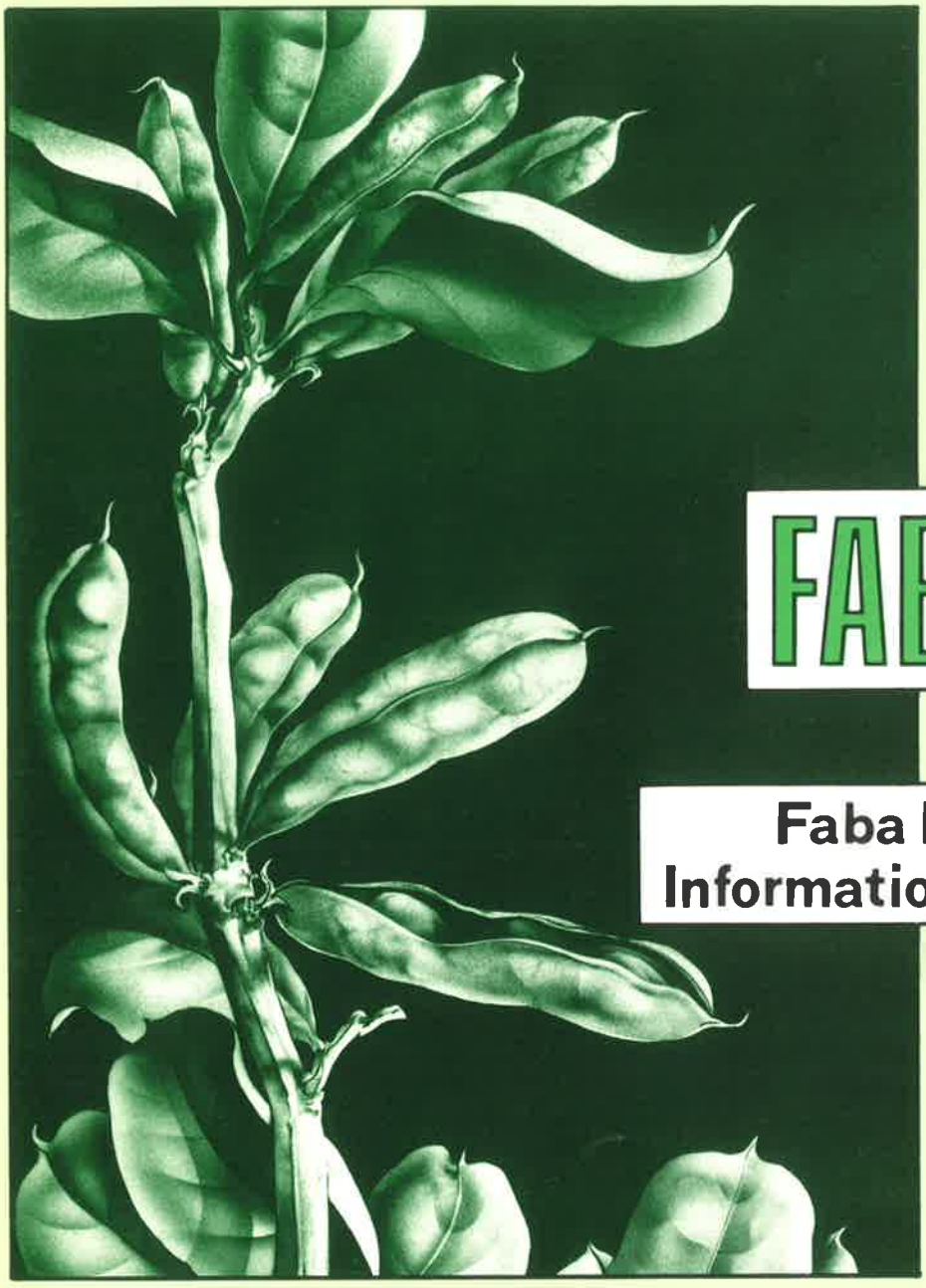

FABIS

Faba Bean Information Service

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INTERNATIONAL CENTER FOR AGRICULTURAL RESEARCH IN THE DRY AREAS

(ICARDA)

ICARDA and CGIAR

Established in 1977, the International Center for Agricultural Research in the Dry Areas (ICARDA) is governed by an independent Board of Trustees. Based at Aleppo, Syria, it is one of 16 centers supported by the Consultative Group on International Agricultural Research (CGIAR), which is an international group of representatives of donor agencies, eminent agricultural scientists, and institutional administrators from developed and developing countries who guide and support its work.

The mission of the CGIAR is to contribute, through its research, to promoting sustainable agriculture for food security in developing countries. The CGIAR conducts strategic and applied research, with its products being international public goods, and focuses its research agenda on problem solving through interdisciplinary programs implemented by one or more of its international centers, in collaboration with a full range of partners. Such programs concentrate on increasing productivity, protecting the environment, saving biodiversity, improving policies, and contributing to strengthening agricultural research in developing countries.

In the context of the challenges posed by the physical, social and economic environments of the dry areas, ICARDA's mission is to improve, through research and training, the welfare of people in the dry areas of the developing world by increasing the production and nutritional quality of food while preserving and enhancing the resource base.

ICARDA serves the entire developing world for the improvement of lentil, barley and faba bean; all dry-area developing countries for on-farm water management, small ruminants and rangelands; and the West Asia and North Africa region for production enhancement of bread and durum wheats, chickpea, and farming systems. ICARDA's research provides global benefits of poverty alleviation through productivity improvements integrated with sustainable natural resource management practices.

FABIS

FABIS Newsletter is produced once a year by ICARDA for the Faba Bean Information Service. It is a forum for communicating research results on faba bean and other Viceae legumes in the genera *Vicia* and *Lathyrus*. Short research articles provide rapid information exchange, and comprehensive reviews are invited regularly on specific areas. The newsletter occasionally publishes reviews of relevant books.

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Review Article

Grasspea (*Lathyrus sativus* L.): The Hardy Pulse Crop

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ETHIOPIA

Grasspea (*Lathyrus sativus* L.) is an important legume in India, Bangladesh, Pakistan, Nepal, China, and to a similar extent in the Middle East, Southern Europe, and parts of Africa and South America (Hang 1989).

In Ethiopia, grasspea (known locally as *guaya)* is usually grown in the vertisols and cambisols of the central highlands at altitudes ranging from 1700 to 2800 m. More than 60% of the production area is concentrated in the northwestern regions of the country (Gojam and Gondar). It accounts for more than 8.3% the country's legume area and 7.6% of production of all pulses grown (Central Statistics Authority 1989). Its relative tolerance to water-logging (in case of flooding), its ability to grow in residual moisture at the end of the rains, its soil-improving capacity through fixation of atmospheric nitrogen, and the low cost involved in its production process make it an important poor person's insurance crop. Double-cropping of grasspea with teff (*Eragrostis tef* (Zucc.) Trotter) or barley (*Hordeum vulgare* L. subsp. *vulgare*) is a common practice in the Fogera plain of south Gondar. Grasspea is also grown intercropped with sorghum (*Sorghum* spp.) or maize (*Zea mays* L.) in the Maksegnit, Dembia and Koladuba areas of north Gondar.

Grasspea, which contains 28% protein, 0.6% fat, 10% moisture, 50% nitrogen-free extract (carbohydrate) and 3% ash (Hang 1989), is used for food, and is usually consumed in the form of sauce (*shiro wet*), roasted grain (*kolo*), boiled grain (*nifro*) and sometimes pancake (*kita*), while the straw is used for livestock feed. Despite all these merits as a crop, lathyrism—a non-reversible neuro-degenerative disease characterized by paralysis of the lower limbs presumably due to a localized lesion in the pyramidal tract (Abera 1989)—is a demerit and discourages its production. The disease, caused by the neurotoxin β-N-oxalyl-L-α,β-diaminopropionic acid (ODAP) present in the seeds, is common in Fogera and Dembia districts of Gondar and Adet area of Gojam, where the crop is produced in large amounts. The problem is usually severe in times of drought when grasspea is the dominant food crop available because of its hardy nature (the production of other food crops is impossible during drought).

Research on this crop with the objective of identifying zero-level or low toxin content grasspea genotypes commenced in 1987/88 at Adet Research Center, as part of the International Network for the Improvement of *Lathyrus sativus* and Eradication of Lathyrism (INILSEL). About 127 grasspea genotypes obtained from the Plant Genetic Resources Center and 8 genotypes obtained from Canada were tested at Adet for their agronomic characters and ODAP content. Two promising genotypes—Acc. No. 46057 with a grain yield of 2.729 t/ha and ODAP content of 0.25% and Acc. No. 201513 with a grain yield of 2.632 t/ha and ODAP content of 0.27%—were identified. We intend to release these as cultivars as an interim measure until more low-toxin lines are screened.

References

Abera Bekele. 1989. Nutritional situation in Ethiopia. Potential for *Lathyrus sativus*. Pages 191–197 *in* The Grasspea Threat and Promise. Proceedings of the International Network for the Improvement of *Lathyrus sativus* and Eradication of Lathyrism. Third World Medical Research Foundation, New York.

Central Statistics Authority. 1989. Agricultural sample survey on area, production and yield of major crops by sector and season. Bulletin no. 74. Addis Ababa, Ethiopia.

Hang, Y.D. 1989. Post production methods of vitamin supplementation of *Lathyrus sativus.* Pages 205–210 *in* The Grasspea Threat and Promise. Proceedings of the International Network for the Improvement of *Lathyrus sativus* and Eradication of Lathyrism. Third World Medical Research Foundation, New York.

Research Articles

Breeding and Genetics

Effectiveness of Two Grid Systems of Mass Selection in Faba Bean

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Abstract

The effectiveness of two grid systems of mass selection (moving hexagonal grid of honeycomb selection under low density versus the fixed grid under high density) was evaluated in faba bean. Faba bean cv Polycarpe was crossed to nine pollinators of different origin. The F_1 hybrids and the parent cv Polycarpe were evaluated in a honeycomb design without competition. The six higher-yielding F_1s were further evaluated in the F_2 generation under the same conditions. The F_2 population was selected by the honeycomb mass selection and the seeds of each of the seven selected plants (six originating from the highest-yielding F_2 population and one from the second highest yielder) were divided and half was further selected using honeycomb mass selection without competition and the other half using grid mass selection with competition. After four cycles of selection, four mixtures were made (H1, H2, G1, G2). These mixtures, together with two controls (the parental Polycarpe and cv Effe) were evaluated in a randomized complete block design in two locations for two years. The four selected populations significantly outyielded the common parent Polycarpe in both locations in the year with high average yields and in one location in the year with low average yields. Under solid stand, the annual yield response to honeycomb mass selection was 5.6% (H1 + H2) as compared with 6.1% (G1 + G2) in the fixed grid system. This difference was not significant. It is concluded that the honeycomb mass selection under low density and the fixed grid mass selection under high density were equally effective under the conditions of this experiment.

Key words: *Vicia faba*, faba beans; cross pollination; backcrossing; crossbreds; hybrids; yields; selection; honeycombs.

فعالية نظاميّ شبكات للانتخاب الإجمالي في الفول

الملخص

تم تقييم فعالية نظاميّ شبكات للانتخاب الإجمالي (شبكة متنقلة سُداسية للانتخاب النُخروبي تحت كثافة متدنية إزاء شبكة ثابتة تحت كثافة عالية) في الفول. فقد تم تهجين صنف الفول بوليكارب ب9 مُلقحات من أصول مختلفة. قُيمت هجن الجيل الأول F1 والصنف الأبوي بوليكارب في تصميم نُخروبي بدون منافسة. كما جرى تقييم آخر في الجيل الثاني لست عشائر من الجيل الأول ذي الغلة الأعلى تحت نفس الظروف، ثم انتُخبت عشيرة الجيل الثاني بواسطة الانتخاب الإجمالي النُخروبي وقُسِمت البذور المأخوذة من كل من النباتات المُنتخبة السبعة (ستة منها ناتج من عشيرة الجيل الثاني الأعلى غلة وواحدة من ثاني أعلى غلة)، ثم أُجري انتخاب آخر على نصفها باستخدام الانتخاب الإجمالي النُخروبي بدون منافسة، وعلى نصفها الآخر باستخدام شبكة انتخاب إجمالي مع وجود منافسة. وبعد أربع دورات من الانتخاب، تم إعداد أربع خلطات (H1, H2, G1, G2) . قُيمت هذه الخلطات مع شاهدين (بوليكارب الأبوي والصنف Effe) في تصميم للقطع العشوائية الكاملة في موقعين لمدة سنتين. وقد تفوقت بشكل كبير غلة العشائر المنتخبة الأربع على الأب المشترك بوليكارب في كلا الموقعين في السنة التي أعطت متوسط غلة عالي، وفي موقع واحد في السنة التي أعطت متوسط غلة متدني. وبلغت استجابة الغلة السنوية للانتخاب الإجمالي النُخروبي، تحت كثافة نباتية شديدة، 5.6% (H1+H2) بالمقارنة مع 6.1% (G1+G2) في نظام الشبكة الثابتة. وهذا الفرق ليس كبيراً. وقد استُخلص أن الانتخاب الإجمالي النُخروبي تحت كثافة متدنية والانتخاب الإجمالي في شبكة ثابتة تحت كثافة عالية، متعادلان في الفعالية تحت ظروف هذه التجربة.

Introduction

Faba bean (*Vicia faba* L.) is a partly self-pollinating species. Average cross-fertilization has been estimated at 30% (Bond 1987), but occasionally exceeds 50% (El-Sherbeeny

1970). Thus, faba bean has an intermediate system of reproduction, and may, therefore, be handled either as a self-pollinating or as a cross-pollinating species.

Mass selection, when applied to faba beans either within landrace populations or in segregating populations, has given little improvement in yield (Hawtin 1982; Bond 1971). However, Gardner (1961) proposes the grid system of mass selection in maize (*Zea mays* L.) and reports an annual gain of 3.9% over four generations. By the 13th generation, a total gain of 38% (or 2.9% per selection cycle) was attained in his material (Gardner 1969). Successful use of the method is also reported in maize by Johnson (1963) and in cotton by Verhalen et al. (1975).

Fasoulas (1973) identified interplant competition and soil heterogeneity as factors which render single-plant selection for yield ineffective with most selection methods. Thus, he developed the so-called honeycomb mass selection at low plant density, based on moving hexagonal grids, the size of which is varied according to the selection pressure (Fasoulas 1988). Bos (1981) studied the relative efficiency of honeycomb mass selection compared with other procedures of mass selection on winter rye (*Secale cereale* L.) and reported that three generations of honeycomb selection reduced culm length by 6.1% and increased kernel yield by 4.3%. Mitchell et al. (1982) obtained significant responses to selection for yield in tetraploid wheat (*Triticum turgidum* L. subsp. *durum* (Desf.) Husn.) when they applied the honeycomb mass selection method. Kyriakou and Fasoulas (1985) report an annual yield response of 8.9% in a rye population.

The present study was undertaken to evaluate the effectiveness of honeycomb mass selection at low plant density (Fasoulas 1973, 1988) compared with the fixed-grid mass selection at relatively high plant density (Gardner 1961) in faba bean.

Material and Methods

The genetic material used for the evaluation of the two mass-selection procedures was developed as follows. Nine F_1 hybrids were developed by crossing Polycarpe (a small-seeded cultivar well adapted in Greece) with nine genetically diverse pollinators of different origin, named A-158, A-159, A-160, A-162, A-163, A-165, A-166, A-167 and A-168. The nine F_1s and the common parent Polycarpe (Pol) were evaluated in a honeycomb design without competition (distance between plants 0.9 m). An equal number of seeds from the two plants with the highest yield from each of the six higher-yielding F_1s (Table 1) was seeded the following year in an R-7 honeycomb design (Fasoulas and Fasoula 1995) with an inter-plant distance of 0.9 m. This population was selected by the honeycomb mass selection (Fasoulas 1981) and seeds of the seven highest-yielding F_2-selected plants were the material used for the evaluation of the two methods. Six of these plants originated from the highest-yielding F_2 population (Pol × A-168) and one from the second in the rank (Pol × A-159) (Table 1). The seeds of each selected plant were divided and one half was advanced following the honeycomb mass-selection procedure (Fasoulas 1981), while a mixture containing an equal number of seeds from the other half was further selected using the grid mass-selection procedure (Gardner 1961).

Table 1. Number of plants surviving to maturity and mean grain yield per plant (% of the common parent cv Polycarpe) in nine F_1 hybrids and in six F_2 populations of faba bean.

Cross	F_1		F_2	
	No. plants collected	Yield (%)	No. plants collected	Yield (%)
Pol × A-166	16	183	112	92
Pol × A-160	12	167	112	98
Pol × A-163	15	143	112	75
Pol × A-167	16	141	112	91
Pol × A-159	11	136	112	103
Pol × A-168	9	121	112	123
Pol × A-162	15	117		
Pol × A-158	19	117		
Pol × A-165	15	95		
Polycarpe (control)	54	100	112	100
Average mean yield (% of the control)		135.5		97

In fall 1984, two experiments were carried out on the farm of the Aristotelian University of Thessaloniki, Greece. The first experiment was established according to R-7 honeycomb selection design (Fasoulas 1988). The distance between the plants was 0.9 m and the total number of hills seeded was 980. The second experiment consisted of 14 rows with 57 plant positions in each. In the first year, the distance between the rows was 0.7 m and between the plants within the rows 0.3 m. In the following cycles of selection, the distances were 0.5 m and 0.2 m, respectively. A total of 798 hills was seeded in each cycle. The yield of each plant was recorded separately, and the highest-yielding plants were selected from each of the two experiments. In both experiments, cultural practices were those common in the area. Seeds produced by the plants selected from the honeycomb design were seeded the following year in a non-replicated-O (NR-O) honeycomb design, and progeny of the

plants selected from the grid experiment were further advanced by the grid system. The same procedure was followed for four cycles of selection (F_3, F_4, F_5 and F_6). The selection pressure applied in each cycle is shown in Table 2. Two selection pressures (1.6 and 2.7% for honeycomb, and 5 and 10% for the fixed-grid system) were applied in the last cycle (F_6). This was to test whether plants selected at a higher selection pressure (1.6 and 5% for honeycomb and fixed grid) were superior to the additional ones selected at lower selection pressure (2.7 and 10%, respectively). Thus, four groups of selected plants were obtained. The first group was a mixture of equal numbers of seeds from each of the plants selected from the honeycomb design with 1.6% selection pressure at the F_6 (H1). The second was a mixture of equal numbers of seeds from each of the additional plants selected from the honeycomb design at 2.7% selection pressure (plants selected under 1.6% selection pressure were not included, H2). Similarly, the G1 mixture comprised plants selected with 5% selection pressure with the grid system, and the G2 mixture comprised seeds from only the additional plants selected with 10% selection pressure.

These four seed mixtures and cvs Polycarpe and Effe (controls) were tested in a randomized complete block design with four replicates in two locations (Larissa and Thessaloniki) for two years. Plots consisted of four 6-m rows. The distance between rows was 0.5 m, between entries 1 m, and between replicates 2 m. One-hundred seeds were seeded per row, and the final plant density was 22 plants/m^2. The yield of each entry was determined and the results were subjected to analysis of variance and means evaluated with Duncan's Multiple Range Test.

Plant height, pod length and number of seeds per pod were measured in five randomly selected plants from each of the two middle rows per entry in both locations during the first year. In addition, the number of days needed from sowing to the first flower and to the date when 50% of the plants had at least one flower were recorded for each of the entries and locations. Finally, the 1000-seed weight was measured in one sample per treatment and location, and therefore no statistical analysis was applied.

Results and Discussion

All F_1s, except Pol × A-165, outyielded the common parent Polycarpe (Table 1). The superiority of the F_1s over the common parent ranged from 17 to 83%, with an overall average of 35.5%. F_1 heterosis of such magnitude has previously been documented in faba bean (Bond 1971; Lawes et al. 1983). A high level of inbreeding depression, however, was observed in the F_2. The average mean yield of the six F_2s studied was close to the yield of the control Polycarpe (Table 2). Thus, any improvement realized in subsequent generations should be attributed to the selection applied.

Table 2. Number of plants surviving to maturity, plant density (plants/m^2), selection pressure used (% selected plants) and number of plants selected in four generations of faba bean advanced under two selection schemes.

Generation	Honeycomb				Grid			
	No. plants collected	Plant density	Selection pressure	No. plants selected	No. plants collected	Plant density	Selection pressure	No. plants selected
F_3	812	1.28	2.7	17	792	4.8	2.8	17
F_4	960	1.28	1.6	15	594	10.0	2.8	16
F_5	959	1.28	1.6	15	999	10.0	2.8	25
F_6	871	1.28	1.6 & 2.7	20 & 32	836	10.0	5.0 & 10.0	40 & 40

The H1 mixture derived after four cycles of selection outyielded the control Polycarpe in both locations and both years (Table 3). In addition, the mixtures H2, G1 and G2 yielded significantly more than the control in both years only in Larissa, while in Thessaloniki they outyielded the control only during 1988/89 (the year with high average yields). Cultivar Effe, however, was always the best line among the genetic material studied. Furthermore, no significant differences were observed among the four mixtures selected by the two grid systems of mass selection. Thus, yield response to honeycomb mass selection was as high as that to the grid mass selection (Table 3). The annual progress under solid stand realized by both the honeycomb (5.6%) and the grid mass selection (6.1%) systems is one of the highest reported for faba bean.

Fasoulas (1988) suggests that the moving hexagonal grid of honeycomb selection under low plant density should be more effective than the fixed grid under high plant density. This was supported by the higher interplant distance applied in honeycomb designs and by the fact that the distance separating an evaluated plant from the remaining plants is minimal and the same for all evaluated plants in

Table 3. Mean grain yield per plot and annual progress (in % of cv Polycarpe) of the selected material by two mass-selection methods in two locations and two years.

Material	1988/89				1989/90				Mean annual progress (%)
	Thessaloniki		Larissa		Thessaloniki		Larissa		
	Mean yield (kg/plot)	Annual progress (%)	Mean yield (kg/plot)	Annual progress (%)	Mean yield (kg/plot)	Annual progress (%)	Mean yield (kg/plot)	Annual progress (%)	
H1	5.8 a	3.4	2.9 a	8.8	3.2 a	6.8	5.6 a	6.9	6.5
H2	5.8 a	3.3	2.8 a	7.1	2.6 ab	1.8	5.5 a	6.1	4.6
G1	5.9 a	4.1	2.8 a	7.8	3.2 ab	6.3	5.4 a	6.0	6.1
G2	5.7 a	3.1	3.1 a	10.4	3.0 ab	5.1	5.4 a	5.7	6.1
Polycarpe	4.9 b		2.0 b		2.4 b		4.2 b		
Effe	6.1 a		4.7 c		3.4 a		7.4 c		
CV (%)	8.9		15.4		16.6		7.9		
LSD	0.76		0.74		0.74		0.66		

Numbers followed by different letters are statistically different at $P = 0.05$

honeycomb selection, while in fixed grids it is different. Our findings, however, indicate that the two grid systems were equally effective. Roupakias et al. (1996) report that the cross Pol × A-37, identified as superior when eight crosses were evaluated in F_1 and F_2 under low plant density in a honeycomb design, had a higher breeding value than the one (Pol × A-58) chosen after evaluation of the same F_1s and F_2s under high plant density with adjacent controls. In addition, the plants selected from Pol × A-37 under both plant densities were not significantly different from one another. The same was observed in the present study. Thus, one could argue that the fixed-grid mass-selection procedure was favored by the fact that the two generations critical to selection (i.e. F_1 and F_2) were evaluated and selected in the absence of competition. This, however, has to be verified by further experimentation.

The results show that the effect of individual selection practised on spaced plants carries over when the progenies of these plants are grown under competition. Although this is in agreement with data reported by other researchers (Lungu et al. 1987; Kulkarni 1991), this is not always the case (Kyriakou and Fasoulas 1985; Danos and Roupakias, unpublished data). We think that this is largely due to the genetic make-up of the material under selection and the controls used. The cultivars of various crops cultivated today can be divided into three groups according to their performance under spaced and dense stands. The first group is cultivars with good performance under both stands (YC), the second group is cultivars that perform well only under competition (yC), and the third group is the ones with good performance only in the absence of competition (Yc). The first group of cultivars is preferred by the breeder because they have better buffering capacity (Kyriakou and Fasoulas 1985). This may indicate that the breeder has a better chance for success if populations that include plants with good performance in both spaced and dense stands can be identified for concentration of selection efforts. Thus, one could suggest the use of at least one genotype of the YC type in the crossing program and evaluation of the F_1s and F_2s without competition and in comparison with a YC-type cultivar. This could enable the identification of promising populations in an early generation, which could be further advanced successfully by any effective breeding methodology.

All the selected mixtures (H1, H2, G1, G2) had larger seeds than the control cv Polycarpe (1000-seed weight, H = 659 g, G = 595 g and Pol = 355 g; Tables 4 and 5). In addition, the selected materials were about six days earlier than Polycarpe. On average they were also taller than Polycarpe, but this difference was not always significant (Tables 4 and 5). Finally, the selected mixtures had significantly longer pods than the common parent Polycarpe and more or less the same number of seeds per pod (Tables 4 and 5).

It is concluded that honeycomb mass selection under low density and the fixed-grid mass selection under high density are equally effective after four cycles of selection in a population of faba beans.

Acknowledgements

Financial assistance from the Greek Ministry of Agriculture is gratefully acknowledged.

Table 4. Means of six traits in four selected mixtures and two faba bean cultivars grown in Thessaloniki.

Material	Days to		Height (cm)	Pod length	Seeds/pod	1000-seed
	1st flower	50% flowering		(cm)		weight (g)
H1	132.0 b	138.8 c	106.4 a	8.2 ab	3.3 a	650
H2	131.5 b	138.8 c	116.3 a	7.8 b	3.3 a	660
G1	133.5 b	141.0 b	115.1 a	8.3 ab	3.5 a	615
G2	133.0 b	140.3 b	109.9 a	7.8 b	3.4 a	605
Polycarpe	139.5 a	146.5 a	108.4 a	5.6 c	3.3 a	390
Effe	125.0 c	129.0 d	88.1 b	8.7 a	2.9 b	1015

Numbers followed by different letters are statistically different at $P = 0.05$.

Table 5. Means of six traits in four selected mixtures and two faba bean cultivars grown in Larissa.

Material	Days to		Height (cm)	Pod length	Seeds/pod	1000-seed
	1st flower	50% flowering		(cm)		weight (g)
H1	132.8 c	141.0 d	81.8 bc	7.3 b	2.6 b	685
H2	133.8 c	141.8 cd	83.5 bc	7.1 b	2.6 b	640
G1	135.8 b	143.8 bc	85.5 ab	7.3 b	3.0 a	605
G2	136.0 b	144.0 b	90.6 a	6.8 b	2.8 ab	555
Polycarpe	141.8 a	151.0 a	76.8 c	4.7 c	2.8 ab	320
Effe	128.5 d	135.5 e	69.5 d	3.8 a	2.8 ab	990

Numbers followed by different letters are statistically different at $P = 0.05$.

References

Bond, D.A. 1971. Breeding methods in field beans *(Vicia faba* L.). Pages 119–126 *in* Report of the Meeting of the Eucarpia Fodder Crops Section, Lusignan, 1970.

Bond, D.A. 1987. Recent developments in breeding field beans (*Vicia faba* L.). *Review of Plant Breeding* 99: 1–26.

Bos, I. 1981. The relative efficiency of honeycomb selection and other procedures for mass selection in winter rye (*Secale cereale* L.). PhD thesis, Agricultural University, Wageningen.

El-Sherbeeny, M.H. 1970. Studies on pollination, fertilization and pod-seting in the field bean and their bearing on breeding the crop. MSc Thesis, University of Cairo. [Reference in Hawtin, 1982].

Fasoulas, A. 1973. A new approach to breeding superior yielding varieties. Deptartment of Genetics and Plant Breeding, Aristotelian University, Thessaloniki, Greece, Publication 3.

Fasoulas, A. 1981. Principles and methods for plant breeding. Department of Genetics and Plant Breeding, Aristotelian University, Thessaloniki, Greece, Publication 11.

Fasoulas, A. 1988. The honeycomb methodology of plant breeding. Department of Genetics and Plant Breeding, Aristotelian University, Thessaloniki, Greece.

Fasoulas, A.C. and V.A. Fasoula. 1995. Honeycomb selection designs. *Plant Breeding Reviews* 13: 87–139.

Gardner, C.O. 1961. An evaluation of effects of mass selection and seed irradiation with thermal neutrons on yield of corn. *Crop Science* 1: 241–245.

Gardner, C.O. 1969. The role of mass selection and mutagenic treatment in modern corn breeding. Pages 15–21 *in* Proceedings of the 24th Annual Corn and Sorghum Research Conference, Chicago, vol. III.

Hawtin, G.C. 1982. The genetic improvement of faba been. An overview of breeding methods. Pages 15–32 *in* Faba Bean Improvement (G. Hawtin and C. Webb, ed.). Martinus Nijhoff, The Hague, Netherlands.

Johnson, E.C. 1963. Effects of mass selection for yield in a tropical corn variety. Pages 82 *in* Annual Meeting of the American Society of Agronomy, Denver, CO. (Abstr.)

Kyriakou, D.T., and A.C. Fasoulas. 1985. Effects of competition and selection pressure on yield response in winter rye (*Secale cereale* L.). *Euphytica* 34: 883–895.

Kulkarni, R.N. 1991. Three cycles of honeycomb selection for herb yield in davana *(Artemisia pallens* Wall.). *Euphytica* 52: 99–102.

Lawes, D.A., D.A. Bond and M.H. Poulsen. 1983. Classification, origin, breeding methods and objectives. Pages 23–76 *in* The Faba Bean (*Vicia faba* L.). (P.D.

Hebblethwaite, ed.). Butterworths, London, UK.
Lungu, D.M., P.J. Kaltsikes and E.N. Larter. 1987. Honeycomb selection for yield in early generations of spring wheat. *Euphytica* 3: 1–9.
Mitchell, J.W., R.J. Baker and D.R. Knott. 1982. Evaluation of honeycomb selection for single plant yield in durum wheat. *Crop Science* 22: 840–843.
Roupakias, D.G., A. Zesopoulou, S. Kazolea, G. Dalkalitses, A. Mavromatis and T. Lazaridou. 1997. Effectiveness of early generation selection under two plant dencities in faba bean (*Vicia faba* L.). *Euphytica* 93: 63–70.
Verhalen, L.M., J.L. Baker and R.W. McNew. 1975. Gardner's grid system and plant selection efficiency in cotton. *Crop Science* 15: 588–591.

Performance of Faba Bean Genotypes over Years in North India

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Abstract

The performance of 50 faba bean genotypes was studied during 1993 and 1994 at the CCS Haryana Agricultural University, Hisar, India. The genotypes exhibiting early flowering during 1993 were 24251, VH 123-2 and DB 24 and during 1994 were HB 180 and 24251. The early-maturing genotypes during 1993 were Pant 3 and VH 86, and during 1994 were AL 5, Pant 3 and KV 12. The genotypes exhibiting high yield during 1993 were No 3, TP 215 and Almora 1, and during 1994 were HB 180, HB 65 and 296 WF.

Key words: *Vicia faba*; faba beans; genotypes; flowering; precocity; maturation; crop performance; yields; India.

Introduction

Faba bean (*Vicia faba* L.) is an important crop which can be used as pulse, vegetable, fodder, green manure and as a cover crop. The evaluation of germplasm is an important step for developing high-yielding and early varieties. Therefore, the present investigation was undertaken to study the performance of 50 faba bean genotypes.

Material and Methods

The experiment was laid out in a randomized block design with three replications at the experimental farm of CCS Haryana Agricultural University, Hisar. The experimental material, comprising 50 indigenous and exotic faba bean genotypes, was sown on 30 October 1993 and 12 November 1994. Single-row plots of 3.5 m length were raised for each genotype with 50 cm between rows and 20 cm between plants. Five randomly selected plants were used for recording five metric traits. Analysis of variance was carried out following standard procedures (Panse and Sukhatme 1967).

Results and Discussion

The analysis of variance revealed significant genotypic differences during both years. The pooled analysis also showed significant G×E interaction. Therefore, mean values are not pooled, but are shown separately for both years for the best and worst genotypes for each character (Tables 1 and 2). The genotypes exhibiting best performance during 1993 (Table 1) were 24251, VH 123-2 and DB 24 for early flowering; Pant 3 and VH 86 for early maturity; KV 12-2, No 3 and TP 215 for plant height; No 3 and KV 12-2 for pod-bearing length; and Minika, 24251 and PDV 84-54 for 100-seed weight.

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كفاءة طرز وراثية من الفول على مدى سنوات في شمالي الهند

الملخص

دُرست كفاءة 50 طرازاً وراثياً من الفول خلال 1993 و1994 في CCS التابع لجامعة هاريانا الزراعية في هيسار بالهند. وكانت الطرز الوراثية التي أظهرت إزهاراً مبكراً خلال 1993 هي 24251, VH 123-2 و24 DB، وخلال 1994 كانت HB 180 و24251، وكانت الطرز الوراثية مبكرة النضج خلال Pant3 1993، و86 VH، وخلال 1994 كانت AL 5, Pant 3 و12 KV، وكانت الطرز الوراثية التي أعطت غلة عالية خلال 1993 هي: No 3, TP 215 و1 Almora، وخلال 1994 هي: HB 180, HB 65 و296 WF.

The genotypes exhibiting best performance during 1994 (Table 2) were HB 180 and 24251 for early flowering; AL 5, Pant 3 and KV 12 for early maturity; Faiz 6, VH 44 and 8055420 for plant height; HB 156, DB 17 and VH 86 for pod-bearing length; and, PDV 84-54 and Minika for 100-seed weight.

From these results, it is apparent that the genotypes mentioned may be used in any breeding program for the improvement of the respective characters.

References

Panse, V.G. and P.V. Sukhatme. 1967. Statistical Methods for Agricultural Workers. ICAR, New Delhi.

Table 1. Mean performance of best and worst genotypes for different characters during 1993.

Genotype	Days to first flower	Days to maturity	Plant height (cm)	Pod bearing length (cm)	100-seed weight (g)
Minika	68.3	162.7	93.1	53.0	36.2
24251	59.7	161.0	83.3	53.9	35.6
VH 134	62.3	156.0	85.2	40.4	16.7
HB 62-3	60.7	157.3	74.5	49.3	19.1
No 3	63.3	159.0	95.3	58.1	20.7
VH 131	95.0	166.3	75.8	44.0	26.6
Faiz 2	64.0	155.0	74.3	48.1	20.0
8055420	88.0	165.3	82.6	38.6	25.3
KV 12-2	66.0	160.3	98.5	56.1	23.8
VH 123-2	59.7	158.0	89.3	49.3	25.1
TP 215	60.3	160.7	95.3	54.2	24.3
Pant 3	67.3	152.0	70.7	42.3	17.3
HB 65	63.3	160.7	70.2	48.5	21.4
PDV 84-54	62.0	160.3	72.1	32.2	31.7
DB 24	60.0	155.3	74.3	38.4	27.2
Topless 6	64.0	162.7	37.6	27.1	22.1
VH 86	66.3	153.3	78.3	43.2	19.7
293 WF	72.7	167.3	79.2	43.6	22.2
296 WF	74.0	167.3	64.6	34.4	26.3
DB 17	75.7	160.7	72.5	44.5	16.0
Almora 1	66.0	159.7	67.2	34.0	24.3
Topless 7-1	64.0	163.3	42.0	18.9	22.4
Topless 10	76.0	166.3	36.1	16.2	24.9
289 WF	88.7	170.3	70.3	23.1	22.9
Topless 1	71.7	164.0	38.8	23.3	20.5
Topless 3-1	85.7	165.0	29.0	22.1	18.3
Topless 9	106.0	165.3	42.0	30.5	15.2
Mean	70.77	161.31	70.08	39.53	23.54
CD (5%)	4.78	1.83	7.87	4.79	1.99
Range	59.7 –106.0	152.0 –170.3	29.0 –98.5	16.2 –58.1	15.2–36.2

Table 2. Mean performance of best and poor genotypes for different characters during 1994.

Genotype	Days to first flower	Days to maturity	Plant height (cm)	Pod bearing length (cm)	100-seed weight (g)
Minika	53.67	148.00	93.60	55.33	40.00
Faiz 1	60.67	148.33	86.40	47.40	21.17
24251	52.33	149.33	90.73	58.67	34.00
AL 5	64.33	144.0	73.26	46.46	20.53
Topless 2-1	74.00	157.00	56.73	38.07	23.57
No 13	61.00	146.00	80.40	64.87	21.50
HB 180	52.00	145.00	78.13	65.27	26.37
8055420	70.67	154.00	108.47	62.27	27.13
VH 44	56.00	150.00	109.13	66.73	25.33
Faiz 6	57.00	146.33	110.00	65.13	21.30
KV 12-2	57.33	147.33	94.87	68.60	25.43
Pant 3	60.00	144.00	76.20	50.80	18.00
HB 65	56.67	150.00	95.93	57.53	23.33
KV 12	62.33	144.67	88.07	58.40	26.00
PDV 84-54	55.33	153.00	93.07	45.33	45.33
Topless 6	57.00	150.00	49.60	30.73	22.20
VH 86	59.33	145.00	92.47	74.27	21.17
296 WF	58.33	152.00	102.13	53.07	26.17
DB 17	58.33	146.33	89.60	75.47	19.27
Topless 7-1	61.33	148.33	51.87	27.93	22.30
Topless 10	67.00	156.00	55.93	28.60	22.17
Topless 5-2	62.67	154.33	54.53	26.47	23.47
PC 82-28	61.00	145.00	88.27	58.20	24.17
Topless 1	61.67	158.00	63.60	31.13	24.17
HB 156	59.67	146.00	92.80	79.40	27.50
Topless 3-1	70.67	157.00	57.07	29.53	22.47
Topless 9	67.33	152.00	65.40	46.93	18.37
Mean	60.65	149.52	81.49	52.32	24.90
CD 5%	2.18	1.72	7.40	6.28	2.42
Range	52.00	144.00	49.60	26.47	18.00
	-74.00	158.00	-110.00	-79.40	-45.3

Male Sterility in Grasspea

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Abstract

Grasspea is a neglected legume but has the potential to become an important crop in dry regions. Grasspea lines L880283 and L900436 were reciprocally crossed and the progenies were evaluated for expression of male fertility/sterility. When line L900436 was used as the male parent, F_1 progenies were sterile, but F_1 progenies of the reciprocal cross were all fertile. Fertile and sterile F_1 progenies were back-crossed with parental lines, and two other breeding lines, and the progenies were evaluated for male fertility/sterility under field conditions. Back-cross progenies (BC_1) with sterile F_1 progenies segregated into combinations which indicated that both cytoplasmic and genetic systems of male sterility may be operating along with environmental influence in the control of expression of male sterility.

Key words: *Lathyrus sativus*; male infertility; crossbreds; hybridization; backcrossing; reciprocal crossing; genotype environment interaction; progeny; evaluation.

Introduction

Grasspea (*Lathyrus sativus* L.) is a well known drought- and flood-tolerant pulse crop grown extensively in the Indian sub-continent. Despite this, relatively little effort has been extended to improve this hardy crop. The main reason has been the knowledge that excessive consumption of grasspea can lead to the neurological disorder called lathyrism. A water-soluble, non-protein amino acid called β-N-oxalyl-L-α,ß-diaminopropionic acid (ODAP) has been identified as the neurotoxic factor and is inherited quantitatively (Tiwari and Campbell 1996).

Although the present research thrust of this highly neglected crop is directed toward lowering the neurotoxin content, research in other aspects of crop improvement is equally important. Male sterility mechanism is extensively utilized in other field crops to facilitate hybrid breeding (McVetty et al. 1990; Reddy et al. 1978). Male sterility in grasspea could be successfully utilized in population improvement programs and in exploiting some of the non-additive gene action on characters affecting production (Campbell et al. 1994). Therefore, this study was conducted to study genetics of male sterility in the grasspea line L880283.

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العقم الذكري في الجلبان (*Lathyrus sativus* L.)

الملخص

يُعتبر الجلبان بقولاً مهملاً، إلا أنه يتمتع بإمكانات تؤهله لكي يكون محصولاً هاماً في المناطق الجافة. هُجنت سلالتا الجلبان L 880283 وL 900436 تهجيناً تبادلياً، وتم تقييم الأنسال من حيث التعبير عن الخصوبة/العقم الذكري. فعندما استخدمت السلالة L 900436 كأب ذكري، كانت أنسال الجيل الأول F1 عقيمة، إلا أن أنسال الجيل الأول للتهجين التبادلي كانت جميعها خصبة. هُجنت أنسال الجيل الأول العقيمة والخصبة تهجيناً رجعياً بالسلالات الأبوية وسلالتي تربية أخرتين كما قُيمت الأنسال من حيث الخصوبة الذكرية/العقم تحت الظروف الحقلية. انعزلت أنسال التهجين الرجعي (BC1) مع أنسال الجيل الأول F1 العقيمة في توليفات، مما يشير إلى أن كلا نظامي العقم الذكري السيتوبلازمي والوراثي قد يعملان إلى جانب التأثير البيئي، على مكافحة التعبير عن العقم الذكري.

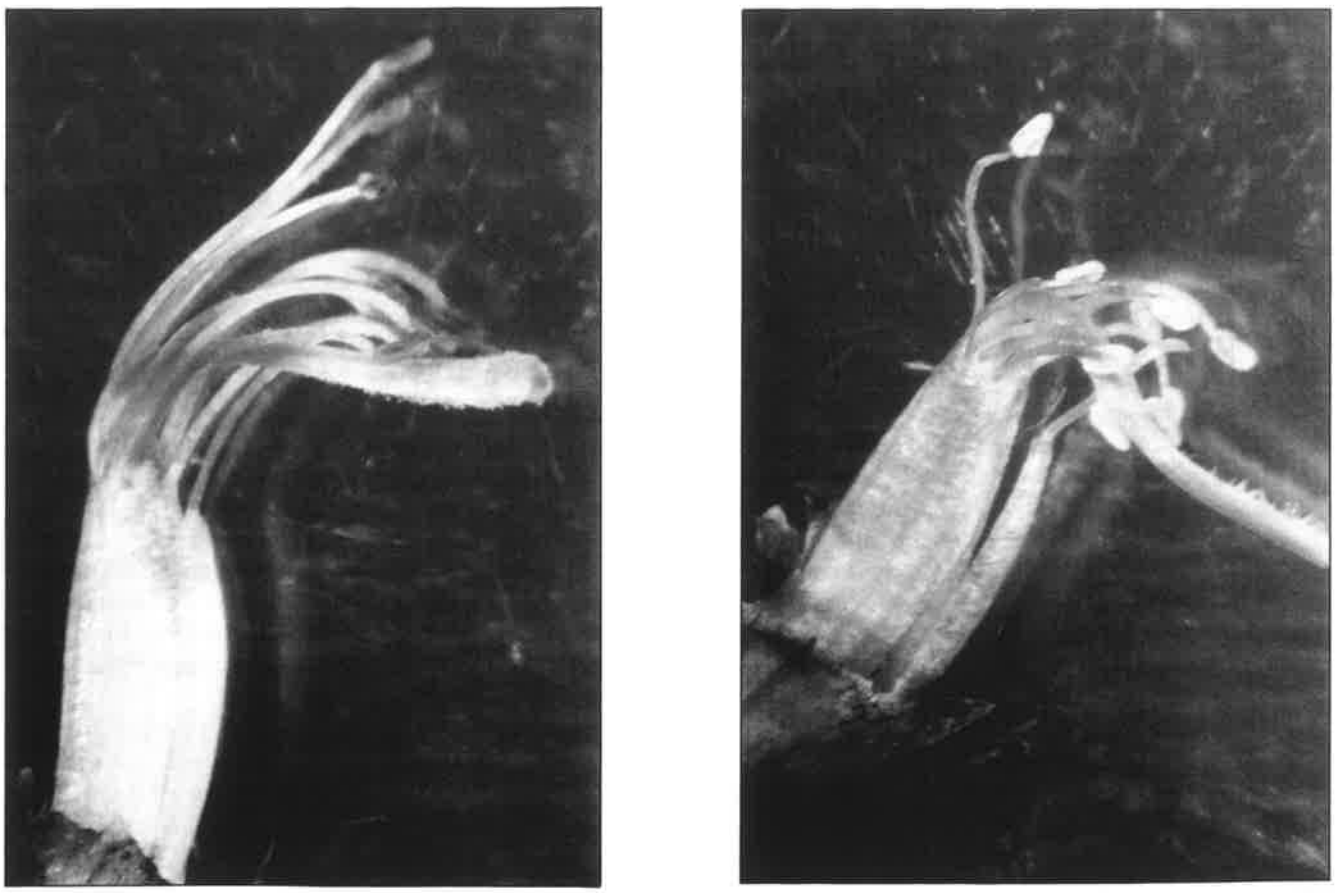
Material and Methods

Grasspea lines L880283 and L900436 produce hermaphrodite flowers and are self fertile. L880283 (PGR 19761) was originated from a German line and L900436 (V 8603) from Bangladesh (Tiwari 1994). These lines were reciprocally crossed and progenies were evaluated for the expression of male fertility/sterility both in the field and in a growth room. The first parents recorded in all the cross combinations were the female parents. Growth-room conditions were maintained at 15°C, 80% relative humidity, high intensity fluorescent light of 380 µmol $m^{-2}s^{-1}$ and 16 h light/8 h dark. Field studies were conducted at Agriculture and Agri-Food Canada, Research Center, Morden, Manitoba. Back-crosses were made with the sterile F_1 progenies of the cross L880283 × L900436 and the fertile F_1 progenies of reciprocal cross L900436 × L880283 with parental lines and two other breeding lines L720060 and LS90235 under field con-

ditions. Seeds from these back-crosses were harvested and the progenies were further evaluated to determine expression of male fertility/sterility under field conditions in summer 1993. Anthers of individual flowers were visually and microscopically examined to determine whether they were fertile (normal, yellow) or sterile (short, translucent).

Results and Discussion

All F_1 progenies of the cross L880283 x L900436 were sterile whereas all the progenies of reciprocal cross were fertile in the growth room. Examination of individual flowers revealed that anthers of the fertile plants were normal and full of pollen grains whereas the anthers of sterile plants were translucent and had short filaments (Fig. 1). However, when grown under field conditions, 25% of the plants were fertile among the F_1 progenies of the cross L880283 X L900436 (Table 1). This difference in the expression of sterility mechanism in the growth room and under field conditions could be due to environmental differences. The F_1 progenies of the reciprocal cross L900436 x L880283 were all fertile both under field conditions and in the growth room. Back-cross (BC_1) progenies of the fertile F_1 to L900436, L880283, L720060 and L90235 were all fertile, while those with sterile F_1 progenies segregated in different combinations (Table 1). An indefinite pattern of segregation for fertility of the back-cross progenies indicated that both cytoplasmic and genetic sterility mechanisms were operating, and that the restorer populations both within and between lines were heterogenous. Further work will be needed to determine the effect of the environment in the expression of male sterility in the cross L880283 x L900436.



(a) (b)

Fig. 1. Grasspea flower: (a) male fertile full of pollen grains; (b) male sterile with translucent anthers and short filaments.

Table 1. Male sterility observations in *Lathyrus sativus* in the growth room and under field conditions.

Cross	Number of plants evaluated		
	Total	Fertile	Sterile
Growth Room			
L900436 × L880283	30	30	0
L880283 × L900436	8	0	8
Field			
L900436 × L880283	26	26	0
L880283 × L900436	26	6	20
Back-crosses (field)			
Fertile F_1			
(L900436 × L880283) × L900436	9	9	0
(L900436 × L880283) × L880283	11	11	0
(L900436 × L880283) × L720060	10	10	0
(L900436 × L880283) × LS90235	19	19	0
Sterile F_1			
(L880283 × L900436) × L900436	26	2	24
(L880283 × L900436) × L880283	43	25	18
(L880283 × L900436) × L720060	41	10	31
(L880283 × L900436) × LS90235	29	7	22

Reddy et al. (1978) reported a single recessive allele to be responsible for male sterility in pigeon pea (*Cajanus cajan*). Chekalin (1972) showed male sterility in grasspea to be controlled by the interaction of a pair of recessive genes and a sterile cytoplasm which could be modified by environmental conditions. Srivastava and Somayajulu (1981) reported a form of genetic male sterility in grasspea where a short filament condition was found to be stable and closely linked with pollen sterility. Similar to our observation, Quader (1987) reported both cytoplasmic and genetic types of male sterility and the presence of two types (monogenic and digenic) of restorers in grasspea. However, contrary to our observations, he reported the sterility mechanism to be stable under different environmental conditions.

References

Campbell, C.G., R.B. Mehra, S.K. Agrawal, Y.Z. Chen, A.M. Abd El Moneim, H.I.T. Khawaja, C.R. Yadav, J.U. Tay and W.A. Araya. 1994. Current status and future strategy in breeding grasspea (*Lathyrus sativus* L.) *Euphytica* 73: 167–175.

Chekalin, N.M. 1972. Studies on genetic nature of induced male sterility in grasspea (*Lathyrus sativus* L.). *Genetica* 8: 20–26.

McVetty, P.B.E., S.A. Edie and R. Scarth. 1990. Comparision of the effect of *nap* and *pol* cytoplasms on the performance of intercultivar summer lilseed rape hybrids. *Canadian Journal of Plant Science* 70: 117–126.

Quader, M. 1987. Male sterility in *Lathyrus sativus* L. *Bangladesh Journal of Botany* 16: 9–13.

Reddy, B.V.S., J.M. Green and S.S. Bisen. 1978. Genetic male sterility in pigeon pea. *Crop Science* 18: 362–364.

Srivastava, Y.C. and P.L.N. Somaya Julu. 1981. Male sterility in *Lathyrus*. *Indian Journal of Genetics and Plant Breeding* 41: 164–166.

Tiwari, K. R. and C.G. Campbell. 1996. Inheritance of neurotoxin (ODAP) content, flower and seed coat colour in grasspea (*Lathyrus sativus* L.). *Euphytica* 91: 195–203.

Tiwari, K.R. 1994. Inheritance of the neurotoxin ß-N-oxalyl-L-α, ß-diaminopropionic acid (ODAP) in grasspea seeds. MSc Thesis. University of Manitoba, Canada.

Intraspecific Diversity of *Vicia sativa* L. and *Vicia angustifolia* Reichard Deduced from Seed Protein Electrophoresis

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Abstract

Seed proteins of 82 accessions of *Vicia sativa* and *V. angustifolia* from different geographical regions were analyzed by SDS-polyacrylamide gel electrophoresis. Based on the combination of legumin bands, six protein types within *V. sativa* and three protein types within *V. angustifolia* were recognized. In addition to remarkable intraspecific variability, these two species differ from one another in their seed protein profiles. The protein type variation observed within *V. sativa sensu stricto* could be not correlated with the described intraspecific taxa. No association was found between geographical origin of *V. sativa* accessions and their protein types. The region with the highest diversity of protein types in the area of former USSR was the Caucasus. This storage-protein investigation confirms the extent of intraspecific differentiation within *V. angustifolia*. Accessions of *V. angustifolia* subsp. *angustifolia* and *V. angustifolia* subsp. *segetalis* had different protein patterns. Protein electrophoregrams may be useful for identifying *V. angustifolia* accessions of Asiatic origin.

Key words: *Vicia sativa; Vicia angustifolia,* seed; proteins; electrophoresis.

Introduction

In the *Vicia sativa* complex, seed protein electrophoresis has been used in taxonomical investigations (Potokina and Eggi 1991) and for cultivar identification (Gavrilyuk and Eggi 1993). The *V. sativa* complex contains plants with different chromosome numbers (2n=10, 12, 14). Variation of seed protein profile among the various chromosomal types in *V. sativa* complex was examined by Ladizinsky (1982). No band or bands of protein profile were related to a specific karyotype or chromosome number. Data describing the extent of variation in seed protein electrophoregrams within species of this complex, within populations of different geographical origin are lacking. However, such information would assist plant collectors and curators in detecting local centers of diversity of this important fodder crop and may be useful to set up strategies of sampling of *V. sativa* germplasm.

Two species from the *V. sativa* complex, *V. sativa* L. *sensu stricto* (s.s.) and *V. angustifolia* Reichard, are characterized by extremely high levels of intraspecific variation. Taxonomical classification of morphological diversity within *V. sativa* s.s. is proposed by Repyev and Stankevich (1989), who described 2 subspecies, 7 varietes and 12 forms based on the variability of morphological features (size and shape of pods, flower color and seed coat pattern).

التنوع البينوعي لـ *Vicia sativa* L. و*Vicia angustifolia* Reichard المستنتج من الرحلان الكهربائي لبروتين البذور

الملخص

تم تحليل بروتين بذور 82 مدخلاً من البيقية الشائعة *Vicia sativa* والبيقية ضيقة الأوراق *V. angustifolia* من مناطق جغرافية مختلفة، بواسطة الرحلان الكهربائي باستخدام طريقة SDS-PAGE. وقد تم التعرف بالاعتماد على توليفة من خطوط لون البقلين، على ستة أنواع من البروتين ضمن البيقية الشائعة وثلاثة أنواع ضمن البيقية ضيقة الأوراق. وبالإضافة إلى التباين الكبير البينوعي، يختلف هذان النوعان عن بعضها البعض في أشكال الحزم المتوضعة من البروتين في كل منهما. إن تباين نوع البروتين الملاحظ ضمن البيقية الشائعة على نطاق ضيق لايمكن أن يرتبط بالتصنيفات البينوعية الموصوفة. إذ لم يكن هناك تلازم بين الأصل الجغرافي لمدخلات البيقية الشائعة وأنواع بروتينها. وكانت المنطقة التي تتميز بأعلى معدلات التنوع الحيوي في أنواع البروتين في منطقة الاتحاد السوفييتي السابق، هي القوقاز. وتؤكد هذه الدراسة حول مخزون البذور مدى الاختلافات البينوعية ضمن *V. angustifolia*. وللمدخلات من *V. angustifolia* subsp. *angustifolia* و*V. angustifolia* subsp. *segetalis* أنماط من البروتين مختلفة. وقد تكون خريطة توزع البروتين مفيدة في تحديد مدخلات *V. angustifolia* ذات الأصل الآسيوي.

At least two groups within *V. angustifolia* can be recognized based on the morphological, ecogeographical and karyotypical differences (Mettin and Hanelt 1964): *V. angustifolia* subsp. *angustifolia* and *V. angustifolia* subsp. *segetalis* (Thuill.) Gaud. Hollings and Stace (1974) treat *V. angustifolia* and *V. segetalis* as separate species and report that only *V. angustifolia* is native in central and nothern Europe, while *V. segetalis* is cultivated in those regions and occurs as an escape from cultivation. Both taxa are included by many authors in *V. sativa* subsp. *nigra* (L.) Ehrl. (Ball 1968; Davis and Plitmann 1970), which is considered as the most widespread and polymorphic of all the *V. sativa* complex taxa (Maxted 1995). *Vicia angustifolia* and *V. segetalis* are highly variable cytologically. Conspicuous karyotype variation was found within *V. angustifolia* (Hollings and Stace 1974; Ladizinsky 1978), and two different chromosomal numbers—2n=12 and 2n=10—are reported by Hollings and Stace for *V. segetalis*. Seed-protein electrophoresis could contribute significantly to our understanding of the genetic diversity within these *Vicia* crops and their taxonomic relationships.

The present study was conducted to investigate the intraspecific variations of *V. sativa* and *V. angustifolia* seed proteins by SDS polyacrilamide gel electrophoresis (PAGE) and to examine possible associations between storage-protein polymorphism, geographical origin and taxonomic status.

Material and Methods

The seed-protein profile was examined in 50 accessions of *V. sativa* and 32 accessions of *V. angustifolia*. Some of these were obtained from the Vavilov Institute of Plant Industry (VIR, Russia) collection, others were collected during collection missions to the Caucasus (Azerbaijan, Nagornyi Karabakh, Daghestan), Crimea and Central Asia (Turkmenistan). We also included four cultivars of common vetch bred in Russia and Ukraine: Byelorozovaya, Byelotzerkovskaya, Lgovskaya 28 and Lgovskaya 65.

The protein was analyzed by the SDS-PAGE system of Laemmli (1970) using a 12.5% polyacrilamide gel. Total seed protein was extracted from 4 mg of seed meal with 0.15 ml of 1 *M* TRIS-HCl buffer (pH = 6.8) containing 10% SDS. Mercaptoethanol (0.3 ml) was then added to the extract. The electrophoresis was conducted at 90 V for the first 30 min and 250 V for a futher 3 h. The molecular-weight standards used were: BCA (65 kD), egg albumin (45 kD), chemotripsinogen (25 kD), and cytochrome C (12.5 kD).

Four groups of bands in the storage-protein electrophoregrams of *V. sativa* could be recognized. These were designated zone I, II, III and IV (Fig. 1). Their identification was done using immunoblotting and the comparison of the electrophoretic patterns with and without marcaptoethanol (Gavrilyuk and Eggi 1993). Each band of spectra was numbered according to the uniform conventional glycine-scale used for legume electrophoregrams registration at VIR (Tarlakovskaya et al. 1990). Zone I includes albumin bands; zone II, vicilin and legumin ones; zone III, acid polypeptides of legumin; and zone IV, basic polypeptides of legumin (Fig. 1). The variation of the banding patterns in the zone IV (molecular range of 23 to 19 kD) is important for species identification within the *V. sativa* complex. (Potokina and Eggi 1991). For this reason, we analyzed the band combinations in zone IV.

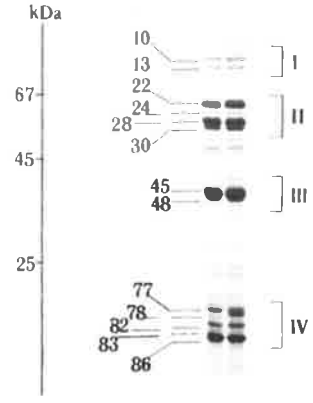

Fig. 1. Bands identification in electrophoretic banding patterns relative to seed storage protein in *V. sativa*.

Results and Discussion

Within zone IV, six banding patterns for *V. sativa* and three banding patterns for *V. angustifolia* were recognized. They were designated as protein types s1, s2, s3, s4, s5, s6 and ang1, ang2, ang3, respectively (Figs 2 and 3). The geographical distribution of the *V. sativa* protein type accessions is shown in Table 1. The most common protein types, s1 and s2, were widely distributed throughout Europe, East Asia and Africa, but were not found in Central Asia. Protein types s3, s4 and s5 were detected in natural populations from the Caucasus and Central Asia. Protein type s6 occurred in the accessions from Central Asia and in the cultivars, which were bred in Russia and Ukraine.

Generally, geographical clines of protein type frequencies for *V. sativa* s.s. were not present in the material. A

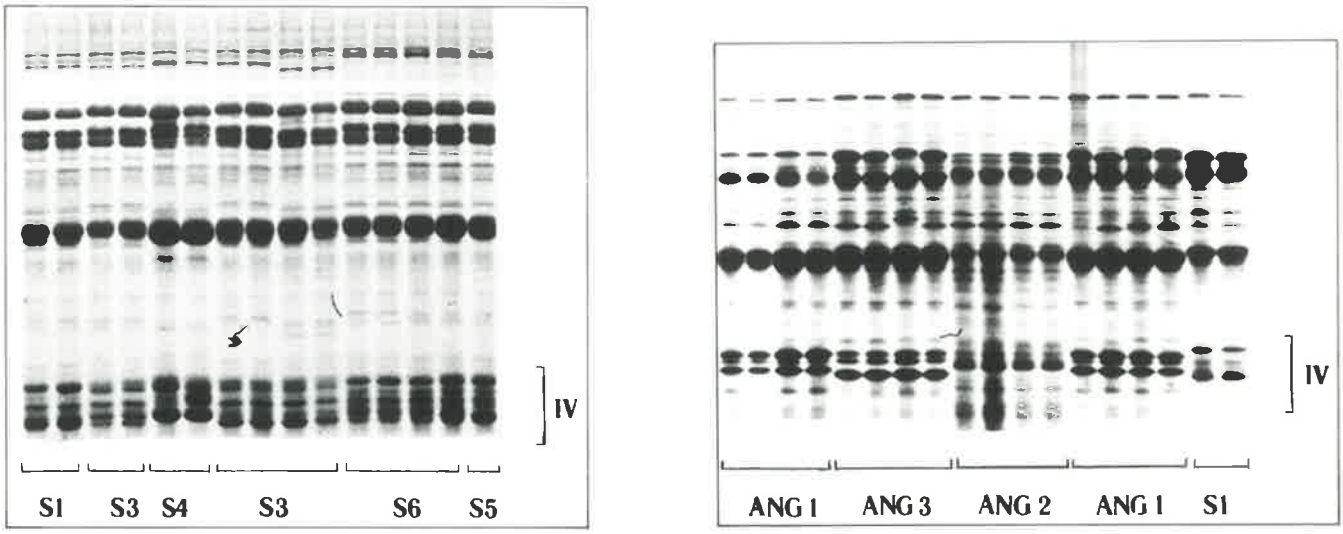

Fig. 2. Different banding pattern types observed within *V. sativa* and *V. angustifolia.*

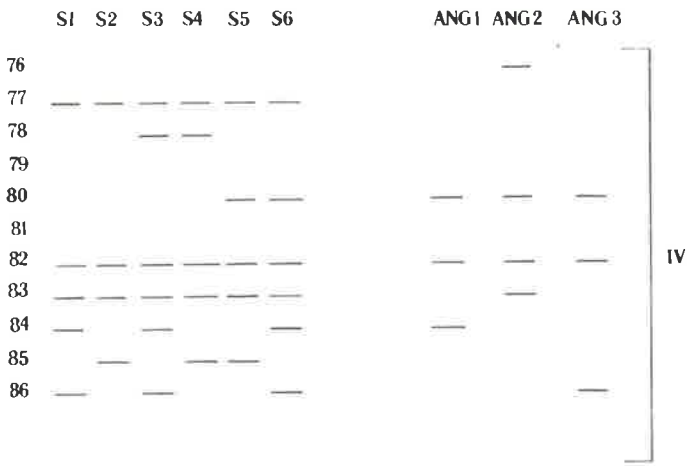

Fig. 3. Interpretive drawing of the banding pattern types observed within *V. sativa* and *V. angustifolia.*

large number of local accessions, especially from wild populations of Turkey, Iran, Afganistan, Spain and Portugal, should be analyzed to obtain more precise information. In the area of the former USSR, the region with the highest diversity of accessions with different protein types were the Caucasus. Here, the richest source of such diversity represents accessions from local natural populations, which were collected according to the principle "one plant one pod." The diversity of protein types in the accessions maintained in germplasm collections seemed to be lower. Some differences in the frequency distribution of the protein types were recognized between the accessions originating from Europe and Central Asia (Table 1). Similar results were obtained by immunochemical investigation of storage globulins in *V. sativa* accessions of European and Asiatic origin (Tarlakovskaya 1979).

Table 1. Origin, protein types and number of *V. sativa* strains examined.

Origin	Code†	No. of strains	Protein types					
			s1	s2	s3	s4	s5	s6
Asia minor	C	9	6	3	-	-	-	-
Europe	C	29	19	10	-	-	-	-
North Africa	C	8	4	4	-	-	-	-
Caucasus	C	54	70	30	-	-	-	-
Caucasus	N	41	10	4	14	11	2	-
Central Asia	N	34	-	-	13	2	3	16
Cultivars	C	55	19	2	-	-	13	21

† Code: C - strains from VIR collection; N - strains from natural populations.

Although some variation in the number of bands and their position in the profiles was found, a general uniformity of seed protein pattern of *V. sativa* s.s. was evident. These results confirm that the seed protein profile as a conservative stable trait is a powerful tool in identifying biological units in complex species (Ladizinsky and Hymowitz 1979). The protein type variation observed within *V. sativa* s.s.

could not be correlated with the described intraspecific taxa. No band or positions of band could be related exclusively to subspecies or varietes within *V. sativa.*

In contrast to *V. sativa*, the intraspecific differentiation of *V. angustifolia* was shown quite clearly using seed-protein electrophoresis. All of the *V. angustifolia* subsp. *angustifolia* accessions examined had ang2 protein type except for one accession from Nagornyi Karabakh (Table 2). Within *V. angustifolia* subsp. *segetalis*, two kinds of protein type accessions (ang1 and ang3) were detected. The accessions of ang3 type were unique to Central Asia and Japan. In Turkmenistan, plants of ang1 and ang3 protein types occasionally formed mixed populations (Fig. 4). In this way, one of three well-defined protein types within *V. angustifolia* could be related to subsp. *angustifolia* and the two others to subsp. *segetalis*. Such remarkable seed protein variation could probably be explained by karyotype polymorphism and large geographical distribution of this species.

Table 2. Protein types detected in the strains of *V. angustifolia*

Cataloge number	Origin	Number of seeds	Protein type	Taxon
33652	Russia	4	ang 2	subsp. *angustifolia*
35983	Russia	4	ang 2	subsp. *angustifolia*
36057	Byelorussia	4	ang 2	subsp. *angustifolia*
35218	Ukraine	4	ang 2	subsp. *angustifolia*
35126	Turkmenia	4	ang 2	subsp. *angustifolia*
35165	Japan	4	ang 2	subsp. *angustifolia*
n.p.†	Russia	10	ang 2	subsp. *angustifolia*
n.p.	Russia	4	ang 2	subsp. *angustifolia*
36059	Russia	4	ang 1	subsp. *segetalis*
36176	Russia	4	ang 1	subsp. *segetalis*
35988	Russia	4	ang 1	subsp. *segetalis*
35966	Russia	4	ang 1	subsp. *segetalis*
34954	Russia	4	ang 1	subsp. *segetalis*
36189	Byelorussia	4	ang 1	subsp. *segetalis*
35975	Ukraine	4	ang 1	subsp. *segetalis*
34872	Azerbaijan	4	ang 1	subsp. *segetalis*
n.p.	Russia	40	ang 1	subsp. *segetalis*
n.p.	Russia	16	ang 1	subsp. *segetalis*
n.p.	Ukraine	20	ang 1	subsp. *segetalis*
n.p.	Ukraine	20	ang 1	subsp. *segetalis*
n.p.	Nafornyi Karabakh	4	ang 1, ang 2	subsp. *segetalis*
35992	Kirghizia	4	ang 1	subsp. *segetalis*
353884	Kirghizia	4	ang 1	subsp. *segetalis*
35194	Turkmenia	4	ang 3	subsp. *segetalis*
36118	Turkmenia	4	ang 3	subsp. *segetalis*
n.p.	Turkmenia	20	ang 1, ang 2	subsp. *segetalis*
n.p.	Turkmenia	10	ang 3	subsp. *segetalis*
n.p.	Turkmenia	10	ang 1	subsp. *segetalis*
n.p.	Turkmenia	10	ang 1, ang 3	subsp. *segetalis*
35110	Japan	4	ang 3	subsp. *segetalis*
34781	Japan	4	ang 1	subsp. *segetalis*
34956	Japan	4	ang 1	subsp. *segetalis*

† n.p. = natural population.

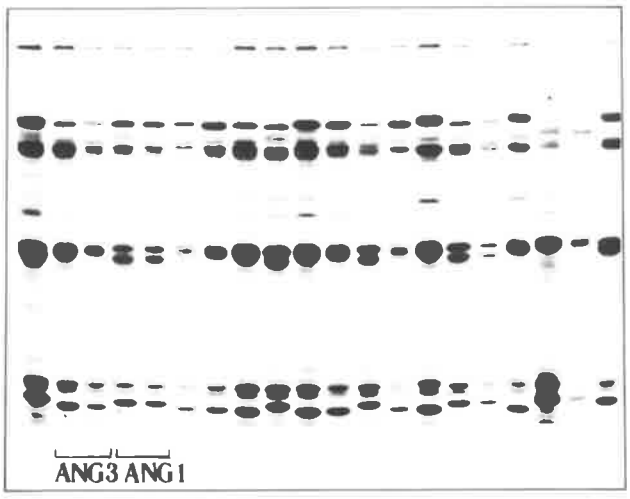

Fig. 4. Protein variability observed in a local population of *V. angustifolia* subsp. *segetalis* from Turkmenia.

Conclusions

As a result of these studies it has been shown that, though there is remarkable intraspecific variability in their protein electrophoregrams, *V. sativa* and *V. angustifolia* are distinguishable by their seed protein profiles. No relationship seems to exist between protein patterns of *V. sativa* accessions and their geographic origin or taxonomic status. The seed protein polymorphism observed for *V. angustifolia* may allow the identification of the two subspecies within this species as well as accessions of Asiatic origin.

References

Ball, P.W. 1968. *Vicia* L. Pages 129–134 *in* Flora Europaea, Vol. 2. Cambridge University Press, Edinburgh.

Davis, P.H. and U. Plitmann. 1970. *Vicia* L. Pages 274–325 *in* Flora of Turkey (P.H. Davis, ed.), Vol. 3. University Press, Edinburgh.

Gavrilyuk, I.P. and E.E. Eggi. 1993. Globulins as markers in solution of problems of phylogeny, distant hybridization and cultivar identification of legumes. Pages 193–231 *in* Molecular Biological Aspects of Applied Botany, Genetics and Plant Breeding (V.G. Konarev, ed.). Kolos, Moscow, Russia.

Hollings, E. and C.A. Stace. 1974. Karyotype variation and evolution in the *Vicia sativa* aggregate. *New Phytology* 73: 195–208.

Ladizinsky, G. 1978. Chromosomal polymorphism in wild population of *Vicia sativa* agg. *Caryologia* 31: 235–241.

Ladizinsky, G. 1982. Seed protein polymorphism in *Vicia* sativa agg. (Fabaceae). *Plant Systematic and Evolution* 141: 1–5.

Ladizinsky, G. and T. Hymowitz. 1979. Seed protein electrophoresis in evolutionary and taxonomic studies. *Theoretical and Applied Genetics* 54: 145–151.

Laemmli, U.K. 1970. Cleavage of structure proteins during assembly of the head of bacteriophage T4. *Nature* 22: 680–685.

Maxted, N. 1995. An Ecogeographical study of *Vicia* subgenus *Vicia.* IPGRI, Rome, Italy.

Mettin, D. and P. Hanelt. 1964. Cytosystematische Untersuchungen in der Artengruppe um *Vicia sativa* L. *Kulturpflanze* 12: 163–225.

Potokina, E.K. and E.E. Eggi. 1991. Electrophoresis of seed storage proteins in the solution of the problems on *Vicia sativa* agg. taxonomy. *Bulletin of Applied Botany, Genetics and Plant Breeding* 139: 112–123.

Repyev, S.I. and A.K. Stankevich. 1989. Classification of the cultivated forms of common vetch (*Vicia sativa* L.). *Bulletin VIR* 190: 7–11.

Tarlakovskaya, A.M. 1979. Seed proteins as markers of species and genera in Vicieae Bronn. *Bulletin VIR* 92: 53–59.

Tarlakovskaya, A.M., E. Eggi, I.P. Gavrilyuk and J. Belyaeva. 1990. Identification of pea cultivars using seed protein electrophoresis. Methodical Instructions. VIR, Leningrad, Russia.

Agronomy and Mechanization

Influence of Seed Size and Population on Seed Yield and Other Yield Characteristics of Faba Bean

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Abstract

In field experiments at Shambat Research Station, Sudan, over two seasons (1987 and 1988), small and large seeds of faba bean variety BF 2/2 were planted alone and with their bulk in comparison with three mixtures of large and small seeds in the proportions of 1:2, 1:1 and 2:1 with differences in competitive capacities assessed at two population levels (16.7 and 25.0 plants/m^2). Initial seed size had a large effect on interplant competition. Grain yield/ha from large-seeded plants increased, while that from small-seeded plants decreased when the two were grown together. Varying the proportion of large to small seeds planted affected the grain yield/ha and number of pods/plant. The number of pods/plant was by far the most important yield component influenced by seed size in 1987. Increasing the population from 16.7 to 25.0 plants/m^2 increased seed yield/ha by 7.3 and 18.5% in 1987 and 1988, respectively, and the reverse was true for seeds/plant and number of pods/plant. This suggests that plant uniformity obtained by uniform seed size may be a basic requirement in achieving higher yield levels.

Introduction

Seed size, both volume and density, has been recognized as a factor affecting plant growth and development since the early 1900s (Kiesselbach and Helm 1917). More vigorous seedlings produce more vigorous plants as the season progresses, unless some environmental factor differentially affects the performance of the plants. Many workers report a positive relationship between seed size and seed yield and biomass of sweet clover (*Melilotus officinalis* (L.) Lam.) (Haskins and Gorz 1975), subterranean clover (*Trifolium subterraneum* L.) (Black 1957), snap bean (*Phaseolus vulgaris* L.) (Clark and Peck 1968) and soybean (*Glycine max* (L.) Merr.) (Smith and Camper 1975). The last of these found that plants grown from either large or small soybean seeds planted in separate rows exceeded the yield of plants produced from a mixture of large and small seeds in the same row.

However, other studies have revealed little relationship between seed size and yield of soybean (Sato and Kamiyama 1959; Singh et al. 1972; Johnson and Ludders 1974), alfalfa (*Medicago sativa* L.) (Cooper et al. 1979), sunflower (*Helianthus annus* L.) (Robinson 1974) and rape (*Brassica naupus* L.) (Major 1977). Salih and Salih (1980) and Salih (1981) report no effect of seed size on grain yield and yield components of both faba bean (*Vicia faba* L.) and dry bean (*Phaseolus vulgaris* L.)

تأثير حجم البذور والكثافة النباتية على الغلة البذرية وخواص الغلة الأخرى للفول

الملخص

زرع في تجارب حقلية في محطة بحوث شمباط بالسودان، وعلى مدى موسمين زراعيين (1987 و1988)، فول صغير البذرة وفول كبير البذرة من الصنف BF2/2 كل على حدة ثم مع مجموعاتهما بهدف إجراء مقارنة بين ثلاث خلطات من البذور الكبيرة والصغيرة بنسب 2:1، 1:1، 1:2، إلى جانب فروق في القدرات التنافسية المحددة بمستويين من الكثافة النباتية (16.7 و25.0 نبات/م2). وكان للحجم الأولي للبذور تأثير كبير على المنافسة بين النباتات، فقد ازدادت الغلة الحبية/هـ للنباتات ذات البذور الكبيرة في حين تناقصت للنباتات ذات البذور الصغيرة عندما زرعا مع بعضهما البعض. وإن تباين نسبة النباتات الكبيرة البذور إلى الصغيرة البذور قد أثر على الغلة/هـ وعدد القرون/النبات، وكان عدد القرون/النبات هو مكون الغلة الأكثر أهمية الذي تأثر بحجم البذور في 1987. وقد أدى ازدياد الكثافة النباتية من 16.7 إلى 25.0 نبات/م2 إلى ارتفاع الغلة البذرية/هـ بنسبة 7.3 و18.5% في 1987 و1988 على التوالي، والعكس صحيح أيضاً بالنسبة للبذور/النبات وعدد القرون/النبات. وهذا يوحي بأن وحدة النبات القائمة على حجم موحد للبذور قد يكون مطلباً أساسياً للحصول على مستويات أعلى من الغلة.

Faba beans are highly variable in their response to plant population. At Zeidab, Salih (1979) studied the effect of four plant densities (1, 2, 3 and 4 seeds/hill at a hill spacing of 20 × 60 cm). He found that seed yield per hectare at 2, 3 and 4 seeds/hill was greater than yield of 1 seed/hill by 25, 34 and 39%, respectively. At Shambat, Taha et al. (1983) and Salih (1987) report that plants at a density of 3 seeds/hill out-yielded those at 1 and 2 seeds/hill by 12.7 and 10.2%, respectively, in 1983 and 11.3 and 6.8%, respectively, in 1987.

Faba bean is normally planted as ungraded seeds. As yields increase, seed size could become an increasingly important factor in maximizing yields. Competition that causes stunting of plants and fewer pods results in inefficient use of environmemtal resources.

The studies reported here were conducted to obtain quantitative data on the effect of seed size and plant population on the productivity of faba bean.

Material and Methods

Two lots of faba bean seed, designated as small and large in 1987 (33.3 vs 51.4 g/100 seeds) and 1988 (31.4 vs 48.9 g/100 seeds), were obtained by sieving and hand-selecting from bulk samples of variety BF 2/2. The small-seed and large-seed lots made up 22 and 39, respectively, in 1987 and 20.5 and 33.3%, respectively, in 1988, of the total number of seeds of the bulk samples in both seasons.

The experiments were sown on 28 October 1987 and 1 November 1988 at Shambat Research Farm, Sudan. The soil was heavy clay with a pH of 8.5.

A split-plot design was used for this experiment. The main plots were two population levels, 16.7 and 25.0 plants/m^2. Seed-size treatments were the subplots and these were:

(a) bulk or original seed lot;
(b) small seed;
(c) large seed;
(d) a 1:2 mixture of small to large seed;
(e) a 1:1 mixture of small to large seed;
(f) a 2:1 mixture of small to large seed.

The seeds were planted into a flat seedbed by hand to achieve an even distribution within the row.

The two population levels, 16.7 and 25.0 plants/m^2, were obtained by sowing 2 and 3 seeds/hill, respectively, on hills 20 cm apart, in rows 60 cm apart. Subplots were 3.6 × 6.0 m, of which 2.4 × 5.2 m was harvested for seed.

Each experiment received 10 irrigations at 7- to 10-day intervals, each plot being covered by 3–5 cm water at each irrigation.

Total seed yield was recorded for every subplot. Pods/plant, seeds/pod and 100-seed weight were determined from 20 plants/subplot selected at random. Plant stand was determined by counting the number of plants in each subplot at harvest.

Results and Discussion

Levels of significance for the components of variation, seed sizes, plant populations and their interactions for 1987 and 1988 are shown in Table 1. The effects of seed size and plant population on the different traits were not consistent in the two seasons. They tended to be independent of each other for all traits, with the exception of number of seeds/pod in 1987 and 100-seed weight in 1988, as indicated by the presence of non-significant f-values obtained from the interaction of seed size × plant population.

Table 1. Significance of effect (f-values) of faba bean seed size and population experiment on yield and yield components†.

Character	Components of variation		
	Seed size and their proportion	Plant population	Interaction seed size × population
	1987		
Pods/plant	*	**	ns
Seeds/pod	ns	ns	**
100-seed weight	*	ns	ns
Plant density	ns	**	ns
	1988		
Seed yield/ha	*	**	ns
Pods/plant	ns	**	ns
Seeds/pod	ns	*	ns
100-seed weight	*	ns	*
Plant density	ns	**	ns

† There was no significant effect of seed size, population or their interaction on variation in seed yield per plant in either year, or on seed yield/ha in 1987.

ns, *, ** not significant, and significant at $P = 0.05$ and $P = 0.01$, respectively.

Seed-size categories and their seed proportion treatments had a significant effect on seed yield/ha in 1988 (Table 2). Larger seeds out-yielded the ungraded (bulk) seeds, small seeds and the proportion of small to large seeds of 2:1 by 18.2, 10.2 and 18.6%, respectively.

The superior seed yield production of plants from larger seeds resulted mainly from additional pods/plant and heavier seeds. Increasing the plant population from 16.7 to 25.0 plants/m^2 increased seed yield/ha significantly (P = 0.05) in 1988 (Table 2). Murinda and Saxena (1985) found that increasing the plant population from 16.7 to 33.3 plants/m^2 increased seed yield in only one of four seasons studied.

The plant populations for all seed-size grades and proportions were equal and higher in 1988 (Table 4) than in 1987 (Table 3). The lowest plant populations were obtained in 1987 from plots planted with the small seed and the 1:2 proportion of small to large seeds.

The effect of plant population on the number of seeds/pod was not significant in either year (Table 3). The fewest seeds/pod was obtained from plants grown at a density of 16.7 plants/m^2. In general, however, number of seeds/pod was little influenced by crop density as found by Ishag (1971) and Pandey (1981).

Table 2. Effect of two seed sizes and their mixtures and two population densities† on seed yield (kg/ha) of faba bean, Shambat, 1987 and 1988.

Seed size	1987		Mean	1988		Mean
	P1	P2		P1	P2	
Ungraded (bulk)	2265	2729	2497	2021	2641	2331
Small	2517	2626	2571	2344	2771	2558
Large	2789	3158	2973	2692	3009	2850
1:2 small to large	2443	2542	2492	2579	2928	2754
1:1 small to large	2428	2473	2450	2526	3156	2841
2:1 small to large	2535	2620	2577	1899	2743	2321
SE (±)	183.6		129.8	202.4		143.1
Mean	2496	2691	2593	2344	2875	2609
SEM (±)	142.4			19.8		

† P1 = 16.7 plants/m^2; P2 = 25.0 plants/m^2.

Bulk seed gave the lowest seed yield/plant in both seasons (Tables 3 and 4). Large seeds and the proportions of 1 small:2 large and 1 small:1 large gave the highest seed-yield means in 1987 and 1988.

Plant population had no significant effect on seed yield/plant or 100-seed weight in either season (Tables 3 and 4). The seed yield/plant tended to be greater at the lower plant population of 16.7 plants/m^2 in 1987 (Table 3). Seed-size grades and the mixture treatments had no significant effect on number of pods/plant, except in 1987 when the larger seeds and the proportions of 1 small:2 large and 1 small:1 large had the most pods, while the ungraded seed and the proportion 2:1 had the fewest pods (Table 3). In 1988, the number of pods/plant was almost the same in all treatments, except the 1:1 mixture (Table 4).

In both seasons, there was a consistent trend for the number of pods/plant to decrease as the number of plants/m^2 increased (Tables 3 and 4).

At harvest, the established plant stands were less than the desired plant density in both seasons. The plant stand percentages at 16.7 and 25.0 plants/m^2 were 82 and 78, respectively, in 1987, and 92.8 and 94.4, respectively, in 1988.

Seed size had no effect on plant stand in either season.

Seed-size categories and their proportion treatments had no significant effect on number of seeds/pod in either season (Tables 3 and 4).

In conclusion, it can be said that under the conditions of this study, the planting of large seeds may be advantageous, but factors such as the economics involved should be taken into account. Where population or other factors are varied, the results might not be the same.

The effects of interplant competition were as large as the result of variation in initial seed size. Methods for the selection of individual plants for agronomic traits in segregating populations under similar conditions should take this source of variation into account for improving efficiency.

Plant uniformity may be a basic factor in achieving higher yields by increasing population. Uniformity in the faba bean population normally contributes to greater seed yield/hectare. The planting of uniformly-sized seeds contributes significantly to the development of a uniform plant population.

Table 3. Effect of two seed sizes and their mixtures and two population densities† on some yield components of faba bean, Shambat, 1987.

Seed size	Seed yield/ plant (g)			Pods/plant			Seeds/pod			100-seed wt (g)			Plant density (per m^2)		
	P1	P2	$\bar{X}$	P1	P2	$\bar{X}$	P1	P2	$\bar{X}$	P1	P2	$\bar{X}$	P1	P2	$\bar{X}$
Ungraded (bulk)	14.7	13.8	14.2	13.6	15.2	14.4	2.57	2.13	2.35	38.5	36.0	37.2	14.0	21.4	17.7
Small	17.5	19.8	18.6	19.3	19.3	19.3	2.28	2.47	2.37	38.2	39.7	38.9	13.3	17.2	15.3
Large	18.5	23.7	21.1	23.3	17.2	20.2	1.67	2.66	2.16	41.3	41.5	41.4	14.3	21.0	17.6
1:2 small to large	21.7	22.1	21.9	21.4	21.5	21.4	2.43	2.25	2.34	40.2	40.5	40.4	13.9	19.6	16.7
1:1 small to large	19.4	24.3	21.8	23.6	16.2	19.9	1.99	2.92	2.45	40.0	38.8	39.4	12.6	18.3	15.5
2:1 small to large	16.7	18.9	17.8	21.0	15.3	16.5	2.13	2.41	2.27	39.0	42.5	40.8	14.0	19.7	16.9
SE (±)	2.52		2.06	2.02		0.14	0.17		0.12	0.15		0.81	1.22		0.86
Mean	18.0	20.4	19.2	19.8	17.4	18.6	2.18	2.47	2.32	39.5	39.8	39.7	13.7	19.5	16.6
SEM (±)	1.06			0.11			0.09			0.48			0.27		

† P1 = 16.7 plants/m^2; P2 = 25.0 plants/m^2.

Table 4. Effect of two seed sizes and their mixtures and two population densities on some yield components of faba bean, Shambat, 1988.

Seed size	Seed yield/ plant (g)			Pods/plant			Seeds/pod			100-seed wt (g)			Plant density (per m^2)		
	P1	P2	$\bar{X}$	P1	P2	$\bar{X}$	P1	P2	$\bar{X}$	P1	P2	$\bar{X}$	P1	P2	$\bar{X}$
Ungraded (bulk)	11.9	11.0	11.4	11.9	11.3	11.6	2.57	2.61	2.59	40.0	40.0	40.0	15.3	24.1	19.7
Small	13.9	11.4	12.7	14.0	10.9	12.4	2.58	2.69	2.64	41.3	39.2	40.3	16.4	24.2	20.3
Large	17.1	11.0	14.0	15.2	10.2	12.7	2.61	2.55	2.58	42.3	42.0	42.2	15.5	24.0	19.7
1:2 small to large	16.9	11.3	14.1	13.9	10.2	12.6	2.94	2.56	2.75	42.3	40.3	41.3	15.3	22.1	18.7
1:1 small to large	15.9	13.5	14.7	14.8	12.6	13.7	2.60	2.64	2.62	39.3	41.0	40.2	15.2	23.3	19.2
2:1 small to large	11.6	12.7	12.2	12.2	12.0	12.1	2.60	2.53	2.56	38.0	41.0	39.5	15.6	24.1	19.8
SE (±)	1.34		0.95	1.03		0.73	0.08		0.05	0.83		0.59	0.52		0.37
Mean	14.5	11.8	13.2	13.7	11.2	12.5	2.65	2.60	2.62	40.5	40.6	40.6	15.5	23.6	19.6
SEM (±)	0.63			0.09			0.01			0.21			0.46		

References

Black, J.M. 1957. The early vegetative growth of three strains of subterranean clover (*Trifolium subterraneum* L.) in relation to size of the seed. *Australian Journal of Agricultural Research* 8: 335–351.

Clark, B.E. and N.H. Peck. 1968. Relationship between the size and performance of snapbean seeds. *New York State Agric. Exp. Station Bulletin* 819.

Cooper, C.B., R.L. Ditterline and L.E. Welty. 1979. Seed size and seeding rate effects upon stand density and yield of alfalfa. *Agronomy Journal* 71: 83–85.

Hasins, F.A. and H.J. Gorz. 1975. Influence of seed size, planting depth, and companion crop on emergence and vigor of seedling in sweetclover. *Agronomy Journal* 67: 652–654.

Ishag, H.M. 1971. Effect of spacing between holes and number of seeds per hole on yield and yield components. *In* Annual Report 1970/71. Hudeiba Research Station, Ed Damer, Sudan.

Johnson, D.R. and V.D. Ludders. 1974. Effect of planted seed size on emergence and yield of soybean (*Glycine max* (L.) Merr.). *Agronomy Journal* 66: 117–118.

Kiesselbach, T.A. and C.A. Helm. 1917. Relation of size of seed and sprout value to the yield of small grain crop. *Neb. Agric. Exp. Sta. Res. Bulletin* 11.

Major, D.J. 1977. Influence of seed size on yield and yield components of rape. *Agronomy Journal* 69: 541–543.

Murinda, M.V. and M.C. Saxena. 1985. Agronomy of faba beans, lentils and chickpeas. Pages 229–244 *in* Proceedings Faba Beans, Kabuli Chickpeas and Lentils in the 1980s, An International Workshop, 16–20 May 1983 (M.C. Saxena and S. Varma, eds). ICARDA, Aleppo, Syria.

Pandey, R.K. 1981. Time of sowing—a major factor for high seed yields of faba bean in northern India. *FABIS Newsletter* 3: 43–44.

Robinson, R.G. 1974. Sunflower performance relative to size and weight of achenes planted. *Crop Science* 14: 616–618.

Salih, F.A. 1979. Faba bean seed rate trial. *In* Annual Report 1978/79. Hudeiba Research Station, Ed Damer, Sudan.

Salih, F.A. 1981. Influence of seed size on yield and yield components of dry beans (*Phaseolus vulgaris* L.). Zeitschrift Fuer Acker- und Pflanzenbau (*Journal of Agronomy and Crop Science*) 150: 19–26.

Salih, F.A. 1987. Effect of nitrogen application and plant population per hill on faba bean (*Vicia faba* L.) yield. *FABIS Newsletter* 17: 27–30.

Salih, F.A. and H.S. Salih. 1980. Influence of seed size on yield and yield components of broad bean (*Vicia faba* L.). *Seed Science and Technology* 8: 175–181.

Sato, J. and K. Kamiyama. 1959. Studies on seed production of soybean. *Proceeding of the Crop Science Society of Japan* 24: 317–318.

Singh, J.N., S.K. Tripathi and P.S. Negi. 1972. Note on the effect of seed size on germination growth and yield of soybean (*Glycine max* L. Merr.). *Indian Journal Agricultural Sciences* 42: 83–86.

Smith, R.J. and H.M. Camper, Jr. 1975. Effects of seed size on soybean performance. *Agronomy Journal* 67: 681–684.

Taha, M.B., G.E. Mohamed, F.A. Salih and H.S. Ibrahim. 1983. Effect of date of planting and plant population on faba bean yield. Sudan 1983/84 crop season. Paper presented at the Fifth Annual Coordination Meeting. ICARDA/IFAD Nile Valley Project on Faba Bean, 13–17 September 1984, Cairo, Egypt. ICARDA, Aleppo, Syria.

Pests and Diseases

Evaluation of Faba Bean Cultivars for Resistance to Black Root Rot (*Fusarium solani*) in Ethiopia

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Abstract

The production of faba bean in the major growing areas of Ethiopia is constrained by several biotic and abiotic stresses. One of the biotic factors is root rot caused by *Fusarium solani*. According to surveys made in Jiru, Gebre Gurach, Fiche, Debre Tsigie and Ghoa Tsione in northwest Shoa, the incidence of root rot was 75, 14, 65 33 and 55%, respectively. The disease incidence is higher on vertisol (black clay soil) than on red soil. Three-hundred faba bean accessions were screened for resistance to wilt/root rot. A total of 45 accessions was resistant/tolerant to the disease. Some accessions (e.g. Bichena 86-1, Bichena 86-3, 79533024-2ZPN-1 and Eth 86-120-2) showed resistance, with 3–7% mortality.

Key words: *Vicia faba; Fusarium solani;* faba beans; disease resistance; root rots; Ethiopia.

تقييم أصناف من الفول لمقاومة تعفن الجذور الأسود (*Fusarium solani*) في إثيوبيا

الملخص

يعوق إنتاج الفول في مناطق زراعته الرئيسية بإثيوبيا العديد من الإجهادات الأحيائية واللا أحيائية. ويعتبر تعفن الجذور الذي يسببه *Fusarium solani* واحداً من العوامل الأحيائية. وطبقاً للدراسات الحصرية التي أجريت في جيرو، جبر جوراش، فيشي، ديبر تسيجي، غوا تسيوني في شمال غربي شوا، بلغت نسبة الإصابة بتعفن الجذور 75، 14، 65، 33، و55% على التوالي. وكانت الإصابة بالمرض أعلى في الترب الفيرتيزولية (الترب الطينية السوداء) منها في الترب الحمراء. تمت غربلة 300 مدخل من الفول لمقاومة الذبول/تعفن الجذور، فكان 45 منها مقاوماً/متحملاً للمرض. وأظهرت بعض المدخلات مثلاً (Bichena 86-1,Bichena 86-3, 79533024-2ZPN-1 و(Eth 86-120-2 مقاومة، مع نسبة موت 3-7%.

Introduction

Faba bean (*Vicia faba* L.) is the most important grain legume grown in Ethiopia. However, its yield is very low in farmers' fields. This is mainly because farmers use mostly local cultivars that are either poor yielders or susceptible to different diseases and other biotic and abiotic stresses (Habtu and Dereje 1985; Salt 1981). In Ethiopia, various diseases have been recorded on faba bean. Among these, soil-borne fungi such as wilt and root rots cause about 20–50% yield losses annually, and under severe conditions this figure reaches 60–70% in farmers' fields (Stewart and Dagnatchew 1967; Habtu and Dereje 1985). Among the root-rot-causing fungi, *Rhizoctonia solani* Kuhn, *Fusarium solani* (Mart.) Sacc. em. Snyder & Hansen *p.p.*, *F. oxysporum* Schlecht. em. Snyder & Hansen, and *Pythium* sp. have been reported (Habtu and Dereje 1985; Uticar and Sulaiman 1976). *Fusarium solani*, which causes black root rot, is considered the most important soil-borne fungus in many parts of the country (Stewart and Dagnatchew 1967; Habtu and Dereje 1985). The ecological factors that influence or favor this disease are high soil moisture, low nutrient supply, wet soil, medium altitude, and occurrence of other diseases and pests (Salt 1981; Uticar and Sulaiman 1976). Developing cultivars resistant to this disease is highly desirable for economic and environmental reasons (Burke and Silberayel 1965; Salt 1981; Bruchl 1983).

Accordingly, the objective of this research work was to evaluate indigenous and exotic faba bean germplasm against black root rot in the field and greenhouse.

Material and Methods

Isolation and multiplication

Five isolates of *Fusarium solani* were obtained from a wide range of naturally infected roots of susceptible local cultivars collected from Ambo, Inewari, Holetta, Selale and Bichena areas. One-centimeter portions from infected roots were washed with tap water and dried on filter paper (Cooper 1953). Pith and outer cortex were removed to avoid decay, and parts of the xylem ring were used exclusively. The root pieces were disinfected, dried on filter paper, plat-

ed on potato dextrose agar (PDA), then incubated for three days at 20–25°C. Pure cultures were obtained by transferring pieces of mycelium from the outside of the colonies to test tubes with PDA (Dixon and Doodson 1970). From these, monospore cultures were produced. For multiplication purposes, high concentrations of microconidia were grown on a czpek-dox solution of 3-day-old culture in a number of test tubes and petri dishes (Dixon and Doodson 1970; Habtu and Dereje 1985).

Blotter-paper screening

Blotter-paper technique (Roberts and Kraft 1971; Nene et al. 1981) was used to confirm the results of the field experiments Each of the test entries was planted in plastic pots (30 cm diameter) containing sterilized sand. After seven days, seedlings were carefully uprooted and soaked in distilled water. The root tips were removed and dipped for 5 min in spore suspension (10^6 spores/ml) prepared from a two-week old culture of *F. solani*. Inoculated lines were kept separately on a five-layer blotter paper on enamel trays sufficiently moistened (> 90%) and incubated for 8–10 days at 25°C in a greenhouse.

Host resistance in field

A mixture of highly susceptible faba bean lines (Kassa and Coll 1978) was planted by hand in a sick-plot developed during the short rainy season to increase inoculum density (Nene et al. 1981). In addition, pure culture of *Fusarium solani* was prepared and mixed with autoclaved faba bean seed. After two weeks of incubation the culture was distributed evenly on the sick-plot during planting of the test material (Nene et al. 1981). Three-hundred faba bean lines were received from the highland pulse coordinator (origin: Ethiopian gene bank and the International Center for Agricultural Research in the Dry Areas, Aleppo, Syria), and planted by hand during the main season in plots of two 4-m rows, 40 cm apart, with 40 seeds/row. A susceptible check was planted after every two test rows (Zaumeyer and Meners 1975). The design was a randomized complete block with four replicates. Data collected during the study were: root rot incidence (% mortality), plant stand (%), yield (kg/ha), 100-seed weight (g) and pods/plant.

Results and Discussion

Isolation and multiplication

The fungi associated with root rots were *Fusarium solani*, *F. oxysporum*, *Rhizoctonia* spp., *Pythium* spp. and, to a lesser extent, *Sclerotium rolfsii* Sacc. The most widely occurring fungus isolated was *F. solani*.

Host resistance

Ninty-six cultivars of faba bean showed resistant and tolerant reactions to black root rot when tested in the field and with the blotter-paper technique (Table 1).

Table 1. Reaction of some of the faba bean accessions tested to black root rot.

Line	Field test					Lab test
	Plant stand (%)	Mortality (%)	Yield (kg/ha)	1000-seed wt (g)	Pods/ plant	Mortality (%)
Eth 86-120-2	98.9	5	–	433	15	5
Bichena 86-1	98.9	4	3470	390	16	4
Bichena 86-3	98.8	5	3910	401	15	6
83Latt 30-168-2DZR-2	97.4	7	4371	396	15	6
79533024-2-2PN-1	97.3	7	5010	433	16	3
L82085-1-2-3-1	98.1	6	4970	358	19	7
NC580CS-5	89.3	10	3311	437	14	12
CD20DK-338-2-2	69.9	23	2143	379	11	25
S83386-1	98.2	5	5031	600	14	7
282098-11-14	76.3	21	2080	301	13	19
74TA59-7-3-1	77.6	14	5344	349	14	10
PGRC/E Acc No208102	70.3	22	2013	481	12	20
L82087	65.2	21	2130	600	14	19
PGRC/E Acc No27354	79.3	18	3520	397	15	16
PGRC/E Acc No027355	80.1	16	3400	389	15	15
PGRC/E Acc No027361	89.7	10	3100	439	16	15
PGRC/E Acc No203128	88.7	12	3200	320	15	10
Local check	45.6	27.9	1773	150	8	48.3

Accessions like Bichena 86-1, Bichena 86-3, Eth 86-120-2 and L82085-1-2-3-1 were highly resistant to root rot. Multilocational testing for root rots was carried out through active cooperation between the highland pulses research program at Holetta and the Plant Protection Research Center at Ambo, and sources of resistance have been found. More than 45 root-rot-resistant sources are available (Saeed et al. 1987). These root-rot-resistant materials are being incorporated into high-yielding faba bean lines (Uticar and Sulaiman 1976). Faba bean lines such as Bichena 86-1, Bichena 86-3 and Eth 86-120-2 were resistant at several locations in multilocation testing.

Conclusions

Black root-rot-resistant/tolerant faba bean lines tested have been given to the breeders. The F_1 generation of 20 crosses has been generated. These crosses will be conducted in three ecological zones (Ambo, Inwari and Selale). There is also a need for testing of agronomy like seed bed and fertility improvement in the black clay soil.

References

Bruchl, G.W. 1983. Nonspecific genetic resistance to soil borne fungi. *Phytopathology* 73: 948–951.

Burke, D.W. and M.J. Silberayel. 1965. Testing beans for field tolerance to fusarium root rot. *Phytopathology* 55: 1045–1046.

Cooper, W.E. 1953. Variation in the pathogenicity of *Sclerotium rolfsii* Sacc. isolates. *Phytopathology* 43: 289–290.

Dixon, G.R. and J.K. Doodson. 1970. Methods of inoculating pea seedlings with fusarium wilt. *Journal of the National Institute of Agrocutural Botany* 12: 577–584.

Habtu Assefa and Dereje Gorfu. 1985. A review of food legume disease in Ethiopia. Pages 345–401 *in* A Review of Crop Protection Research in Ethiopia (Tsedeke Abate, ed.), 4–7 February 1985, Addis Ababa, Ethiopia. IAR, Addis Ababa, Ethiopia.

Nene, Y.L., M.P. Haware and M.V. Reddy. 1981. Chickpea diseases. Resistance screening techniques. ICRISAT Information Bulletin No. 10: 5–10.

Roberts, D.D. and Y.M. Kraft. 1971. A rapid technique for studying fusarium wilt of peas. *Phytopathology* 61: 342–343.

Saeed, E.M.A., S.O. Freigoun, M.E. Omer and S.B. Hanounik. 1987. Effect of wilt and root rot disease complex on some quality parameters of faba bean (ful masri) seeds. *FABIS Newsletter* 19: 23–26.

Salt, G.A. 1981. Factors affecting resistance to root rot and wilt diseases. Pages 259–270 *in* Faba Bean Improvement (G. Hawtin and C. Webb, eds). Matinus Nijhoff, The Hague, The Netherlands.

Stewart, R.D. and Dagnatchew Yirgou. 1967. Index of Plant Disease in Ethiopia. *Expt. Bull.* No. 30. HISU College of Agriculture. Debre Zeit.

Uticar, P.G. and M. Sulaiman. 1976. Note on fusarium wilt resistance in peas. *Indian Journal of Mycology and Plant Pathology* 6: 68–70.

Zaumeyer, W.J. and J.P. Meners. 1975. Desease resistance in beans. *Annual Review of Phytopathology* 13: 313–314.

Studies on the Control of *Orobanche crenata*. I. Use of *Azotobacter* spp. and *Escherichia coli* Transformants to Break Dormancy of *Orobanche crenata*

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Abstract

Crude DNA preparations from *Vicia faba* were used to prepare transformants of *Azotobacter* spp. and *Escherichia coli*. The transformants produced *Vicia*

دراسات حول مكافحة الهالوك *Orobanche crenata* I-استخدام محورات *Azotobacter* spp. و*Escherichia coli* لقطع السُبات في الهالوك

الملخص

استخدمت تحضيرات DNA الخام المأخوذة من الفول *Vicia faba* لتحضير محورات من *Azotobacter* spp. و*Escherichia coli*. وقد أنتجت تلك المحورات منشطات شبيهة بما تنتجه *Vicia faba* عملت على تحفيز إنبات بذور العشب الطفيلي، الهالوك. كانت محورات *A. chroococcum* و*E. coli* الأكثر فعالية، في حين كانت محورات *A. vinelandii* غير فعالة، وأظهرت المستعمرات الفردية لنوعي البكتيريا الأولين نشاطاً متبايناً. ويُرجح أن تتمتع هذه المحورات بإمكانات عوامل المكافحة الحيوية وذلك لأنها تسبب إنباتاً بلا عائل لبذور العشب الطفيلي.

***faba*-like stimulants which promoted the germination of seeds of the parasite *Orobanche crenata*. Transformants of *A. chroococcum* and *E. coli* were most active, while those of *A. vinelandii* were inactive, and individual colonies of the former two bacterial species exhibited variable activity. It is proposed that such transformants have potential as biocontrol agents by causing non-host germination of parasitic weed seeds.**

Key words: *Vicia faba*; faba beans; *Azotobacter*; *Escherichia coli*; *Orobanche crenata*; DNA; seed; germination; dormancy breaking; weed control; Egypt.

Introduction

Parasitic weeds (e.g. *Orobanche* spp.) cause considerable damage to many crops in tropical and sub-tropical regions (Parker 1986, 1994). However, the control of *Orobanche* by various techniques is dificult and of limited success. It is also evident that chemicals such as gibberellins, kinetin, vitamins, sugars and amino acids are unable to break the dormancy of *Orobanche* spp. seeds *in vitro* (Chabrolin 1938; Edwards et al. 1976; Garas et al. 1974; Khalaf 1982). The naturally occurring compound Strigol, from cotton, which stimulates the germination of *Orobanche* spp. cannot be used to promote non-host "suicidal" germination in soil as its synthesis is too complex to be economical and it has poor stability under soil conditions (Mangnus and Zwanenburg 1989). Considerable progress has been made to synthesize and modify a new series of novel Strigol analogues as a new approach for controlling *Orobanche* spp. and *Striga* spp. parasitism (Zwanenburg et al. 1994; Welzel et al. 1994); however, such efforts have not been entirely successful.

Therefore, attention has turned to developing genetically modified bacteria for breaking the dormancy of *Orobanche* spp. seed *in vitro*, which might be developed eventually as a biocontrol agent against *Orobanche* parasitism. Little has been done on the application of genetic-engineering techniques to breaking the dormancy of *Orobanche* spp. seed *in vitro*. Khalaf and Ali (1991) attempted, unsuccessfully, to produce *Vicia faba* stimulant(s) for inducing *O. crenata* Forsk. germination by developing genetically modified *Azotobacter* strains. Meanwhile, Bouillant et al. (1996) suggest that the bacteria *Azospirillum brasilense* could be a good tool for reducing parasitic weed infestation with a potential plant growth promoting rhizobacteria (PGPR), since their results indicated that *A. brasilense* isolated from soil of a sorghum field was able, *in vitro*, both to inhibit the germination of *Striga* seeds and to promote the growth of sorghum.

The objective of the current research was to investigate the application of different methods of transforming selected bacteria of *Azotobacter* spp. and *Escherichia coli*, with faba bean (*Vicia faba* L.) DNA associated with the production of *Orobanche* germination stimulant(s), leading to transformants able to trigger the germination of *Orobanche crenata* seed *in vitro*, and in soil.

Material and Methods

Plant and bacteria material

Faba bean (cv Giza 2, 45 days old) roots were used as a source of DNA, while *Azotobacter chroococcum*, *A. vinelandii* and *Escherichia coli* wild-type strains were used as recipients. *Azotobactyer* spp. were supplied by the Department of Microbiology, Faculty of Agriculture, Ain Shams University, Egypt. *Escherichia coli* was obtained from the Carolina Biological Supply Company. Specific media were selected for the growth and transformation of *Azotobacter* spp. (*Azotobacter* growth medium, AM, Page and Von-Tigerstrom 1979; *Azotobacter* transformation medium, TM, Click et al. 1986) and nutrient broth medium (Nb) for *Escherichia coli* (Allen 1953).

Isolation of DNA for *Orobanche* stimulant(s) production from *Vicia faba*

High molecular weight DNA of *V. faba* root was extracted by the method of Bendich and Bolton (1967). The roots (100 g fresh weight) were cut up and placed in a mortar with a solution (10 ml) of 1% sodium dodecyl sulfate (SDS) and 0.1 *M* disodium EDTA, and saline sodium citrate (30 ml, 0.15 *M* sodium chloride and 0.01 *M* sodium citrate, SSC). The roots were ground at room temperature until a homogeneous paste was obtained, which was then transferred to an equal volume of chloroform containing 1% octanol. The mixture was shaken rapidly for 5 minutes and centrifuged at 4000 rpm for 10 minutes to separate the phases. The upper aqueous layer, which contained the DNA, was poured into a pre-heated bottle and incubated for 5 minutes at 70–80°C in a water bath. This extract was quickly cooled in an ice bath and sufficient sodium chloride added to give a final concentration of 1 *M*. An equal volume of chloroform containing 1% octanol was added to the extract, then shaken, and centrifuged for 10 minutes. The aqueous layer was transferred to a clean sterilized tube, and two volumes of cold (–20°C) absolute ethanol layered over the extract. The layers were slowly mixed and the DNA fibers were collected by centrifugation. The DNA precipitate was dissolved in 35 ml

SSC (0.1%) buffer by gently shaking, and purification of DNA carried according to Charles (1970).

Transfer of *Vicia faba* DNA for *Orobanche* stimulant biosynthesis to bacteria

***Azotobacter* procedure (Page and Von-Tigerstrom 1978)**
Azotobacter chroococcum and *A. vinelandii* wild-type strains were induced for competence in Mo- and Fe-deficient nitrogen-free medium (TM) for 22–24 h at 30°C by rotary shaking. Purified *V. faba* DNA and *A. chroococcum* and *A. vinelandii* suspensions (1 ml) were added together to form competent cells in 10 ml Burk buffer (TM). After 20 minutes, the transformed cells were plated (0.5 ml) on Burk nitrogen-free medium using suitable dilution (10^1 to 10^4; Table 1). The plates were incubated at 30°C for seven days and a selection of active transformants made using an *Orobanche* germination bioassay technique (described below).

***Escherichia coli* transformation procedure**
Crude *V. faba* DNA was mechanically fragmented using a microsyringe through which the DNA solution was passed several times. The fragmented DNA (1 ml) was added to bacterial culture (1 ml) grown on nutrient broth for 24 h. The mixture was incubated for one hour on ice, half an hour at 24°C and then re-incubated for one hour on ice (Hoekstra et al. 1980). Samples of the transformation mixture (0.5 ml) were diluted with sterilized water (10^1 to 10^4; Table 1), then plated onto nutrient agar and incubated for seven days at 30°C. The selection of transformants active in the *Orobanche* germination assay were made.

***Orobanche* germination assay technique**

Sterilized No. 1 Whatman filter paper (5.5-cm diameter) was placed on punctuated growing bacteria colonies. *Orobanche crenata* seeds were soaked in distilled water for 10 days, sterilized with sodium hypochlorite (5%, 2 min), and then washed with sterilized distilled water. The seeds were transferred using a sterilized brush-tip onto the sterilized filter paper covering the growing colonies. Each treatment was applied three times in a complete randomized block design and the plates were incubated at 25°C for 10 days. The germination of *O. crenata* was examined by using a binocular microscope (50×). The number of germinated seeds, together with total number of seeds used in each dish, were recorded and the mean percentage germination calculated. The data was subjected to analysis of variance methods (Snedecor and Cochran 1967).

Selection of transformants of *Azotobacter* and *Escherichia coli*

The transformed bacteria active below the germinated *Orobanche* seeds were identified, scored and isolated separately for each strain of their specific medium. The isolated transformants of *Azotobacter* spp. and *E. coli* were retested to select the efficient transformants by using the *Orobanche* germination bioassay technique.

Results and Discussion

Activity of *Azotobacter* spp. and *Escherichia coli* transformants on the *in-vitro* germination of *Orobanche crenata*

Transformation of *Azotobacter* spp. and *E. coli* by crude DNA from *V. faba* revealed significant effect in the stimulation of the *in-vitro* germination of *O. crenata* (Table 1). *Azotobacter vinelandii* transformants appeared devoid of any activity. *E. coli* transformants showed some activity, but the greatest activity was obtained with transformants of *A. chroococcum*. Original untransformed cultures of the organisms showed no activity. Dilutions of the cultures of *A. chroococcum* transformants showed significant increases of activity in the tests for *O. crenata* germination; no such effect was observed with *E. coli* (Table 1).

The germ tube of stimulated *O. crenata* was stunted, coupled with dissolution of the seed coat of both germinated and ungerminated seeds, irrespective of whether germination was attempted with *A. chroococcum* or *E. coli* (Fig 1).

Table 1. Activity of *Azotobacter chroococcum* and *Escherichia coli* transformants on the germination of *Orobanche crenata* seed *in vitro*.

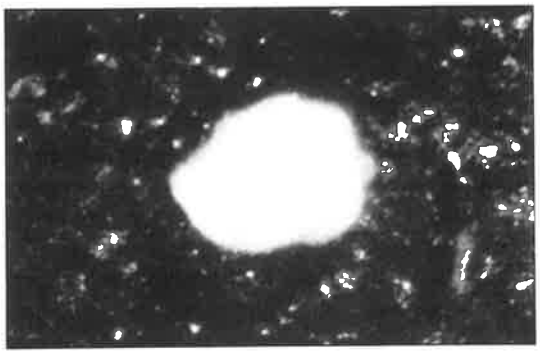
Dilution of bacterial transformants	Germination of *O. crenata* seed (%)	
	A. chroococcum	*E. coli*
10^4	22.22	6.67
10^3	41.76	0
10^2	6.44	6.60
10^1	0	8.89
10^0 (orignal culture)	6.44	4.76
Control (untransformed bacteria)	0	0
LSD (5%)	1.09	0.39

Activity of selected colonies of *Azotobacter* spp. and *Escherichia coli* transformants on *in-vitro* germination of *Orobanche crenata*

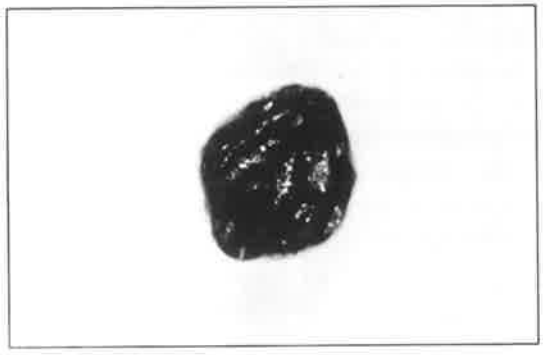
The activity of selected colonies of *Azotobacter* spp. and *E. coli* transformants at various dilutions is summarized in Table 2. Selected colonies of *A. vinelandii* failed to stimulate any germination of *O. crenata*, and only diluted sub-cultures of colonies of *A. chroococcum* exhibited activity. This is in contrast with the activity shown by the initial mixed culture of *A. chroococcum* found in the preliminary test (Table 1), even after dilution by 10^4. Sub-cultures of selected colonies of *E. coli* appeared to show much greater activity even after dilution by 10^3 and 10^4 than might be expected from the preliminary tests (Table 1). The selected colonies of transformed bacteria varied greatly in their ability to stimulate the germination of *O. crenata*. It is difficult to reconcile the greater activity of individual colonies of *E. coli* (Table 2), with the low activity of the initial transformed culture (Table 1); however, this might be explained partly by assuming that inactive transformants secrete *O. crenata* germination inhibitors and are subsequently eliminated during the selection of active colonies or reduced by dilution of the culture. As noted above, germination stimulation by selected colonies was characterized by short germ tube and loss of the seed coat (Fig. 1).

The present work was focused on studying the possibility of using genetically engineered bacteria of *Azotobacter* spp. and *Escherichia coli* strains as producers of *Orobanche crenata* germination stimulant(s), which might be eventually useful as biocontrol agents of *Orobanche* infestation. Subsequently, the advantage of selecting *Azotobacter* spp. in this study may be due to their added ability to act as biofertilizer to decrease the impact of using the synthetic fertilizer, while *E. coli* already exists in the soil as a result of using animal dung.

Transformation of *Azotobacter* spp. and *Escherichia coli* strains showed significant stimulatory effect on the germination of *Orobanche crenata* seed *in vitro*. However, such data contrasts with our earlier results (Khalaf and Ali 1991), which showed that *A. chroococcum* had no stimulatory action on the germination of *O. crenata in vitro*, this might

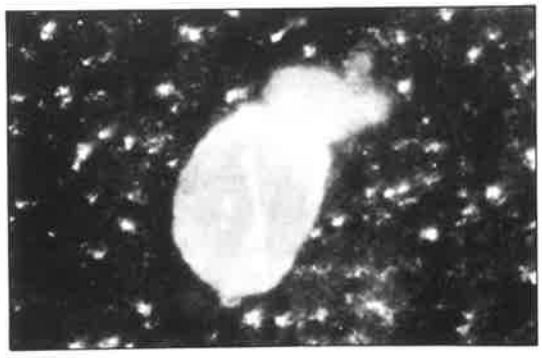


Control
(treated with untransformed bacteria)



Control
(untreated with bacteria)

Normal germination



Abnormal germination

Fig. 1. Different types of *O. crenata* germination associated with disappearance of seed coats, after treatment with *A. chroococcum* and *E. coli* transformants.

Table 2. Activity of selected colonies of *Azotobacter chroococcum* and *Escherichia coli* transformants on the germination of *Orobanche crenata in vitro.*

Concentration (cells/ml) of selected bacteria transformant	Colony reference number	Germination of *O. crenata* (%)
A. chroococcum		
	I	8.0
	II	4.0
10^2	III	0
	IV	0
	V	0
10^1	I–V	0
	I	9.0
10^0	II	13.0
(original culture)	III	8.0
	IV	6.7
	V	20.6
E. coli		
	I	26.4
	II	0
10^4	III	18.2
	IV	0
	V	0
10^3	I–V	0
	I	11.8
10^2	II	15.9
	II	15.9
	III	27.3
	IV	9.1
	V	0
	I	19.1
	II	1.8
10^1	III	0
	IV	0
	V	0
10^0	I	16.4
(original culture)	II–V	0
Controls (untransformed culture)	I–V	0

be attributable to different transformation techniques, different selected bacteria, or both.

The reduction of germ-tube length of germinated *O. crenata* seed after incubation *in vitro* with *A. chroococcum* and *E. coli* transformants might be related to the presence of both germination stimulant and inhibitor in the exudates of both bacteria. Alternatively, the stunting of the *Orobanche* germ tube might be associated with the presence of high levels of nitrogen, either from nitrogenous compounds in the growth medium of *E. coli* or nitrogen fixation by *A. chroococcum*. These suggestions are supported by the results of Van-Hezewij et al. (1991) who found that nitrogen in the form of ammonium or urea reduced, in most cases, the germination rates and length of the germ tube of *Orobanche* seeds. Also, Nandula et al. (1996) revealed that *in vitro* nitrogen-containing nutrient inhibited the radicle lengths of both *O. aegyptiaca* Pers. and *O. ramosa* L. to a greater extent by NH_4NO_3 and NH_4Cl than by KNO_3. Whitney (1979) points out that the ratio of the stimulant to inhibitor liberated by *Vicia faba* roots appears to affect the radical elongation of *Orobanche* seed, and probably the process of germination itself.

The disappearance of the seed coat of the germinated *Orobanche* seed *in vitro* might be attributable to the fact that *A. chroococcum* and *E. coli* transformants can secrete certain enzymes which are capable of degrading the seed coat of *Orobanche* seeds.

Thus, it may be concluded that the transfer of *V. faba* DNA containing *Orobanche* germination stimulant(s) to bacteria has given some useful leads for breaking the dormancy of *O. crenata in vitro*. However, while the present data are preliminary, the technique has considerable potential as a new technique for the biological control of *Orobanche* spp. parasitism in the long term.

References

Allen, O.N. 1953. Experiment in Soil Bacteriology, First ed. Burgess, USA.

Bendich, A.J. and E.T. Bolton. 1967. Relatedness among plants as measured by the DNA-agar technique. *Plant Physiology* 42: 959–967.

Bouillant, M.L., M. Kabir, G. Alexandre, C. Jacoud, L. Miche and R. Bally. 1996. Inhibition of *Striga* seed germination by bacteria isolated from soil under sorghum field. Pages 900–905 *in* Advances in Parasitic Plant Research. Sixth International Parasitic Weed Symposium, 16–18 April, Cordoba, Spain.

Chabrolin, C.H. 1938. La germination des graines de l'*Orobanche* speciosa. *C.R. Acad. Sci. Paris* 206: 1990–1992.

Charles, P. 1970. Isolation, preparation and characterization of deoxyribonucleic acids. *In* Uptake of Informative Molecules by Living Cells. (L. Ledoux, ed.). North Holland, Amsterdam.

Click, B.R., H.E. Brooks and J.J. Pasternak. 1986. Physiological effects of plamid DNA transformation on

Azotobacter vinelandii. *Canadian Journal of Microbiology* 32: 145–148.

Edwards, W.G., R.P. Hiron, and A.I. Mallet. 1976. Aspects of the germination of *Orobanche crenata* seeds. *Zeitshrift für Pflanzenphysiologie* 80: 105–111.

Hoekstra, W.P.M., H.E. Bergmans and E.M. Zuidweg. 1980. Transformation in *Escherichia coli*. Studies on the nature of donor DNA after uptake and integration. *Genetical Research* 35: 279–289.

Garas, N.A., C.M. Karssen and J. Bruinsma. 1974. Effect of growth regulating susbstances and root exudates on the seed germination of *O. crenata*. *Zeitschrift für Pflanzenphysiologie* 71: 108–114.

Khalaf, K.A. 1982. Some studies on the control of *Orobanche crenata* parasitism of *Vicia faba*. PhD Thesis, University of London.

Khalaf, K.A. and A.M. Ali. 1991. Preliminary attempts of producing genetically engineered *Azotobacter chroococcum* transformants able to produce *Orobanche* stimulant(s). *FABIS Newsletter* 28/29: 35–36.

Mangnus, E. and B. Zwanenburg. 1989. Design and synthesis of germination stimulant(s) for seeds of *Striga* and *Orobanche* spp. Pages 157–165 *in* Proceedings of the International Workshop on *Orobanche* Research, 19–22 August, Obermarchtal, Germany.

Nadula, V.K., C.L. Foy and J.H. Westwood. 1996. Environmental influences on germination of *Orobanche*. Pages 409–414 *in* Advances in Parasitic Plant Research. Sixth International Parasitic Weed Symposium, 16–18 April, Cordoba, Spain.

Page, W.J. and M. Von-Tigerstrom. 1978. Induction of transformation competence of *Azotobacter vinelandii* iron-limited cultures. *Canadian Journal of Microbiology* 24: 1590–1594.

Page, W.J. and M. Von-Tigerstrom. 1979. Optimal conditions for transformation of *A. vinelandii*. *Journal of Bacteriology* 139: 1058–1061.

Parker, C. 1986. Scope of the agronomic problems caused by *Orobanche* species. *In* Biology and Control of *Orobanche*. Proceeding of a Workshop, 13–17 January, Wageningen, The Netherlands.

Parker, C. 1994. The present state of the *Orobanche* problem. Pages 17–126 *in* Biology and Management of *Orobanche*. Proceedings of the Third International Workshop on *Orobanche* and Related *Striga* Research, Royal Tropical Institute, 8–12 November, The Netherlands,.

Snedecor, G.W. and W.G. Cochran. 1967. Statistical Methods. Iowa State University Press, Iowa, USA.

Van-Hezewijk, M.G., K.H. Linke, J.A.C. Verkleij and A.H. Pieterse. 1991. The effect of ammonium fertilizer in combination with bean. Pages 470–483 *in* Fifth International Symposium on Parasitic Weed, 24–30 June, Nairobi, Kenya.

Welzel, P., K. Frischmuth, C. Bergmann and K. Wegmann. 1994. Structure–activity relationships of Strigol analogues: results and conclusions. Pages 198–208 *in* Biology and Management of *Orobanche*. Proceedings of the Third International Workshop on *Orobanche* and Related *Striga* Research, Royal Tropical Institute, 8–12 November, The Netherlands.

Whitney, P.J. 1979. Broomrape seed germination stimulants and inhibitors from host roots. Pages 182–192 *in* Proceedings of the Second International Symposium on Parasitic Weeds, North Carolina, USA.

Zwanenburg, B., E.M. Mangnus, and J.W.F. Thuring. 1994. Strigol analogues: design, synthesis and biological. Pages 187–197 *in* Biological and Management of *Orobanche*. Proceedings of the Third International Workshop on *Orobanche* and Related *Striga* Research, 8–12 November, Royal Tropical Institute, The Netherlands.

Studies on the Control of *Orobanche crenata*. II. Effectiveness of *Azotobacter* spp. and *Escherichia coli* Transformants in Biological Control of *Orobanche crenata* on *Vicia faba* under Soil Conditions

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Abstract

Studies on the influence of culture temperature of *Azotobacter chroococcum* and *Escherichia coli* transformants on the germination of *Orobanche crenata* seed *in vitro*, indicated that *A. chroococcum* and *E. coli* cultured between 20 and 30°C were more efficient for inducing the germination of *O. crenata* seeds *in vitro* than organisms cultured at lower temperature (15°C). Selected active *A. chroococcum* and *E. coli* transformants cultured at 25°C and added to soil already infested with *O. crenata* seed showed promising results for controlling *O. crenata* parasitism on faba bean, irrespective of whether the transformants were applied once or twice to the soil. However, two applications of *E. coli* transformants were slightly more effective in reducing *O. crenata* infestation than was the application of *A. chroococcum* transformants. Preliminary data suggest that using genetically modified *A. chroococcum* and *E. coli* transformants, as producers of *O. crenata* germination stimulant(s), might be useful for biological control of *O. crenata* on faba bean.

Key words: *Vicia faba;* faba beans; *Azotobacter; Escherichia coli; Orobanche crenata;* seed; germination; *in vitro* culture; temperature; biological control; weed control; Egypt.

Introduction

Methods of controlling broomrape (*Orobanche* spp.) have long been considered unsuccessful. However, recent studies have highlighted the possibility of using genetically engineered bacteria as producers of *Orobanche* germination stimulant(s), which might eventually be useful as an indirect biocontrol agent against *Orobanche* infestation. Khalaf and Ali (1991) made a preliminary attempt to control *O. crenata* Forsk. parasitism on faba bean by developing genetically engineered *Azotobacter* spp. strains, which might be able to produce *Orobanche* stimulant(s) in addition to their ability to fix nitrogen, but the results were unsatisfactory.

Petzoldt (1979) reports that the occurrence of bacterial nodules (*Rhizobium* spp.) might provide a mechanism by which *Orobanche* spp. may bypass the root rhizodermis and achieve infection. Kasasian (1973) attempted to control *O. crenata* infestation by increasing legume nodules on *Vicia faba* L. roots, but showed that inoculating faba bean seeds with *Rhizobium leguminsarum* failed to reduce the infection of *O. crenata* on *Vicia faba*. Conversely, Khalaf (1982) found that *Vicia faba* root-nodule extracts have no stimulatory effect on the germination of *O. crenata* seeds *in vitro*, suggesting that there is no particular relationship between the presence and development of root nodules and *V. faba*

دراسات حول مكافحة الهالوك *Orobanche crenata* II- فعالية محورات *Azotobacter* spp. و*Escherichia coli* في المكافحة الحيوية للهالوك على الفول *Vicia faba* تحت ظروف التربة

الملخص

أشارت الدراسات حول تأثير درجة حرارة زراعة محورات *Azotobacter chroococcum* و*Escherichia coli* على إنبات بذور الهالوك في المختبر، إلى أن *A. chroococcum* و*E. coli* المستنبتة بين 20 و30 مْ كانت أكثر كفاءة على استحداث إنبات بذور الهالوك في المختبر من الكائنات الدقيقة المستنبتة بدرجة حرارة أدنى (15مْ). وقد أظهرت محورات *A. chroococcum* و*E. coli* المنتخبة الفعالة، والمستنبتة على درجة حرارة 25 مْ، والمضافة إلى تربة مُصابة أصلاً ببذور الهالوك، نتائج مبشرة لمكافحة تطفل الهالوك على الفول، بغض النظر عما إذا أضيفت المحورات مرة أو مرتين إلى التربة. إلا أن إضافة محورات *E. coli* مرتين كانت فعالة أكثر بقليل في تخفيض الإصابة بالهالوك من إضافة محورات *A. chroococcum*. وتوحي البيانات الأولية بأن استخدام محورات *A. chroococcum* و*E. coli* المعدلة وراثياً كمنتجة لمنشطات إنبات الهالوك، قد تكون مفيدة في المكافحة الحيوية للهالوك على الفول.

parasitization by *O. crenata*. However, we have observed (Khalaf et al. 1997) that *Azotobacter* spp. and *Escherichia coli* transformants may well be useful as biocontrol agents against *O. crenata* infestation.

Thus, the objective of the present investigation was to study the biological control of *Orobanche crenata* on *Vicia faba* by developing genetically modified bacteria (*Azotobacter chroococcum* and *Escherichia coli*) wild-type strains which might be able to produce *Vicia faba* stimulant(s) for *O. crenata* germination, at the same time, taking advantage of the *Azotobacter* ability to fix nitrogen and decrease the need for synthetic nitrogen fertilizer (misuse of which causes pollution to the soil and environment).

Material and Methods

This work deals with the effect of *Azotobacter chroococcum* and *Escherichia coli* transformants on the germination of *Orobanche crenata in vitro* at different temperatures, in relation to their response as a biocontrol agent against *O. crenata* parasitism on faba bean under greenhouse conditions. Cultures of both transformed bacteria were obtained corresponding to the procedures as described earlier (Khalaf et al. 1997).

Effect of culture temperature of *Azotobacter chroococcum* and *Escherichia coli* transformants on *O. Crenata* germination *in vitro*

For each of the transformants (*Azotobacter chroococcum* and *Escherichia coli*), 20 ml of liquid medium was inoculated with 0.05 ml of the relevant transformant colony. The inoculated media were incubated in a shaking water bath incubator at 30°C for 72 h, then plated on *Azotobacter* growth medium (AM; Page and Von-Tigerstrom 1979) and nutrient agar medium (NA), and incubated at 30°C for 10 days. Then, aliquots (1 ml) from both liquid mediums were transferred to another 19 ml of both media (AM and nutrient broth, Nb). Serial transfers were carried out from 30 to 25°C, 25 to 20°C and 20 to 15°C, with 72 h of incubation at each temperature, then the growing transformants were plated.

Bioassay of *Orobanche* germination was carried out after incubation of the transformants. Sterilized *Orobanche* seeds were placed in petri dishes containing sterilized Whatman filter paper No. 1, and left for 10 days at the four temperatures (treatments). The germination of *O. crenata* was assessed by using a binocular microscope (50×). Three replicates were used for each treatment. The data obtained were subjected to two factors completely randomized design (Snedecor and Cochran 1967). The number of germinated seeds, together with the total number of seeds used in each dish, were recorded and mean percentage germination calculated.

Since the *in-vitro* studies showed that the optimum temperatures (20 to 30°C) were suitable for the growth of the transformed *Azotobacter chroococcum* and *Escherichia coli*, and that they induced high germination rate of *O. crenata* seeds, it was decided to examine the effect of both *A. chroococcum* and *E. coli* transformants as biocontrol agents for *O. crenata* parasitism in soil.

Response of *Azotobacter chroococcum* and *Escherichia coli* transformants for biological control of *Orobanche crenata* under soil conditions

Single active colonies of *A. chroococcum* and *E. coli* of four dilutions of both bacterial transformants (10^4 to 10^1) were grown in specific broth media (AM and Nb). The cultures of both strains were incubated at 30°C for 10 days with gentle shaking and then examined for biological control potential on *O. crenata* under greenhouse conditions as follows: 48 pots (30×30 cm^2) were filled with a mixture of Nile clay soil and compost (9:1) and infested with *O. crenata* seeds (0.1 g/pot) at 5 cm below the soil surface. The first set (24 pots) was inoculated thoroughly with *A. chroococcum* solution (100 ml/pot), while the second set (24 pots) was inoculated with *E. coli* solution (100 ml/pot) at the same level as the *O. crenata* seeds. One month later, half of each set of the pots (i.e. 12 pots) was re-inoculated with the same bacterial culture solution (100 ml/pot). Three pots were used for each treatment. After 15 days from the second bacterial inoculation, all treatments were sown with faba bean cv Giza 2 (4 seeds/pot) at 3 cm depth. The combination of four selected sets of each of *A. chroococcum* and *E. coli* transformants were also used (3 pots each). An additional 6 pots were used as infested and healthy controls (3 pots each). The untransformed cultures of *A. chroococcum* and *E. coli* (3 pots each) were applied once to the soil exactly as per transformants. The pots were arranged in a completely randomized design. The treatments were maintained under greenhouse conditions and watered as required. After 120 days from sowing (pod stage), *Orobanche* samples were taken for evaluating the biological control on *V. faba*. The number, fresh and dry weights (g, 105°C, overnight) of *Orobanche* tubercles attached to faba bean roots (12 plants) for each treatment were estimated. Timing for emergence of *Orobanche* shoots from the surface was also noted. The data obtained were subjected to analysis of variance methods (Snedecor and Cochran 1967).

Results and Discussion

Effect of culture temperature of *Azotobacter chroococcum* and *Escherichia coli* transformants on the germination of *Orobanche crenata in vitro*

The influence of *Azotobacter chroococcum* and *Escherichia coli* transformants cultured at different temperatures on the germination of *O. crenata* seeds *in vitro* is shown in Table 1. The two transformed bacteria species grown at different temperatures, ranging from 15 to 30°C, were significantly different in their ability to induce the germination of *O. crenata* seed *in vitro*. Incubation of the *A. chroococcum* and *E. coli* at 25°C led to the highest germination of *Orobanche* seed (13–33%), compared with other culture temperatures or with the untransformed controls, (the latter failed to induce *O. crenata* germination). However, both transformed bacterial species grown at 20°C significantly induced the germination of *O. crenata* seed at levels (5.1–28.2%) similar to those at 25°C. The *Azotobacter chroococcum* grown at 30°C was more effective in inducing the germination of *O. crenata* seeds (16–25%) than the *E. coli* (6–12%). Culture of *A. chroococcum* and *E. coli* at low temperature (15°C) resulted in significantly decreased germination of *O. crenata* seeds (3–14%) compared with other cultural temperatures.

Response of *Azotobacter chroococcum* and *Escherichia coli* transformants as biocontrol agents against *Orobanche crenata* infestation on faba bean

The effects of different selected *A. chroococcum* and *E. coli* transformants for controlling *O. crenata* parasitism on *Vicia faba* under greenhouse conditions are summarized in Tables 2 and 3, respectively. *Azotobacter. chroococcum* and *E. coli* transformants gave promising results for the biological control of *O. crenata* parasitism on faba bean under soil conditions. However, applying *E. coli* transformants was more effective in reducing *Orobanche* infestation than was *A. chroococcum*, whether the soils were inoculated with the transformants once or twice.

Table 2 shows the use of genetically transformed *A. chroococcum*, wild-type strain, in an attempt to prematurely germinate *O. crenata* in soil to decrease parasitisim. The different selected *A. chroococcum* transformants significantly inhibited *Orobanche* parasitism in faba bean compared with the infested control, whether the bacteria were inoculated once or twice. However, inoculation of the soil once with *A. chroococcum* transformants appeared to be slightly more effective for controlling *O. crenata* parasitism than did two inoculations. The effectiveness of *A. chroococcum* on controlling *Orobanche* infestation was characterized by a significant reduction in the number and fresh weight of *Orobanche* tubercles, but had no significant effect on dry weight (Table 2). *Azotobacter chroococcum* transformants did not retard the emergence of *Orobanche* spikes from the soil surface, irrespective of number of applications. These emerged at the same time as the infested control, i.e. 89 days after sowing.

The effectiveness of *E. coli* transformants as a biocontrol agent against *O. crenata* parasitism on faba bean under greenhouse conditions is shown in Table 3. Inoculation of soil with *E. coli* transformants significantly reduced *Orobanche* infestation in faba bean plants compared with

Table 1. Effect of culture temperature of *Azotobacter chroococcum* and *Escherichia coli* transformants on *Orobanche crenata* germination *in vitro*.

Transformant colony no.	Germination of *Orobanche* seeds (%)					
	15°C	20°C	25°C	30°C	Mean (%)	LSD (5%)
A. chroococcum†						
2	10.2	18.0	22.1	19.4	17.4	
3	3.1	19.5	18.4	16.5	14.3	
5	12.1	28.2	32.4	25.5	24.3	
Control (untransformed bacteria)	0	0	0	0	0	
Mean %	6.3	16.5	18.1	15.3		1.37
E. Coli‡						
1	7.3	5.1	13.0	6.6	8.0	
2	13.1	18.8	18.7	12.0	15.0	
3	14.3	22.9	33.1	6.3	19.1	
Control (untransformed bacteria)	0	0	0	0	0	
Mean %	8.6	11.7	16.2	6.2		1.02

† LSD (5%) for Temperature (T) × Colony (C) = 2.75; ‡ LSD (5%) for T × C = 2.04

Table 2. Activity of *Azotobacter chroococcum* transformants as biocontrol agents for *Orobanche crenata* parasitism of *Vicia faba* under greenhouse conditions.

Transformant colonies/used as original concentrations (cell/ml)	Mean *O. crenata* parasitism/plant		
	No. *Orobanche* tubercles	Fresh wt *Orobanche* (g)	Dry wt *Orobanche* (g)
†5 10^{-1} 2‡ (once)	0.45	0.24	0.03
5 10^{-1} 2 (twice)	1.00	0.37	0.05
5 10^{-1} 3 (once)	0.25	0.12	0.08
5 10^{-1} 3 (twice)	0.33	0.22	0.06
5 10^{-1} 4 (once)	0.25	0.61	0.11
5 10^{-1} 4 (twice)	0.58	0.12	0.08
5 10^{-1} 6 (once)	0.33	0.22	0.08
5 10^{-1} 6 (twice)	0.91	0.95	0.12
Combination of the 4 colonies	1.16	1.05	0.08
Infested control	3.33	5.58	0.36
Healthy control	0	0	0
Untransformed cultures of *A. chroococcum* "AM medium"	2.08	1.25	0.18
LSD (5%)	1.24	1.22	N.S

† Colony transformant number; ‡ Sub-colony transformant number.

the infested control, irrespective of number applications. The *E. coli* transformants significantly reduced the number and fresh and dry weights of *Orobanche* tubercles (Table 3). However, inoculation of the soil once with *E. coli* was generally more effective for controlling *O. crenata* infestion than was two applications, with the exception of inoculation of the soil with treatment "2 10^{-2} 6" twice, which competely controlled *Orobanche* infestation in faba bean. No *Orobanche* tubercles were observed attached to faba bean roots; however, like *A. chroococcum, E. coli* transformants also failed to suppress the emergence of *Orobanche* spikes from the soil, irrespective of number of applications. A combination of both transformants also showed significant response for controlling *O. crenata* parasitism compared with the infested control (Tables 2 and 3). The cultural media of both bacteria slightly reduced *O. crenata*, either compared with the transformed treatments or with the infested control. This was somewhat surprising, but the minerals in such cultural media may well be sufficient to account for this.

The data indicated that incubation of *A. chroococcum* and *E. coli* transformants at 20–30°C was preferable for inducing the germination of *O. crenata* seeds. This result would seem to support that given by Kasasian (1973) who concludes that there is a considerably higher level of *Orobanche* infestation at higher temperatures (e.g. 25°C), that there is no germintation of *O. crenata* below 8°C, and that it is poor at 13°C.

Both *A. chroococcum* and *E. coli* transformants showed promising results for the biological control of *O. crenata* on faba bean under greenhouse conditions, mainly through inducing the germination of *O. crenata* seed, coupled with reducing the germ-tube length of the germinated *Orobanche* seed (Khalaf et al. 1997). Applying the *A. chroococcum* transformant to the soil might control *O. crenata* infestation in faba bean by increasing the level of soil nitrogen through nitrogen fixation; this might inhibit the germination rate and stunt the germ tube. However, Van-Hezewijk et al. (1991) found that nitrogen in the form of ammonium or urea reduced, in most cases, the germination rate and length of the germ tube of *Orobanche* seeds, suggesting that the effect of nitrogen application on *O. crenata* in the field is not likely to be associated with a decrease in pH in the rhizosphere of the host (Van-Hezewijk et al. 1994). Pieterse (1989) observed that there was a reduction in the germination rate of *O. crenata* from 58% in the presence 4 m*M* ammonium sulfate. On the other hand, Kasasian (1973) attempted to control *O. crenata* infestation by increasing legume nodules on *V. faba* root; however, inoculating faba bean seeds with *Rhizobium leguminosarum* failed to reduce the *Orobanche* infestation.

The use of *A. chroococcum* and *E. coli* transformants against *Orobanche* parasitism in faba bean and other hosts could be considered practically under field conditions, particularly as such bacteria are likely to be persistent in the

Table 3. Activity of *Escherichia coli* transformants as biocontrol agents for *Orobanche crenata* parasitism of *Vicia faba* under greenhouse conditions.

Transformant colonies/used as original concentrations (cell/ml)	Mean *O. crenata* parasitism/plant		
	No. *Orobanche* tubercles	Fresh wt *Orobanche* (g)	Dry wt *Orobanche* (g)
†2 10^{-2} 2‡ (once)	0.16	0.33	0.02
2 10^{-2} 2 (twice)	1.41	2.08	0.08
2 10^{-2} 3 (once)	0.41	0.55	0.03
2 10^{-2} 3 (twice)	1.25	1.11	0.05
2 10^{-2} 4 (once)	1.75	2.82	0.23
2 10^{-2} 4 (twice)	1.25	0.91	0.04
2 10^{-2} 6 (once)	0.16	0.61	0.08
2 10^{-2} 6 (twice)	0	0	0
Combination of the 4 colonies (10^{-2} 2, 3, 4 & 6)	0.91	1.02	0.12
Infested control	3.33	5.58	0.36
Healthy control	0	0	0
Untransformed cultures of *E. coli* "Nb medium"	2.25	1.41	0.16
LSD (5%)	1.36	1.06	0.23

† Colony transformant number; ‡ Sub-colony transformant number.

soil for a long period (Saber 1986). Thus, it seems that *Orobanche* control can be achieved by applying the bacterial culture solutions to the soil 2–4 weeks prior to sowing the host plants, to induce the germination of *Orobanche* seeds in the absence of their hosts, resulting in their death.

This preliminary evidence suggests that developing genetically modified bacteria should be taken into consideration for biological control of *Orobanche* parasitism in the long term; however, such observations need further investigations.

References

Kasasian, L. 1973. Control of *Orobanche*. *PANS* 19: 368–371.

Khalaf, K.A. 1982. Some studies on the control of *Orobanche crenata* parasitism of *Vicia faba*. PhD Thesis, University of London.

Khalaf, K.A. and A.M.M. Ali. 1991. Preliminary attempts of producing genetically engineered *Azotobacter chroococcum* transformants able to produce *Orobanche* stimulant(s). *FABIS Newsletter* 28/29: 35–36.

Khalaf, K.A., A.M. Ali, K.A. Youssef and S.A. Abd-Al-Aziz. 1997. Studies on the control of *Orobanche crenata*. I. Use of *Azotobacter* spp. and *Escherichia coli* transformants to break dormancy of *Orobanche crenata*. *FABIS Newsletter* 40: 25–30.

Page, W.J. and M. Von-Tigerstrom. 1979. Optimal conditions for transformation of *A. vinelandii*. *Journal of Bacteriology* 139: 1058–1061.

Petzoldt, K. 1979. Bacterial nodules of *Rhizobium leguminosarum* and *Orobanche crenata* germination and penetration on broad beans with an intergrated control program. Pages 260–268 *in* Proceedings, Second International Symposium on Parasitic Weed, North Carolina.

Pieterse, A.H. 1989. The effect of nitrogen fertilizers on the germination of seed of *Striga hermonthica* (Del.) and *Orobanche crenata* Forsk. Pages 115–124 *in* Proceedings *Orobanche* Workshop, 19–22 August, Obermarchtal, Germany.

Saber, M.S.M. 1986. Prolonged effect of land disposal of human wastes on soil conditions. *Water Science and Technology* 18: 371–374.

Snedecor, G.W. and W.G. Cochran. 1976. Statistical Methods. Iowa State University Press, Iowa, USA.

Van-Hezewijk, M.J., K.H. Linke, J.A.C. Verkleij and A.H. Pieterse. 1991. The effect of ammonium fertilizer in combination with bean. Pages 470–483 *in* 5th International Symposium on Parasitic Weed, 24–30 June, Nairobi, Kenya.

Van-Hezewijk, M.J., J.A.C. Verkleij and A.H. Pieterse. 1994. The effect of pH on germination of *Orobanche crenata*. Pages 173–178 *in* Proceedings of the Third International Workshop on *Orobanche* and Related *Striga* Research, 8–12 November, Royal Tropical Institute, The Netherlands.

Seed Quality and Nutrition

Amino Acid Composition of Protein Fractions of Faba Bean

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Abstract

The amino acid composition of the protein fractions of faba bean showed that all the essential amino acids were present, except for sulfur-containing ones, in adequate amounts for human nutrition in all the faba bean cultivars studied. Arginine (7.9–16.4 g/16 g N) was the predominant essential amino acid, while methionine and cystine were present in very small quantities.

Key words: *Vicia faba;* faba beans; amino acids; proteins.

Introduction

Legume seeds constitute an excellent source of protein and energy for improving diets based on cereals and roots in low-income countries (Sgarbieri et al. 1978). In several African countries, particularly Sudan, faba bean (*Vicia faba* L.) is the most important legume seed consumed directly as human food. The most popular way of preparing faba bean in Sudan is stewed faba bean (*fool madamas*), which people eat for breakfast and supper, as well as in sandwiches at any time of the day. Other popular ways of preparing faba beans are as bean cakes (*falafel* or *taamia*) and germinated beans (*fool nabet*).

Faba bean contains over 25% protein and about 51–66% carbohydrates (Kay 1979), in addition to several essential minerals and vitamins. Because of its high lysine content, faba bean protein complements cereal proteins which are low in lysine. Cereal proteins complement faba bean protein by contributing cystine and methionine, which are limiting amino acids in faba bean protein. The aim of this study was to determine the amino-acid composition of the protein fractions of 15 faba bean cultivars.

Material and Methods

Faba bean cultivars

Fifteen faba bean genotypes were obtained from Shambat Research Station (Table 1). The seeds were cleaned and freed from foreign material, washed with distilled water,

الأحماض الأمينية المكونة لأجزاء بروتين الفول

الملخص

أظهرت الدراسة وجود جميع الأحماض الأمينية الأساسية بكميات كافية للغذاء البشري، باستثناء الأحماض الحاوية على الكبريت، في أجزاء بروتين الفول في جميع الأصناف التي تمت دراستها. وكان الارجينين (7.9-16.4 غ/16 غ آزوت) هو الحمض الأميني الأكثر شيوعاً بين الأحماض الأمينية الأساسية، في حين كانا الميثيونين والسيستين موجودين بكميات محدودة جداً.

Table 1. Faba bean cultivars.

Serial no.	Name	Origin
1	Rebaya 30× Giza 1	Bulk of single crosses made at Hudeiba Research station (HRS) (1968/69)
2	Giza×BF 2/2	
3	Selaim×BF 2/2	
4	BF 2/2×1W	
5	1W×Selaim Large	
6	188×Giza 1	
7	NEB 423×BF 2/2	Bulk of a single cross produced at HRS, 1975
8	Syrian Local	A Syrian entry from an ICARDA† breeding nursery.
9	Mass Selection Giza 1	Development at HRS from Giza 1, an Egyptian cultivar.
10	Hudeiba 72	Released by HRS as a selection from the Egyptian cv Rebaya 40.
11	BF 2/2	A selection from cv Baladi at HRS.
12	Selaim Large	Development from cv Selaim at HRS.
13	BM 9/3	Selection from Baladi "Mussiab," a village near HRS.
14	HSB 10	A collection from a field in one of the villages in the Northern State.
15	NEB 424.S	A Selection from an ICARDA-supplied nursery.

† ICARDA = International Center for Agricultural Research in the Dry Areas.

sun-dried and milled into fine powder (0.4 mm screen) and kept in closed glass bottles.

Protein fractionation

Triplicate 2.5 g samples of milled faba beans were placed in 250 ml plastic centrifuge bottles with screw caps. Each sample was extracted with 25 ml of CO_2-free distilled water. Extraction was carried out for 2 hours with continuous shaking. The extract was separated from the residue by centrifugation of 2000 *g* for 30 min. Because the supernatant liquids often contained some suspended particles, a second centrifugation was carried out at 4600 *g* for 20 min. Five milliliters of the clear supernatant liquid were transferred to Kjeldahl flasks to determine the total amount of albumin + glycinin. The remainder of the clear supernatant liquid was brought to 25 ml using distilled water. About 6 drops of 0.1 *N* HCl was added to obtain a pH of 4.5, then the glycinin fractions were precipitated by centrifugation and the clear supernatant liquid was obtained. Five milliliters of this fraction were transferred to Kjeldahl flasks.

The residues of the water-soluble fractions were then extracted successively in a similar manner with 0.1 *M* NaCl solution (adjusted to pH 7). Extraction was continued with 70% ethanol and 0.2% NaOH, and the extracts were collected as described above.

Amino acid analysis

Amino acid analysis was carried out by ion-exchange chromatography. The chromatography was carried out using 4.75-hour lithium buffer solution program on an LKB Model 4400 Amino Acid Analyzer. The hydrolysates were obtained by hydrolyzing the sample in 6 *N* HCl for 22 hours at 105°C.

Statistical analysis

Each sample was analyzed in triplicate and the figures were then averaged. Data were assessed by analysis of variance (Snedecor and Cochran 1987) and by Duncan's Multiple Range Test (Duncan 1955) with a probability of $P \leq 0.05$.

Results and Discussion

Table 2 shows the protein fractions of the faba bean cultivars. These fractions comprised glycinin (17.3–28.8%), albumin (8.8–12.9%), globulin (31.8–43.5%), prolamin (0.9–1.9%), glutelin (11.5–17.1%) and the insoluble residue (7.2–19.6%). The cultivars Syrian Local and BM 9/3 had the largest glycinin fraction, whereas Giza 1 × BF2/2 had the largest albumin fraction. Genotypes 188 × Giza 1 and Hudeiba 72 contained the largest amounts of globulin. The glutelin fraction was highest in BF2/2 × IW and Hudeiba 72. These results showed that the globulin fraction was the predominant fraction for all the genotypes studied, while prolamins were present in very small amounts. Cubero (1984) reports that globulins were the largest fractions followed by albumins and glutelins; the results of this study seem to agree with this, if glycinins and albumins are assumed as one fraction.

Amino acid composition of the protein fractions

The amino acid composition of the glycinin fraction is shown in Table 3. All the essential amino acids were present, except the sulfur-containing cystine and methionine. Arginine was the predominant amino acid (10.5–14.2 g/16 g N), followed by leucine (8.7–10.4 g/16 g N) and lysine (5.3–8.8 g/16 g N). Sjodin (1982) and Griffiths (1983) reported similar results.

The sulfur amino acid cystine in the glycinin fraction was absent, except in NEB424.S, while the amounts for the other sulfur amino acid, methionine, were very low—some of the genotypes contained only traces of methionine.

Table 4 shows the amino acid composition of the albumin fraction. The values for arginine ranged between 7.9 and 16.3 g/16 g N; BM9/3 and HSB10 contained the largest amounts of this essential amino acid. Leucine ranged between 7.3 and 10.7 g/16 g N; BM9/3 contained the largest amount. Lysine ranged from 6.8 to 8.8 g/16 g N and it was present in large amounts in IW × Selaim, NEB423 × BF2/2 and Syrian Local. The sulfur amino acids cystine and methionine were only present in small amounts (0.0–0.8 and 0.2–0.6 g/16 g N, respectively). Syrian Local, BF2/2 and Hudeiba 72 contained relatively high quantities of cystine and methionine.

Tables 5 and 6 show the amino acid composition of the globulin and glutelin fractions, respectively. In these fractions, arginine and leucine were present in high amounts (9.6–13.7 and 8.2–10.5 g/16 g N, respectively) in the glutelin fraction. Selaim × BF2/2 and Selaim Large contained the largest amounts of arginine and leucine in the globulin fraction, while BF2/2 had the highest values for the two amino acids in the glutelin fraction. The sulfur amino acids cystine and methionine were the limiting amino acids in these fractions; however, they were present at relatively high concentration in the globulin fraction in Syrian Local and Hudeiba 72 and in the glutelin fraction in BF2/2 × IW and Syrian Local.

Table 2. Protein fractions for the faba bean cultivars expressed on dry matter basis (%).

Sample	Glycinin	Albumin	Globulin	Prolamin	Glutelin	Insoluble residue	Total protein recovered
Rebaya 30×Giza 1	25.7 (±0.09) b	9.2 (±0.24) ig	41.3 (±0.08) ab	1.4 (±0) cd	11.5 (±0.61) f	7.7 (±0.45) efgh	96.8
Giza 1×BF 2/2	22.6 (±0.09) d	12.9 (±0.09) a	37.9 (±0.18) cde	1.4 (±0) cd	12.8 (±0.09) ef	8.2 (±0.05) defg	95.8
Selaim×BF 2/2	22.9 (±0.11) d	10.9 (±0.16) bc	38.2 (±0.18) cde	1.4 (±0) cd	14.8 (±0.1) bcd	8.6 (±0.07) cde	96.4
BF 2/2×IW	17.3 (±0.11) g	9.1 (±0.19) ig	40.7 (±1.39) abc	1.9 (±0) a	16.9 (±0.17) a	10.9 (±0.04) a	96.8
IW×Selaim	25.1 (±0.38) bc	8.8 (±0.15) g	38.2 (±0.65) cde	1.3 (±0.06) cd	15.4 (±0.18) b	9.3 (±0.06) bc	98.2
188×Giza 1	22.9 (±0.07) d	9.4 (±0.15) fg	43.3 (±0.64) a	1.4 (±0.06) cd	15.1 (±0.25) bc	7.4 (±0.25) gh	99.3
NEB24×BF 2/2	22.6 (±0.74) d	11.8 (±0.24) b	39.8 (±0.44) bcd	1.5 (±0.05) cd	13.5 (±0.18) de	9.9 (±0.19) b	99.0
Syrian Local	28.8 (±0.15) a	10.8 (±0.33) c	31.8 (±0.27) g	1.6 (±0.1) bc	14.6 (±0.06) bcd	8.7 (±0.08) cd	96.1
Mass selection Giza 1	24.8 (±0.29) be	9.9 (±0.09) def	50.0 (±0.2) bcd	1.3 (±0.04) cd	12.7 (±0.1) ef	7.8 (±0.18) defgh	96.5
Hudeiba 72	18.8 (±0.28) f	9.1 (±0.17) fg	43.5 (±0.08) a	0.9 (±0.01) e	17.1 (±0.2) a	7.7 (±0.18) efgh	97.1
BF 2/2	20.9 (±0.14) e	9.9 (±0.09) def	41.3 (±0.24) ab	1.8 (±0.09) ab	13.8 (±0.09) cde	7.7 (±0.18) efgh	95.5
Selaim Large	25.4 (±0.35) bc	9.3 (±0.18) fg	37.3 (±0.2) de	1.6 (±0.1) bc	14.3 (±0.1) bcd	8.4 (±0.25) def	96.3
BM 9/3	28.2 (±0.3) a	10.3 (±0.29) cde	33.8 (±0.49) fg	1.4 (±0) cd	12.0 (±0.32) ef	11.6 (±0.43) a	97.2
HSB 10	25.6 (±0.53) b	10.6 (±0.43) cd	36.2 (±1.05) ef	1.8 (±0.08) ab	14.1 (±0.28) bcde	7.2 (±0.19) h	95.4
NEB 424.S	23.4 (±0.26) cd	9.7 (±0.04) efg	37.0 (±1.95) de	1.4 (±0) cd	14.0 (±0.36) bcde	11.0 (±0.1) a	96.5

Values are means (±0SD). Means not sharing a common letter in a column are significantly different at $P \leq 0.05$ as assessed by Duncan's Multiple Range Test (DMRT)

Table 3. Amino acid composition of the glycinin fraction (g/16 g N).

Sample	Asp	Thr	Ser	Glu	Pro	Gly	Ala	Cys	Val	Met	Iso leu	Leu	Tyr	Phe	Lys	His	Arg
Rebaya 30×Giza 1	11.4	2.9	4.4	16.3	5.5	3.9	3.7	0	4.9	0.1	4.3	8.9	2.5	4.3	5.8	2.3	12.7
Giza 1×BF 2/2	13.4	2.6	3.9	19.5	5.1	4.0	3.8	0	5.2	0.1	4.3	8.8	2.2	4.1	2.5	2.2	12.9
Selaim×BF 2/2	13.2	3.2	4.0	17.2	6.2	4.6	4.6	0	10.1	0.2	4.6	8.7	2.2	4.6	2.4	2.4	10.5
BF 2/2×IW	12.0	2.4	3.6	18.7	5.6	4.0	3.8	0	8.6	Tr	4.3	8.8	2.2	4.3	2.4	2.5	12.0
IW×Selaim	13.7	2.8	4.1	18.6	4.1	4.4	4.1	0	9.5	0.1	4.7	9.5	2.2	4.5	2.7	2.9	12.8
188×Giza 1	14.0	2.8	4.0	21.0	3.6	4.2	4.0	0	9.1	0.1	4.6	9.2	2.1	4.4	6.1	2.5	12.7
NEB423×BF 2/2	13.5	2.8	3.7	21.2	3.5	4.3	4.2	0	9.5	0.1	4.8	9.3	2.0	4.6	6.7	2.5	11.6
Syrian Local	11.0	3.0	4.3	23.1	4.9	4.3	4.2	0	5.7	0.1	4.5	9.2	2.4	4.3	6.2	2.6	12.5
Mass selection Giza 1	09.8	3.0	1.6	19.9	4.1	4.1	3.8	0	4.5	0.2	4.3	8.8	2.5	4.3	6.2	2.4	10.8
Hudeiba 72	13.0	3.5	5.4	24.5	6.4	4.7	4.4	0	6.2	0.1	5.2	10.4	2.9	4.8	7.0	3.0	14.2
BF 2/2	12.4	3.4	5.0	16.0	9.7	4.8	4.6	0	6.5	0.2	5.6	10.3	2.5	4.9	7.3	2.9	12.7
Selaim Large	12.7	3.4	4.7	18.9	3.6	4.5	4.3	0	6.9	0.1	5.2	9.8	2.4	4.6	6.8	2.6	12.8
MB 9/3	11.4	3.8	5.1	16.1	7.0	4.3	4.7	0	6.2	0.3	4.9	8.8	2.6	4.7	7.5	2.5	10.7
NEB 424.S	11.2	5.0	5.4	15.7	2.3	5.0	5.9	0.4	7.1	0.7	5.7	9.3	3.2	5.3	8.8	2.8	10.5
HSB10	10.5	3.7	4.7	15.1	7.4	4.4	4.4	0	5.5	0.4	5.2	8.6	3.0	4.3	6.9	2.6	10.4

Asp = aspartine; Thr = threonine; Ser = serine; Glu = glutamine; Pro = proalanine; gly = glycine; Ala = alanine; Cys = cysteine; Val = valine; Met = methionine; Iso leu = isoleucine; Leu = leucine; Tyr = tyrosine; Phe = phenylalanine; Lys = lysine; His = histine; Arg = argenine.

Table 4. Amino acid composition of the albumin fraction (g/16 g N).

Sample	Asp	Thr	Ser	Glu	Pro	Gly	Ala	Cys	Val	Met	Iso leu	Leu	Tyr	Phe	Lys	His	Arg
Rebaya 30×Giza 1	11.0	3.9	4.8	15.8	6.3	4.3	4.7	0.2	5.8	0.4	5.8	8.8	2.8	4.8	7.9	2.5	9.2
Giza 1×BF 2/2	12.2	3.5	5.1	19.3	6.4	4.3	4.3	0	5.8	0.2	5.5	9.4	2.9	4.7	7.2	2.9	12.3
Selaim×BF 2/2	11.6	3.8	5.2	14.0	12.3	4.8	4.7	0.2	6.1	0.4	5.5	9.1	3.0	4.9	6.8	3.1	11.3
BF 2/2×IW	10.0	4.0	5.3	14.2	18.7	4.9	5.3	0.3	5.6	0.2	5.1	7.3	3.2	4.6	7.9	2.9	8.7
IW×Selaim	12.0	4.4	5.1	12.5	16.3	5.0	5.6	0.3	7.1	0.3	5.6	9.1	3.0	5.3	8.5	3.3	11.1
188×Giza 1	10.2	4.0	4.5	14.2	14.6	4.5	5.1	0.3	5.8	0.3	5.1	7.7	3.0	4.5	7.3	2.8	7.9
NEB423×BF 2/2	11.5	4.7	5.6	15.9	7.3	5.1	5.5	0.4	7.0	0.3	5.8	9.3	3.3	5.4	8.6	3.2	11.0
Syrian Local	14.4	4.3	5.7	14.2	12.0	5.2	5.5	0.8	5.3	0.3	5.6	7.8	3.6	5.0	8.8	3.3	9.6
Mass selection Giza 1	09.3	3.7	4.6	13.3	7.3	4.5	4.7	0	6.4	0.2	5.0	8.6	2.6	4.8	7.8	2.8	9.7
Hudeiba 72	13.4	3.7	4.8	21.0	6.6	4.6	5.0	0.2	6.1	0.6	5.9	9.2	2.7	4.9	7.8	2.5	11.6
BF 2/2	10.3	4.3	4.4	15.4	9.3	4.6	5.4	0.6	6.1	0.6	5.9	7.8	3.1	4.6	7.7	2.7	8.6
Selaim Large	10.7	3.6	4.4	17.5	10.7	4.4	4.4	0.3	6.1	0.2	5.6	9.1	2.9	4.9	7.7	2.9	9.6
MB 9/3	14.9	4.1	6.4	26.1	8.5	5.1	5.0	0	7.3	0.4	6.1	10.7	3.4	5.9	7.2	3.6	16.5
NEB 424.S	13.6	3.6	5.4	21.9	5.6	4.8	4.8	0	6.8	0.4	5.7	9.9	2.9	5.4	7.4	2.9	11.5
HSB10	13.2	3.6	5.2	23.8	5.3	4.4	4.3	0.1	6.4	0.2	5.4	9.5	2.7	5.3	6.8	2.7	13.5

Table 5. Amino acid composition of the globulin fraction (g/16 g N).

Sample	Asp	Thr	Ser	Glu	Pro	Gly	Ala	Cys	Val	Met	Iso leu	Leu	Tyr	Phe	Lys	His	Arg
Rebaya 30×Giza 1	11.7	3.4	4.5	17.4	4.5	4.4	4.5	0.2	5.7	0.2	4.9	10.2	2.7	4.9	6.2	2.6	11.0
Giza 1×BF 2/2	11.0	3.4	5.2	20.1	4.0	4.3	4.3	tr	6.1	0.1	5.3	8.9	2.7	5.2	5.9	2.5	12.6
Selaim×BF 2/2	11.8	3.6	5.2	22.2	4.2	4.6	4.7	tr	6.1	0.2	4.8	8.8	2.8	5.2	6.2	2.5	13.7
BF 2/2×IW	12.6	3.2	5.3	20.5	4.2	4.1	4.2	0.2	5.5	0.2	4.5	8.4	3.0	4.9	5.6	2.6	12.3
IW×Selaim	12.2	3.0	4.5	20.1	4.9	4.1	4.1	0.3	5.5	0.2	4.9	8.5	3.0	4.6	5.9	2.4	11.6
188×Giza 1	10.4	2.9	4.5	19.4	4.4	4.0	4.0	0.4	5.1	0.2	4.5	8.2	2.7	4.4	5.7	2.3	11.2
NEB423×BF 2/2	11.9	2.8	4.6	21.5	5.4	4.1	4.1	0.1	5.3	0.1	4.7	8.6	2.6	4.6	5.8	2.4	11.8
Syrian Local	12.6	3.5	5.1	22.0	7.3	4.8	4.9	0.5	6.1	0.2	5.7	9.8	3.1	5.2	6.5	2.8	12.7
Mass selection Giza 1	12.8	3.5	4.6	19.7	6.2	4.6	4.5	0.4	6.4	0.1	5.3	9.1	2.9	4.6	6.0	2.6	11.6
Hudeiba 72	11.6	3.3	4.6	12.4	5.7	4.3	4.4	0.3	5.7	0.3	5.0	8.9	2.8	4.6	6.5	2.8	10.4
BF 2/2	9.8	3.2	4.6	13.2	7.3	4.1	4.1	0.2	5.3	0.2	5.3	8.8	2.8	4.3	5.7	2.5	9.6
Selaim Large	14.8	4.4	6.9	20.4	6.1	5.1	5.1	0.4	6.6	0.2	6.1	10.5	3.5	5.5	6.9	3.1	12.3
MB 9/3	12.6	3.4	4.7	19.9	6.1	4.7	5.0	0.1	6.4	0.1	5.6	9.8	2.7	2.3	6.6	2.7	10.0
NEB 424.S	12.5	3.8	5.0	16.6	6.8	4.8	2.1	0.1	6.5	0.1	5.5	9.9	2.9	5.5	7.0	3.0	11.0
HSB10	9.7	3.4	4.6	15.4	6.2	4.3	4.6	0.1	5.7	0.1	4.9	9.0	2.7	4.8	6.4	2.8	10.2

tr = trace

Table 6. Amino acid composition of the glutelin fraction (g/16 g N).

Sample	Asp	Thr	Ser	Glu	Pro	Gly	Ala	Cys	Val	Met	Iso leu	Leu	Tyr	Phe	Lys	His	Arg
Rebaya 30×Giza 1	11.3	3.8	4.4	17.9	7.4	4.7	5.0	0.3	6.3	0.2	5.3	9.0	2.9	5.3	5.8	3.1	9.3
Giza 1×BF 2/2	12.7	4.1	4.8	19.8	8.1	5.1	5.2	0.4	6.7	0.1	5.8	10.0	3.4	6.0	6.5	3.2	11.3
Selaim×BF 2/2	9.9	3.7	4.3	16.5	6.8	4.7	4.8	0.3	5.8	0.2	4.9	8.6	2.7	5.0	5.2	2.7	9.6
BF 2/2×IW	12.5	3.8	4.1	20.4	7.4	4.2	4.2	0.5	5.2	0.3	5.0	9.0	3.2	4.7	4.5	3.4	9.2
IW×Selaim	10.7	3.2	3.9	16.4	5.7	4.4	4.3	0.5	5.3	0.2	4.9	9.1	3.0	5.1	4.9	3.1	10.1
188×Giza 1	9.5	2.9	3.5	17.6	7.3	4.3	3.9	0.4	4.7	0.2	4.5	8.0	2.6	4.3	4.8	2.6	8.8
NEB423×BF 2/2	10.7	3.2	5.6	15.7	8.4	4.1	4.1	0.3	5.1	0.3	4.9	7.6	5.1	4.7	4.7	2.7	8.9
Syrian Local	13.7	3.9	4.3	18.3	6.2	4.8	4.9	0.3	5.2	0.3	5.2	9.0	5.5	5.9	5.8	3.0	10.9
Mass selection Giza 1	12.0	3.0	4.1	15.5	6.8	4.5	4.4	0.3	5.7	0.2	5.2	8.9	4.6	4.4	4.9	2.7	9.8
Hudeiba 72	13.3	3.1	3.9	15.4	7.8	4.6	4.5	0.2	6.0	0.1	5.5	9.5	5.1	4.8	5.4	3.1	11.3
BF 2/2	14.3	3.6	4.9	15.1	8.2	5.1	4.5	0.4	6.6	0.3	6.0	10.5	3.0	5.7	6.4	3.3	11.6
Selaim Large	11.5	3.4	4.2	17.7	8.0	4.6	4.7	0.3	6.0	0.1	5.4	9.4	2.7	5.1	5.6	2.9	10.5
MB 9/3	14.5	3.6	4.0	19.1	8.7	5.0	5.0	0.4	6.2	0.1	5.9	10.0	2.7	5.3	5.5	3.0	10.8
NEB 424.S	14.1	3.6	4.0	20.1	7.8	4.7	4.6	0.3	5.9	0.1	539	9.8	2.7	5.0	6.4	2.9	10.5
HSB10	13.6	3.4	3.9	16.6	4.6	4.9	5.0	0.4	5.1	0.1	5.4	9.4	2.7	4.9	4.8	2.9	10.8

Generally, arginine and leucine were the predominant amino acids in the fractions of faba bean, while the sulfur amino acids cystine and methionine were present in only small amounts. Lafiandra et al. (1983) and Sjodin et al. (1981) report higher values for methionine. The low quantities of this sulfur amino acid in this study may be due to the fact that these cultivars contained small quantities of the protein fraction prolamin. There was a negative correlation between sulfur amino acid content and protein percentage. This negative correlation is probably explained by the fact that storage proteins, which are relatively low in sulfur amino acid, constitute a higher proportion of the protein fractions of the seeds (Boulter 1977). This problem can be overcome by complementing the diet based on legumes with other proteins in which the sulfur amino acids are not limiting factors.

References

Boulter, D. 1977. Protein quality from leguminous crops. EUR 5686EN.11.

Cubero, J.I. 1984. Problems and perspectives in breeding for protein content in *Vicia faba* L. *FABIS Newsletter* 9: 1–9.

Duncan, B.D. 1955. Multiple range and multiple F tests. *Biometrics* 11: 1–42.

Griffiths, D.W. 1983. The amino acid composition of high and low protein faba bean (*Vicia faba* L.) varieties and selections. *FABIS Newsletter* 6: 18–24.

Kay, D.E. 1979. Food legumes. TPI Crop and Product Digest No. 3. Tropical Products Institute, London, UK. (p. 35.)

Lafiandra, D., O.B. Polignano and G. Colaprico. 1983. Protein content and amino acid composition of seed in varieties of *Vicia faba* L. *Zeitschrift für Pflanzenzuchtung* 79: 308–314.

Sgarbieri, V.C., R.S. Garruti M.A.C. Moraes and L. Hartman. 1978. Nutritional and sensory evaluation of mixtures of soybean (*Glycine max* L.) and common bean (*Phaseolus vulgaris* L.) for direct use as human food. *Journal of Food Science* 43: 208–212.

Sjodin, J. 1982. Protein quantity and quality in *Vicia faba*. Pages 319–331 *in* Faba Bean Improvement (G. Hawtin and C. Webb, eds). Martinus Nijhoff, The Hague, The Netherlands. Proceeding of the Faba Bean Conference held in Cairo, Egypt, March 7–11, 1981.

Sjodin, J., P. Martensson and T. Maggarosi. 1981. Selection for increased protein content in field bean (*Vicia faba* L.). *Zeitschrift für Pflanzenzuchtung* 86: 210–220.

Snedecor, G.W. and W.G. Cochran. 1987. Statistical Methods, 7th ed. Iowa State University Press, Ames, Iowa, USA. pp. 221–222.

News

Editors' notes

Publication frequency

Starting with this issue, the frequency of publication of *FABIS Newsletter* has been reduced to one issue per year. Researchers may submit their work on faba bean, *Vicia* and *Lathyrus* for publication in *FABIS Newsletter*.

Recent Literature

Proceedings

***Opportunities for High Quality, Healthy and Added-Value Crops to Meet European Demands.* Proceedings of the 3rd European Conference on Grain Legumes, 14–19 November 1998, Valladolid, Spain.** 513 p. European Association for Grain Legume Research (AEP), 12 Avenue George V-75008 Paris, France. E-mail: a.schneider-aep@prolea.tm.fr.

The Proceedings include close to 300 contributions in grain legume research. These topics include human and animal nutrition, crop physiology, food and non-food uses, genetics, diseases, cropping systems, as well as many others.

Forthcoming Events

1999

***Biowork II: International Workshop on Plant Breeding in the Turn of the Millennium*, Viçosa, Minas Gerais, Brazil, 2 March.** www.ufv.br/dft/biowork/index.htm.

***XVI International Botanical Congress*, Saint Louis, Missouri, USA, 1–7 August 1999.** Contact: The Secretary General, XVI IBC, c/o Missouri Botanical Garden, P.O. Box 299, St Louis, MO 63166-0299, USA [Fax +1-314-577-9589; e-mail *ibc16@mobot.org*].

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Journal article: Schubert, I. and R. Rieger. 1990. Alteration by centric fission of the diploid chromosome number in *Vicia faba* L. *Genetica* 81: 67–69.

Article in book: Bos, L. 1982. Virus diseases of faba beans. Pages 233–242 *in* Faba Bean Improvement (G. Hawtin and C. Webb, ed.). Martinus Nijhoff, The Hague.

Article in proceedings: Montoya, J.L. 1988. The production of seed of leguminous crops in Spain. Pages 136–142 *in* Seed Production in and for Mediterranean Countries. Proceedings of the ICARDA/EC Workshop, 16–18 December 1988, Cairo, Egypt (A.J.G. van Gastel and J.D. Hopkins, ed.). ICARDA, Aleppo, Syria.

Book: Agarwal, V.K. and J.B. Sinclair. 1987. Principles of Seed Pathology. CRC Press, Boca Raton, Florida, USA.

Thesis: El-Hosary, A.A. 1981. Genetic studies of some strains of field beans (*Vicia faba* L.). PhD Thesis. Menoufia University, Egypt.

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