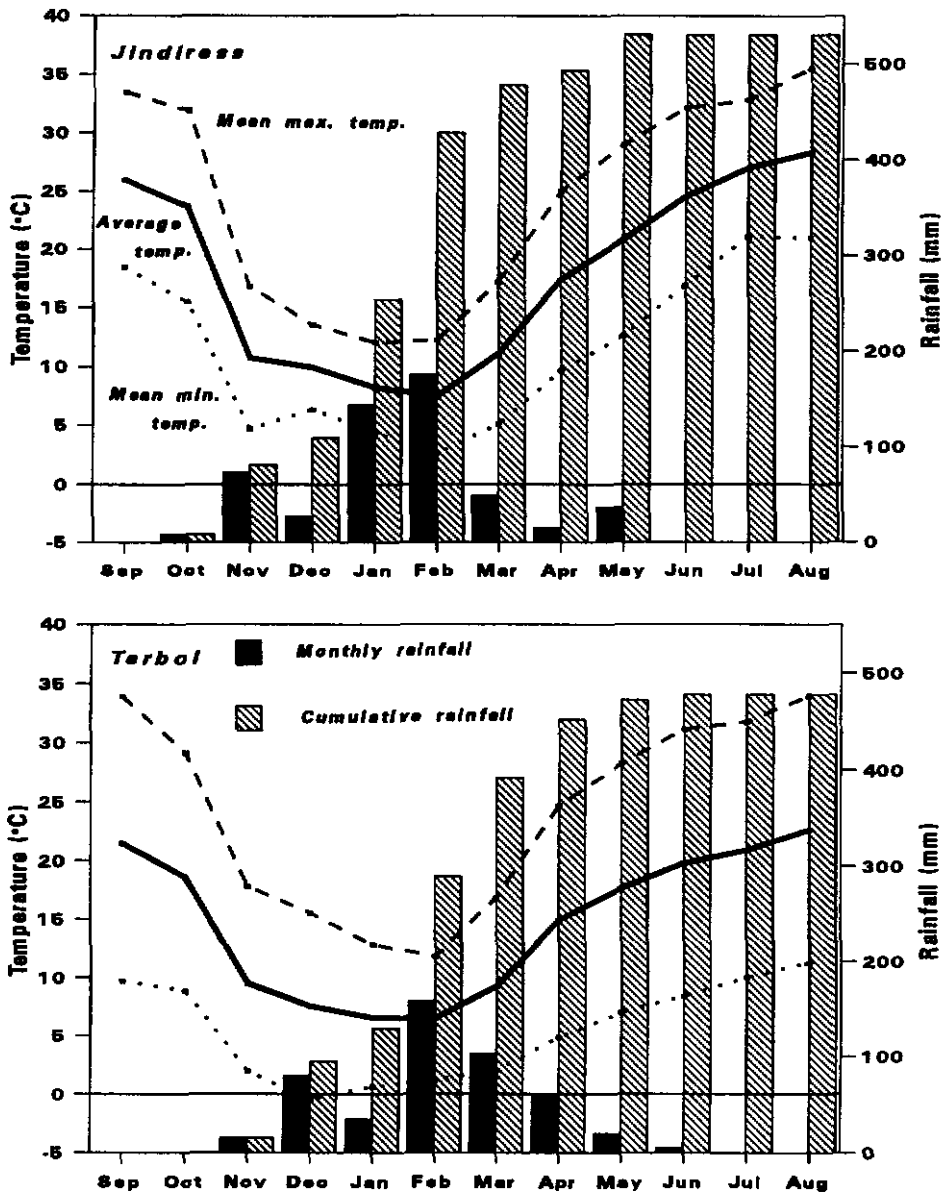
Figure 11.2. Mean monthly average, maximum and minimum temperatures, and monthly total and cumulative rainfall at Jindiress and Terbol during 1993/94.
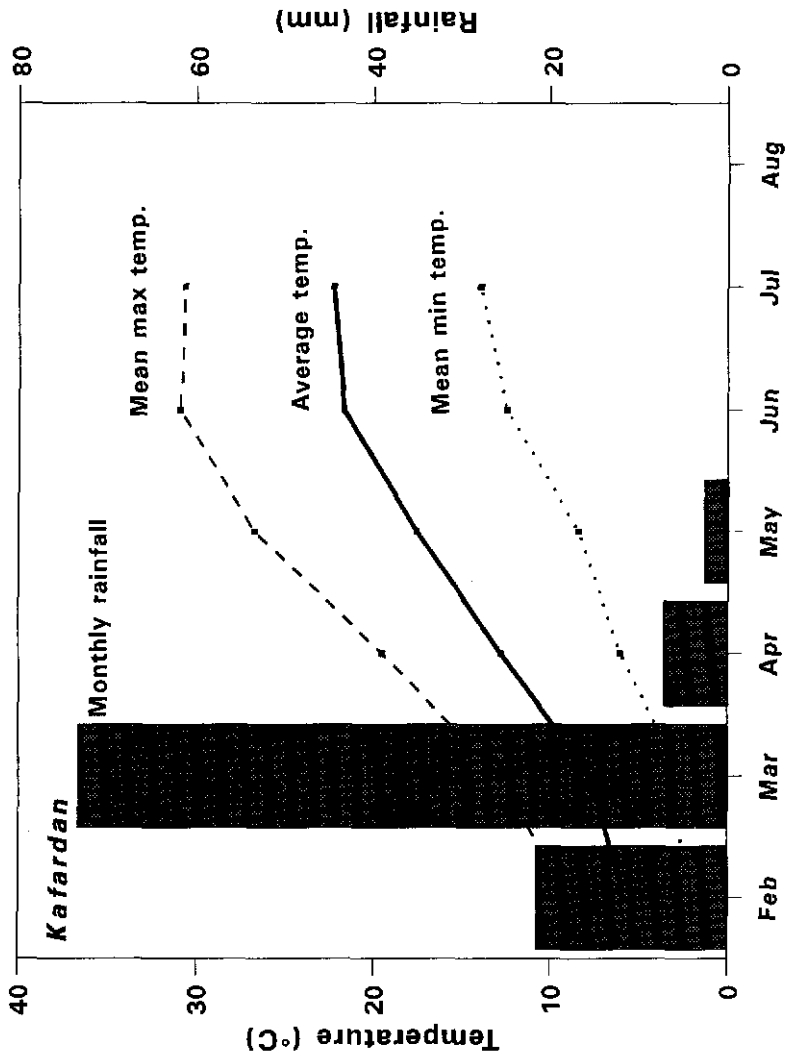
Figure 11.3. Mean monthly average, maximum and minimum temperatures and monthly total rainfall at Kfardan research station from February to July 1994.

12. STAFF LIST - LEGUME GROUP

M.C. Saxena	Germplasm Program Leader
Ali Abd El Moneim	Forage Legume Breeder
M. Baum	Molecular Biologist
W. Erskine	Lentil Breeder
H. Ketata	Training Scientist
K. Makkouk	Virologist
M.T. Mmbaga*	Chickpea Pathologist
K.B. Singh	Chickpea Breeder (ICRISAT)
F. Weigand	Molecular Biologist
S. Weigand	Entomologist
R.S. Malhotra	International Trials Scientist
G.A. Alloush*	Consultant Plant Nutritionist (Tishreen University)
Bassam Bayaa	Consultant Pathologist (Aleppo University
Mamdouh Omar*	Visiting Scientist, Chickpea Breeding
N.P. Saxena*	Visiting Scientist, Crop Physiology (ICRISAT)
Sripada Udupa	Post Doc. Fellow, Molecular Biology
Fadel Afandi	Research Associate
Bruno Ocampo	Research Associate
Suheila Arslan	Research Assistant
Mustafa Bellar	Research Assistant
Joana Haidar*	Training Assistant
Samir Hajjar	Research Assistant
Hassan El-Hassan	Research Assistant
Abdulla Joubi	Research Assistant
Gaby Khalaf	Research Assistant

Siham Kabbabeh	Research Assistant
Hani Nakkoul	Research Assistant
Nabil Trabulsi	Research Assistant
George Zakko	Research Assistant
Imad Mahmoud	Research Assistant
Riad Ammaneh	Senior Research Technician
Pierre Kiwan	Senior Research Technician (Terbol)
S. Kumari	Senior research Technician
Moaiad Lababidi	Senior Research Technician
Raafat Azzo	Research Technician
Bunian A. Karim	Research Technician
Khaled El-Dibl	Research Technician
M.K. Issa	Research Technician
M.I. El-Jasem	Research Technician
Nidal Kadah	Research Technician
Omar Labban	Research Technician
Diab Ali Raya	Research Technician
Seta Unji	Research Technician
Seta Unji	Research Technician
Joseph Karaki	Research Technician (Terbol)
Ghazi Khatib	Research Technician (Terbol)
Aida Naimeh	Research Technician (Terbol)
Mary Bogharian	Senior Secretary
Hasna Boustani	Secretary
Nuha Sadek*	Secretary
Namman Ajanji	Driver-cum-Store Assistant

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* Left during the year 1994