

RECEIVED
Date 11 DEC 1997

FABIS

Faba Bean Information Service

NEWSLETTER No. 38/39
January–December 1996



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 *Viciae* 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. Recent references are published in an annual supplement.

SUBSCRIPTIONS: *FABIS Newsletter* is available on subscription at US\$ 20.00 per year. However, *FABIS Newsletter* is made available free to ICARDA cooperators. To subscribe, please send US\$ check drawn on any branch of a US bank, or a US branch of any other bank, to: FABIS/CODIS, ICARDA, P.O. Box 5466, Aleppo, Syria.

FABIS Coordinating Committee

BRAZIL: Dr H. Aidar, National Center for Research on Rice and Beans, BR-153, km 4—Goiania/Anapolis, Caixa Postal 179, 74000—Goiania, Goias.

CANADA: Dr C. Bernier, Department of Plant Science, University of Manitoba, Winnipeg, Manitoba R3T 2N2.

EGYPT: Dr A. Nassib, Field Crops Institute, Agricultural Research Center, Giza 12619.

FRANCE: Dr J. Picard, 4 Rue du 8 Mai, 36100 Neuvy-Pailloux.

ITALY: Prof. C. de Pace, Istituto di Biologia Agraria, Universita della Tuscia, Viterbo.

SPAIN: Dr J.I. Cubero, Escuela Technica Superior de Ingenieros Agronomos, Departamento di Genetica, Apartado 3048, Cordoba.

SUDAN: Dr F.A. Salih, Agricultural Research Corporation, Shambat Research Station, P.O. Box 30, Khartoum North.

SYRIA: Dr L.D. Robertson/FABIS Editor, ICARDA, P.O. Box 5466, Aleppo.

THAILAND: Dr K. Kogure, JICA Team Office, Faculty of Agriculture, Chiang Mai University, Chiang Mai 50200.

UK: Dr D.A. Bond, Plant Breeding Institute, Maris Lane, Trumpington, Cambridge CB2 2LQ.

FABIS Production Team

L.D. Robertson/Technical Editor

G.R. Manners/Editor

K. Al-Jbaili and Adel Abdel Khaleq/Arabic abstracts

W. Meskine/Typesetter



Faba Bean Information Service

FABIS Newsletter 38/39

1996

CONTENTS

Page	Review Articles
1	Food Legumes in Qinghai Guo Gaoqiu Minyi
2	Nutritional Potential of Faba Bean for Improved Productivity in Ruminants – A Review B.S. Tewatia and A.S. Virk
Research Articles	
<i>Breeding and Genetics</i>	
12	Combining Ability in Faba Bean D.K. Kaul and K.L. Vaid
14	Genetic Principal Components and Classification for Quantitative Characters in Faba Bean Yuan Mingyi, Shen Haining, Zhang Peilan and Liu Yang
18	Induced Leaf Variations in Faba Bean Mohammad Yasin
21	Colchicine Induced Tetraploids of <i>Vicia faba</i> L. Alexey Y. Kravchenko

- 24 Karyotype Study in *Lathyrus sativus* L. cv P-505
Sushil Kumar and D.K. Dubey
- 26 Variability and Correlation Studies in Grasspea (*Lathyrus sativus* L.)
Sushil Kumar and D.K. Dubey
- 30 Inheritance of Seed Weight in Grasspea (*Lathyrus sativus* L.)
K.R. Tiwari and C.G. Campbell
- 33 Divergence among Induced Mutants of Grasspea (*Lathyrus sativus* L.)
Sushil Kumar and D.K. Dubey

Pests and Diseases

- 37 Morphological, Cultural and Pathogenic Variability among Nine Isolates of *Botrytis fabae* from Ethiopia
Dereje Gorfu

Seed Quality and Nutrition

- 42 Rapid Spectrophotometric Method for Reduction of Vicine and Convicine in Faba Bean Seed
G. Sixdenier, F. Cassecuelle, L. Guillaumin and G. Duc

Variety Release Notice

- 45 Qinghai 9, A New Spring-sown Faba Bean Cultivar with Large Seeds and High Yield in China
Yuan Mingyi, Shen Haining, Zhang Peilan and Liu Yang

News

- 46 Editors' Notes
- 46 Agricultural Libraries Receiving ICARDA Publications

Contributors' Style Guide

Review Articles

Food Legumes in Qinghai

Guo Gaoqiu Minyi

*Qinghai Academy of Agriculture and Forestry
Qinghai, CHINA*

Qinghai is situated in the northeast of the Qinghai-Tibet Plateau, 2500–4500 meters above sea level. The cultivation of food legumes including pea (*Pisum sativum* L.), faba bean (*Vicia faba* L.), chickpea (*Cicer arietinum* L.), and lentil (*Lens culinaris* Medik.) has a long history in the province, which is one of the important legume-producing regions of China.

Food legumes are an important part of the agricultural sector, including livestock husbandry, in Qinghai. Because of their nitrogen fixation, the crops play a significant role in improving the soil and are the best break crops in cereal-based crop rotations; they are also widely used as green manure. Since they contain more protein in dried seeds than other crops, the seeds of legumes are used as food for people or feed for livestock, and their fresh stems and leaves also serve as fodder. Having large seeds (100-seed weight \geq 150 g) and good quality, dry seed of faba bean is the main grain export commodity of Qinghai.

Food legumes are mainly cultivated in the eastern farming area, which is the main agricultural production region of Qinghai. Upland fields, 2200–2700 m above sea level, make up two-thirds of the area, and have annual precipitation of over 350 mm, and a crop-growing period of about 150 days. The remaining area is warmer and irrigated. The crop-growing period is about 170 days, and rainfall is about 300 mm in May–August.

The annual area under food legumes is 60,000 ha in Qinghai. Generally, yield of pea is 1650 kg/ha; and that of faba bean is 2700 kg/ha – the highest in China. However, yield could be more than 6000–7000 kg/ha for both crops. Yield of chickpea is usually 2250 kg/ha and that of lentil is 1500 kg/ha.

Collection of food-legume germplasm began in the 1950s. The genetic resources collected comprise over 1000 pea, over 700 faba bean, 396 chickpea, and 250 lentil accessions, held at Qinghai Academy of Agriculture and Forestry (QAAF). Some of these have been utilized in breeding. Cultivars planted over large areas for pea include Dagin, Prairie No. 12, Dake, Prairie 224, Prairie No. 7, and No. 11, and for faba bean are Maya, Ga, Qinghai No. 3, No.

8 and No. 9. In addition, two new lines of chickpea, CP55 and 85-47C, have been released.

Optimum agronomic practices for pea and faba bean, determined through many years' research, have been widely applied by farmers. In the rotation systems, legume crops (pea and faba bean) are forecrops for other crops. The rotations practised are cereal–legume–cereal and cereal–cereal–legume–cereal–cereal (cereal = wheat and barley).

Pea and faba bean are generally sown in late March to early April, and are harvested in late August to early September. Sowing depth is 6–8 cm (on dryland 8–10 cm). Fertilizer applied is 15–22.5 t organic fertilizer/ha and 30–40 kg N/ha; 78–80 kg P₂O₅/ha is given as a basal dressing at sowing; under irrigated conditions the field is watered 3–4 times during growth. The density of pea is 75,000–90,000 plants/ha and of faba bean is 24,000–300,000 plants/ha. Diseases and pests are mainly controlled chemically.

The major limiting factors to production are drought at various stages of growth, especially during the stages of flowering to pod-filling; poor soil; and damage by diseases and insects which are chocolate (red) spot (*Botrytis fabae* Sard.), rust (*Uromyces fabae* (Pers.) de Bary), leaf spot (*Ascochyta fabae* Speg.), root rot (*Fusarium solani* Mart.), broad bean aphid (*Aphis medicaginis* Koch), and faba bean beetle (*Bruchus rufimanus* Boh.) for faba bean, and root diseases and leaf miner (*Phytomyza atricornis* Meigen) for pea.

Agronomic (cultivation) recommendations have been made for chickpea by QAAF and are being used in crop production.

QAAF has carried out research on legumes for 30 years. Now, it is shouldering the legume-improvement program for Qinghai, which includes pea and faba bean breeding and cultivation, chickpea breeding and drought tolerance, and legume germplasm. We are working to develop food legumes in China.

Further reading

- Guo Gaoqiu. 1994. Peas. Pages 129–147 in *High Yield Cultural Techniques on Faba Bean and Pea* (Fu Xiao and Guo Gaoqiu). Jingdun Press, Beijing, China.
- Legumes Lab. 1987. Research on high-yield cultivated synthetic technology of faba bean. QAAF, Xining, Qinghai.

Nutritional Potential of Faba Bean for Improved Productivity in Ruminants – A Review

B.S. Tewatia and A.S. Virk

Department of Animal Nutrition
CCS HAU, Hisar 125 004 (Haryana), INDIA

Abstract

As a rich source of protein and vitamins, faba bean holds good nutritional potential to replace some conventional protein supplements in ruminant feed. The nutrient composition of faba bean is favorable compared with other pulses. Although the lipid content of faba bean is low (0.9–4.2%), linoleic acid constitutes more than 50% of total lipids. Faba bean protein is highly soluble in the rumen, highly degradable, and is comparable to urea. Extrusion of faba bean at 120°C significantly reduced the rumen degradation of protein for its efficient utilization. Protection of faba bean protein using 1.0–1.5 g formaldehyde per 100 g faba bean protein significantly improved the nitrogen retention and body weight gains of growing kids. High levels of formaldehyde improved feed conversion efficiency and nitrogen balance. The presence of antinutritional factors like tannins, trypsin inhibitors and favism-inducing agents in faba bean sometimes limit its use as animal feed; however, no adverse effect on animal health or production was observed. Further, these antinutritional factors can be reduced either by breeding or by various processing techniques. Faba bean can successfully replace 45–60% of conventional protein sources in the diets of growing and lactating ruminants.

Key words: *Vicia faba*; faba beans; ruminants; animal feeding; proximate composition; animal nutrition.

Introduction

Increased livestock populations and the shift of feed resources toward poultry and swine production is widening the gap between feed availability and requirements of Indian ruminants. It has been estimated that the difference between availability and requirements of these animals will be 28.08 million tonnes of concentrates by AD 2000 (Singh 1990). Therefore, there is a need to explore the utilization of newer feed resources to bridge this gap. Faba bean (*Vicia faba* L.) is an under-utilized source of native protein. It is a leguminous crop grown under irrigated conditions; however,

الإمكانية الغذائية للفاول لتحسين إنتاجية المجترات -
مراجعة

المخلص

يوصفه مصدراً غنياً للبروتين والفيتامينات، يمتلك الفول (*Vicia faba* L.) إمكانية غذائية جيدة لكي يحل محل بروتينات تكميلية تقليدية في أعلاف المجترات. ويُعد التركيب الغذائي للفاول موافياً بالمقارنة مع البقوليات الحبية الأخرى. ورغم أن محتوى الفول من المواد الدهنية منخفض (0.9-4.2%)، فإن حمض اللينوليك يشكل أكثر من 50% من إجمالي الدهون. كما يتمتع بروتين الفول بقدرة كبيرة على التحلل والانحلال في كرش المجترات، ويضاهي اليوريا. إن انبثاق الفول بدرجة حرارة 120 مئوية قد خفض كثيراً من قدرة الكرش (المعدة الأولى) على تحلل البروتين لاستخدامه بشكل فعال. إن حماية بروتين الفول باستعمال 1.0-1.5 غ من ألدهيد النمل (formaldehyde) في كل 100 غ من بروتين الفول، أدت إلى تحسين الاحتفاظ بالآزوت وزيادة وزن جسم الجداء النامية إلى درجة كبيرة. كما أدت المستويات العالية من ألدهيد النمل إلى تحسين فعالية تحول العلف وموازنة الآزوت. إن وجود عوامل مضادة للتغذية مثل التانين ومثبطات التربيسين والعوامل المحرضة على التفول (favism) في الفول يحد في بعض الأحيان من استخدامه كعلف للمواشي، علماً أنه لم تلحظ أية تأثيرات عكسية على صحة الحيوان أو إنتاجه. وعلاوة على ذلك، فإن العوامل المضادة للتغذية هذه يمكن تقليلها إما بالتربية أو بواسطة تقنيات معالجة عديدة. ويمكن للفاول أن يحل محل 45-60% من مصادر البروتين التقليدية في غذاء المجترات في مرحلتي النمو والإرضاع.

it can also withstand high water tables and soil salinity (Lockerman et al. 1983) and yield more than conventional pulses. The total area under cultivation in the world is 3.201 million hectares with a production of 4.312 million tonnes (FAO 1990). Faba bean is a rich source of protein (Hove et al. 1978) besides being rich in vitamins and minerals except sodium and chloride (Clarke 1970). Faba bean has a low oil content of 2.0–2.6% (Bjerg et al. 1981) and methionine and cystine are limiting amino acids (Szelenyine et al. 1984). Antinutritional factors like tannin, trypsin inhibitors and favism-inducing agents present in faba bean can limit its feeding value (Marquardt 1983). Therefore, the possibility

of using faba bean as a protein supplement in the diets of ruminants needs to be investigated. This review presents the potential of faba bean for growing and lactating ruminants, including the role and effects of antinutritional factors and their detoxification procedures.

Chemical Composition

Faba bean seed is made up of two components, testa and kernel, of which testa is 12–16.6%. Faba bean is a rich protein (23–32%) source (Evans et al. 1972; Szelenyine et al. 1984). Most of the protein is found in the kernel, and the protein concentration of the testa or seed coat is low (Evans et al. 1972). Faba bean seeds are a poor source of methionine and cystine (Boulter 1980), which combined make up only 2–2.3% of the protein (Szelenyine et al. 1984). Crude fiber content ranges from 6 to 11%, and spring-sown cultivars are reported to have low fiber content (Eden 1968); the testa contributes a higher proportion of crude fiber than the kernel. Lipid concentration of faba bean is low (0.9–4.2%) and the kernel is richer in lipid than the testa (Newton and Hill 1983). The majority of the fatty acids present in faba bean are unsaturated, and linoleic acid makes up more than 50% of the total (Clarke 1970). Water-

soluble sugars are 5.7–10.08% (Sammour 1987). Faba bean is a rich source of vitamins and has tocopherol, riboflavin and choline at 1.0, 3.1 and 1110 mg/kg, respectively (Bolton 1963). Macro- and micro-mineral content, except sodium and chloride, is also comparable with other legume crops (Hove et al. 1978). Seed hulls are rich in calcium, whereas cotyledons contain higher concentrations of phosphorus (Marquardt et al. 1975). Chemical composition reported by different investigators is summarized in Table 1.

Protein degradability

It is well known that digestible crude protein (DCP) values are not satisfactory indicators of the dietary protein needs of ruminants. Dietary protein needs of ruminants can be calculated from rumen-degradable nitrogen (RDN). Faba bean proteins are highly soluble in the rumen and their degree of degradation is comparable with urea for non-protein nitrogen (NPN) (Miller 1980; Cros et al. 1991). In order to use faba bean protein more efficiently in ruminant nutrition, its degradation at the rumen level must be reduced and by-passed without altering intestinal digestion. This can be achieved by extrusion (Cros et al. 1991) or by chemical treatment (Sharma and Nicholson 1975).

Table 1. Proximate (%) and macro nutrient (g/kg) composition of faba bean seeds.

CP	EE	CF	Ash	NFE	Na	K	Ca	Mg	Reference
31.4	1.5	8.0	4.0	55.2	0.1	11.7	1.6	1.3	Eden (1968)
26.5	1.5	9.0	4.0	59.0	0.2	12.2	1.9	1.3	
31.8	0.9	8.5	3.6	55.2	–	–	1.0	–	Marquardt et al. (1975)
25.6	2.5	15.4	3.4	53.1	0.1	12.1	0.8	1.2	Hove et al. (1978)
31.5	–	–	–	–	5.2	1.6	3.2	3.6	Sammour (1987)
27.4	1.3	11.1	3.7	56.5	–	–	–	–	Valentine and Bartsch (1987)
23.7	2.3	11.6	6.7	55.6	–	–	1.8	–	Akbar and Gupta (1990b)
28.4	1.1	–	4.3	–	–	–	–	–	van der Poel et al. (1991)
28.8	2.0	–	4.7	–	–	–	–	–	Cros et al. (1992)
28.9	2.3	12.5	8.9	47.2	–	–	–	–	Fulpagare (1993)
23.9	0.8	13.0	3.8	58.5	–	–	–	–	Virk et al. (1993)

CP = Crude protein; EE = Crude fat, ether extract; CF = Crude fiber; NFE = Nitrogen free extract.

Extrusion

Heat treatment of faba bean at 105°C does not enhance the passage of protein to the duodenum in cattle since this temperature is inadequate for protein protection (McMeniman and Armstrong 1979). However, extrusion at 120°C reduced nitrogen solubility (21.1 vs 74.9%), lowered rumen degradation of dry matter (74.6 vs 80.4%) and protein (70.2 vs 89.2%), and increased the availability of faba bean protein for intestinal digestion in non-lactating Holstein cows (Cros et al. 1991). It is further reported that extrusion increased the amount of dietary protein digested at the post-ruminal level by 110–170% in Holstein cows (Cros et al. 1992). Nutrient digestibility in Holstein cows was not altered by extrusion of faba bean at 195°C (Benchaar et al. 1992), while the flow of amino acids and availability of essential amino acids improved in lactating Holstein cows (Benchaar et al. 1994).

Chemical treatment

Treatment of faba bean protein with formaldehyde at 1.5 g/100 g protein (Sharma and Nicholson 1975) improved the ruminal flow of total and protein nitrogen to blood serum of sheep, and weight gains were higher in the treatment group (93.6 g/day) than in the group fed untreated faba bean (87.9 g/day). Similarly, Virk et al. (1994) observed improved digestibility of dry matter and ether extract with formaldehyde treatment (0.5, 1.0, 1.5 g/100 g protein) in kids. Body-weight gains and N retained as percentage of intake also increased as formaldehyde level increased. However, lower levels of formaldehyde (0.4, 0.5, 1.2 g/100 g) did not improve feed conversion efficiency or nitrogen balance in lambs (Pisulewski and Rys 1975). Similarly, lower levels of formaldehyde (0.43 and 0.54 g/100 g) did not improve nutrient digestibility, milk yield or milk composition in goats fed faba bean (Tewatia et al. 1995).

Iron and aluminum cations in the form of alums (2–40 g metal/kg feed) were used to reduce faba bean protein degradation (Antoniewicz, 1993). Iron alum was as effective as formaldehyde in reducing protein solubility. Autoclaving (120°C for 20–30 minutes) in combination with iron was more effective in reducing the ruminal degradation of faba bean protein.

Rumen metabolic profile

Incorporation of 35% faba bean in concentrate mixture of lactating cows lowered the total volatile fatty acids (91.1 meq/L) compared with soybean meal (105.7 meq/L), but the molar percentage of acetate was higher in the group fed faba bean (Ingalls and McKirdy 1974). However, Ingalls et al.

(1980) did not observe any difference in the molar percentage of acetate, propionate and butyrate when faba bean replaced soybean meal at the 35% level in the diet of Holstein cows. Valentine and Bartsch (1987) observed higher concentrations of total volatile fatty acids in dairy cows fed faba bean (99 meq/L) than in those fed a barley-based diet (90.8 meq/L). Ammonia-nitrogen concentration with faba bean feeding was lower (18.7 meq/100 ml) compared with soybean meal (19.3 mg/100 ml); faba bean protein protection with formaldehyde further reduced the rumen NH₃-N level in Holstein calves (Sharma and Nicholson 1975). Similarly, Virk et al. (1994) observed that as the level of formaldehyde treatment increased (0.5, 0.1, 1.5 g/100 g protein), the level of rumen NH₃-N decreased ($P<0.05$) in kids. However, at lower levels of formaldehyde (0.43, 0.54 g/100 g protein), no effect on NH₃-N concentration was observed in goats fed faba bean (Tewatia et al. 1995). Fulpagare (1993) reports that as the level of faba bean in concentrate increased, ruminal total and ammonia nitrogen increased ($P<0.05$) in lambs. However, Akbar and Gupta (1990a) did not observe any change in total and ammonia nitrogen concentration in buffalo calves fed concentrate mixtures having 0, 20, 40 and 60% protein from faba bean.

Nutrient digestibility

Faba bean inclusion in diets did not alter dry-matter intake (Giovanni et al. 1976; Akbar and Gupta 1990b; Virk et al. 1991); however, greater dry-matter intake was observed in goats fed faba bean compared with controls (Tewatia et al. 1993). Faba bean feeding at various levels did not affect nutrient digestibility (Akbar and Gupta 1990b; Virk et al. 1991; Tewatia et al. 1993). However, Fulpagare (1993) reports that as the level of faba bean increased (0, 25, 100%) in the diet of lambs, the digestibility of dry matter (either extract and crude fiber) increased, while that of nitrogen-free extract decreased. Dry-matter intake and nutrient digestibility reported by various researchers is presented in Table 2.

Growth and meat production

Body-weight gains were not affected in wethers (castrated male sheep) when faba bean replaced soybean meal at the 40% level (Ringdorfer 1990). Similarly, Virk et al. (1991) conclude that faba bean could be an alternative to groundnut cake up to 60% in the diet of kids without adversely affecting body-weight gains. Akbar and Gupta (1990b) observed greater body-weight gains in buffalo calves given more faba bean (45%) in the concentrate mixture. Fulpagare (1993)

Table 2. Dry matter intake and nutrient digestibility in ruminants fed various levels of faba bean.

Species	Diet	DM intake (kg/100 kg BW)	Nutrients digestibility (%)†					Reference
			DM	CP	EE	CF	NFE	
Calves	Soybean meal	2.32	63.0	73.0	-	-	-	Sharma and Nicholson (1975)
	Water-treated faba bean	2.51	54.9	67.9	-	-	-	
	Formaldehyde-treated faba bean	2.35	55.3	66.5	-	-	-	
	Conventional concentrate mixture	2.34	64.5	73.7	58.5	56.6	67.7	
Buffalo calves	15% faba bean in concentrate mixture	2.34	63.8	73.5	57.6	57.1	69.3	Akbar and Gupta (1990b)
	30% faba bean in conc. mix.	2.41	64.1	73.9	53.8	56.8	67.8	
	45% faba bean in conc. mix.	2.31	66.0	70.6	60.2	58.9	70.8	
Kids	Gram straw-conventional concentrate mixture	3.41	58.8	57.3	74.9	33.1	81.6	Virk et al. (1991)
	Gram straw-20% CP of GNC replaced by faba bean	3.06	55.4	58.3	75.8	40.1	83.1	
Lambs	Gram straw-40% CP faba bean	3.18	61.8	63.1	73.0	50.4	83.1	Fulpagare (1993)
	Gram straw-60% CP faba bean	2.85	58.4	56.7	82.5	41.5	83.7	
	Gram straw-concentrate mix.	2.99	61.7	71.0	59.8	48.2	73.0	
	Gram straw-25% faba bean in conc. mix.	2.75	62.9	71.1	61.5	57.4	70.4	
	Gram straw-100% faba bean in conc. mix.	2.65	63.4	73.6	62.4	61.3	70.5	
Goats	Gram straw-concentrate mix.	3.80	62.1	84.3	72.5	51.3	67.1	Tewatia et al. (1993)
	Gram straw-30% faba bean in conc. mix.	4.30	61.5	83.8	70.5	47.4	66.5	
Kids	Gram straw-60% faba bean in conc. mix.	4.10	62.0	83.7	72.0	52.4	66.1	Virk et al. (1994)
	Concentrate mix. (60% faba bean)	3.00	65.7	63.7	74.9	47.8	79.2	
	0.5% formal dehyde-treated concentrate (ftc)	3.30	64.6	59.9	83.4	41.2	77.1	
	1.0% ftc	3.10	68.8	60.9	82.6	46.1	80.8	
	1.5% ftc	3.30	69.8	69.3	90.5	48.6	80.7	

†DM = dry matter; other abbreviations, see Table 1.

replaced conventional concentrate mixture protein at the 0, 25 and 100% levels in the diet of lambs and observed that body-weight gains were greater at 25% (58.9 g/day) and 100% (67.8 g/day) levels than in the control (46.4 g/day). Contrary to these observations, Pichler (1990) observed a decrease in body-weight gains in bulls with faba bean replacement of soybean meal at a level of 50%. Effects of faba bean on growth rate and feed-utilization efficiency as observed by various workers are presented in Table 3.

Faba bean inclusion in the diet had no effect on dressing percentage (Leitgeb 1988; Pichler 1990; Fulpagare 1993) or meat composition (Caballero et al. 1992). However, Giovanni (1984) reports that calf-carcass grading was impaired after faba bean feeding.

Milk yield and its composition

Milk yield was not adversely affected when faba bean replaced soybean and rapeseed (Ingalls and McKirdy 1974), soybean meal and pea (Jutz and Leitgeb 1989) and soybean (Ingalls et al. 1980) in the diets of lactating cattle, or groundnut cake in the diets of lactating goats (Tewatia et al. 1993). Faba bean feeding enhanced milk fat content (Hansen and Anderson 1972). However, Ingalls et al. (1980) and Jutz and Leitgeb (1989) found no effect on milk fat content when faba bean replaced other vegetable protein sources. Milk protein percentage was unaltered (Ingalls and McKirdy 1974) or increased (Tewatia et al. 1993) with faba bean feeding; however, Hansen and Anderson (1972) observed decreased milk protein percentage when faba bean was included at the 60% level. Inclusion of faba bean in the diets of lactating cattle and goats and their effects on milk yield and its composition are presented in Table 4.

Antinutritional factors and their detoxification

Tannins

Most legumes contain appreciable amounts of polyphenolic substances known as tannins, and most of these compounds in faba bean are found in the testa (Kadirvel and Clandinin 1974). Tannic acid content of whole faba bean seed varies from 0.75 to 2.0% (Reddy et al. 1985). The seed coat of colored-flowered varieties may contain up to 8% tannins (Griffiths and Jones 1977), while seeds of white-flowered varieties are practically tannin-free (Sjodin et al. 1981). High tannin content decreases the digestibility of faba bean (Marquardt et al. 1978a), and there is a negative correlation ($r = -0.84$ to -0.94) between tannin content and *in-vitro* dry-

matter digestibility (Garrido et al. 1989). Faba bean tannins also reduce the biological availability and *in-vitro* digestibility of proteins (Lacassagne et al. 1988) and carbohydrates (Deshpande and Salunkhe 1982). However, Griffiths and Jones (1977) observed that the amount of protein precipitated by tannins was low and, in the absence of the testa, 98% of the protein was soluble in pepsin while 95% was soluble in the presence of the testa.

Negative effects of faba bean tannins can be reduced by breeding and various processing techniques. Breeding efforts are being made to develop low-tannin varieties (Bond and Smith 1989). Tannins are mainly located in the seed coat, so they can be removed by dehulling the beans (Reddy et al. 1985). This process also removes much of the fiber of the seed, and the nutritional value of faba bean protein can be better utilized (Sosulski and Dabrowski 1984). Approximately 40% of the total tannins are removed by manual dehulling, which also removes more than 85% of condensed tannins (van der Poel et al. 1991). Tannins from faba bean have also been eliminated by treatment with 4% NaOH solution, autoclaving at 121°C or addition of adsorbents (Garrido et al. 1988). Treatment with NaOH and autoclaving removed 97 and 57% of tannins, respectively. Storing faba bean seeds for 14 months resulted in an 11% decline in tannins (Marquardt et al. 1978b). Reduction of tannins resulted in improved *in-vitro* protein digestibility (van der Poel et al. 1991) and a positive correlation ($r = 0.90-0.99$) between quantity of tannins removed and *in-vitro* protein digestibility (Babiker and El Tinay 1993).

Trypsin inhibitor

Trypsin, a proteolytic enzyme, is inhibited by glycoproteins present in faba bean. Variation in trypsin-inhibitor activity among faba bean cultivars is reported by McNab and Wilson (1977). Trypsin-inhibitor activity in faba bean (11.0 units/mg) is higher than that in lupin (0.9 units/mg), but lower than that in pea (13.1 units/mg; Korol et al. 1986). Whole soybean contained a nine-fold higher concentration of trypsin-inhibitor activity than faba bean (Marquardt et al. 1975). Greater trypsin-inhibitor activity was observed in cotyledons than in the testa (Wilson et al. 1972); however, activity in the testa double that in the cotyledons has also been observed (Marquardt et al. 1975). This suggests that dehulling of beans is unlikely to reduce trypsin-inhibitor activity. Autoclaving at 121°C for 20 minutes resulted in almost complete destruction of trypsin-inhibitor activity (Marquardt et al. 1975). Activity was also reduced when faba beans were initially processed at 100°C for 5 minutes, but yield was reduced by 18% (Borowska 1993).

Table 3. Growth rate and feed utilization efficiency in ruminants fed faba bean.

Species	Diet	Wt gain (g/day)	DM consumed (kg per kg wt gain)	N balance (g/day)	Reference
Calves	16% soybean meal	700	2.6	19.9	MacLeod et al. (1972)
	12% white-fish meal	790	2.6	27.9	
	30% faba bean	740	2.6	22.0	
Calves	Soybean meal	973	6.6	2.7	Sharma and Nicholson (1975)
	Water-treated faba bean	1046	6.9	2.3	
Buffalo calves	Formaldehyde-treated faba bean	1114	6.2	3.1	
	Conventional concentrate mix.	511	7.9	43.4	Akbar and Gupta (1990b)
	15% faba bean in concentrate	501	7.9	46.2	
	30% faba bean in concentrate	573	7.8	54.8	
Kids	45% faba bean in concentrate	583	7.0	54.1	
	Conventional concentrate mix.	47.6	11.9	1.3	Virk et al. (1991)
	20% faba bean in conc. mix.	42.1	11.7	2.1	
Lambs	40% faba bean in conc. mix.	50.6	10.8	2.9	
	60% faba bean in conc. mix.	50.0	9.7	2.1	
	Faba bean	271.0	3.6	-	Caballero et al. (1992)
Kids	Soybean meal	281.0	3.5	-	
	Concentrate mixture having 60% faba bean	36.6	10.2	2.8	Virk et al. (1994)
	0.5% formaldehyde treatment	43.4	9.8	3.8	
	1.0% formaldehyde	59.2	6.9	3.4	
	1.5% formaldehyde	67.9	6.3	4.1	

Table 4. Milk yield and composition as affected by faba bean inclusion in diet of ruminants.

Species	Diet	Milk (L/day)	FCM yield (L/day)	Fat (%)	Protein (%)	Reference
Cattle	Hay-silage-17% faba bean	25.4	19.1	2.4	3.5	Ingalls and McKirdy (1974)
	Hay-silage-35% faba bean	24.9	20.2	2.9	3.4	
	Hay-silage-soybean meal	26.0	19.5	2.3	3.5	
	Hay-silage-rapeseed	26.7	20.3	2.4	3.5	
Cattle	Straw-soybean meal	20.7	20.1	3.9	3.4	Ingalls et al. (1980)
	Straw-35% faba bean	19.1	18.3	3.7	3.5	
Cattle	Wheat-barley-soybean	20.4	21.3	4.3	3.5	Jutz and Leigeb (1989)
	Wheat-barley-faba bean	19.4	19.9	4.2	3.4	
	Wheat-barley-pea	20.2	21.1	4.3	3.4	
Goats	Gram straw-concentrate mix.	0.27	0.30	5.1	3.9	Tewatia et al. (1993)
	Gram straw-30% faba bean in concentrate	0.35	0.38	4.5	4.4	
Goats	Gram straw-60% faba bean	0.33	0.35	4.4	4.2	
	Gram straw-60% faba bean in concentrate	0.34	0.43	5.7	5.1	Tewatia et al. (1995)
	Gram straw-0.43 g formaldehyde/100 g faba bean protein	0.39	0.49	5.7	4.8	
	Gram straw-0.54 g formaldehyde	0.39	0.49	5.8	4.5	

FCM = Fat Corrected Milk (4%).

Hemagglutinins

Activity of hemagglutinins is $3.4\text{--}5.6 \times 10^3$ per gram of whole seed and is mainly concentrated in the kernel (Marquardt et al. 1975). This activity is low compared with other legumes (Palmer and Thompson 1975). Sheep and cattle are tolerant to hemagglutinin activity as their erythrocytes do not agglutinate in the presence of extracts from faba bean (Marquardt et al. 1975). Hemagglutinin activity can be reduced effectively by heat treatment (Marquardt et al. 1976), especially if beans are soaked in water before treatment.

Vicine and convicine

Vicine and convicine contents of faba bean are 0.55 and 0.32%, respectively (Bjerg et al. 1980). The seed coat had lower activity (0.07%) of vicine compared with the cotyledon (0.23–0.6%). These favism-inducing factors have been reduced by heat treatment (Marquardt et al. 1975).

Future lines of investigation

- Larger areas should be used for faba bean cultivation to enhance the production of this protein-rich source, especially since it can withstand high water tables and saline soils.
- Varieties with low content of trypsin inhibitors and favism-inducing factors should be developed.
- Since more than 50% of the total lipids in faba bean are linoleic acid, faba bean can serve as a dietary methane depressant in ruminants. This dietary manipulation will enhance the energetic efficiency and lower the environmental pollution due to methane production caused by ruminant feeding.
- Efforts should be made to lower the protein degradation of faba bean to enhance the net protein utilization, especially in the diets of growing livestock.
- For minimizing antinutritional factors, techniques like transgenic crossing or chemical treatment should be explored.
- Nutritionists should generate more data so that this feed ingredient is used widely by feed manufacturers and livestock producers.

References

- Akbar, M.A. and P.C. Gupta. 1990a. Effect of feeding different levels of faba bean (*Vicia faba* L.) seeds on some rumen metabolic profiles in buffalo calves. *Indian Journal of Animal Nutrition* 7: 219–220.
- Akbar, M.A. and P.C. Gupta. 1990b. Faba bean (*Vicia faba* L.) as a source of protein supplement in the ration of buffalo calves. *Indian Journal of Animal Sciences* 60: 1474–1480.
- Antoniciewicz, A. 1993. The influence of trivalent cations and thermal treatment on ruminal degradability of field beans (*Vicia faba*) and rape seed (*Brassica napus*) protein. *Journal of Animal and Feed Sciences* 1: 263–278.
- Babiker, E.E. and A.H. El Tinay. 1993. Effect of soaking in alkali on tannin content and *in vitro* protein digestibility of faba bean cultivars. *FABIS Newsletter* 33: 33–36.
- Benchaar, C., M. Vernay, C. Bayourthe and R. Moncoulon. 1992. Incidence of bean (*Vicia faba*) extrusion on starch and nitrogen intestinal flow in lactating cows. *Dairy Science Abstract* 54: 6953.
- Benchaar, C., M. Vernay, C. Bayourthe and R. Moncoulon. 1994. Effect of extrusion of whole horse bean on protein digestion and amino acid absorption in dairy cows. *Journal of Dairy Science* 77: 1360–1371.
- Bjerg, B., M.H. Poulsen and H. Sorenson. 1980. Quantitative estimation of favism releasing factors in *Vicia faba* seeds. *FABIS Newsletter* 2: 51–52.
- Bjerg, B., C.N. Knudren, M.H. Poulsen and H. Sorenson. 1981. Elimination of anti-nutritional and favism releasing factors in *Vicia faba* L. *Pulse Crop Newsletter* 1: 36–38.
- Bolton, W. 1963. Poultry Nutrition. UK Ministry of Agriculture, Fisheries and Food Bulletin No. 174, 2nd edn. HMSO, London. (Pages 43–83.)
- Bond, D.A. and D.B. Smith 1989. Pages 285–296 in *Recent Advances of Research in Anti-nutritional Factors in Legume Seeds*. Pudoc, Wageningen, The Netherlands.
- Borowska, J. 1993. Protein preparations from faba bean seeds. *Nutrition Abstracts and Reviews (B)* 63: 4862.
- Boulter, D. 1980. Ontogeny and development of biochemical and nutritional attributes in legume seeds. Pages 127–134 in *Advances in Legume Science*. England.
- Caballero, R., J. Rioperez, E. Fernandez, M.T. Marin and C. Fernandez. 1992. A note on the use of field beans (*Vicia faba*) in lamb finishing diets. *Animal Production* 54: 441–444.
- Clarke, H.E. 1970. The evaluation of field beans (*Vicia faba* L.) in animal nutrition. *Proceedings of the Nutrition Society* 29: 64–73.
- Cros, P., M. Vernay and R. Moncoulon. 1991. *In situ* evaluation of the ruminal and intestinal degradability of extruded whole horsebeans. *Reproductive Nutrition and Development* 31: 249–255.
- Cros, P., M. Vernay, C. Bayourthe and R. Moncoulon. 1992. Influence of extrusion on ruminal and intestinal disappearance of amino acids in whole horsebean. *Canadian Journal of Animal Science* 72: 359–366.
- Deshpande, S.S. and D.H. Salunkhe. 1982. Interaction of tannic acid and catechin with legume starches. *Journal of Food Science* 47: 2080.

- Eden, A. 1968. A survey of the analytical composition of field beans (*Vicia faba* L.). *Journal of Agricultural Science* 70: 299-301.
- Evans, L.E., J.F. Seitzer and W. Bushuk. 1972. Horse beans – a protein crop for Western Canada. *Canadian Journal of Plant Science* 52: 657-659.
- FAO. 1990. Production Year Book 44. Food and Agriculture Organization of the United Nations, Rome. (Page 102.)
- Fulpagare, Y.G. 1993. Nutritional evaluation of bakla (*Vicia faba* L.) on long-term feeding in lambs. PhD Thesis. CCS HAU, Hisar (Haryana).
- Garrido, A., A. Gomez and J.E. Guerrero. 1988. Methods of removing tannins from *Vicia faba* L. *Nutrition Abstracts and Reviews (B)* 60: 4642.
- Garrido, A., A. Cabrera, A. Gomez and J.E. Guerrero. 1989. Pages 160-164 in Recent Advances of Research in Antinutritional Factors in Legume Seeds. Wageningen, The Netherlands.
- Giovanni, R. 1984. Use of field beans without or with tannins in concentrate feeds for growing lambs. *Nutrition Abstracts and Reviews (B)* 54: 5370.
- Giovanni, R., R. Guithemet and R. Toullec. 1976. The effect of replacing soyabean oilmeal by beans as the main source of nitrogen in concentrate feeds for rearing calves. *Nutrition Abstracts and Reviews* 46: 8280.
- Griffiths, D.W. and D.J.H. Jones. 1977. Cellulose inhibition by tannins in the testa of field beans (*Vicia faba*). *Journal of the Science Food and Agriculture* 28: 983-989.
- Hansen, M.S. and P.E. Anderson. 1972. Horse beans (*Vicia faba* L.) for dairy cows. *Bulletin Fra Forsogslaboratoriet, Copenhagen* 396: 1-32.
- Hove, E.L., S. King and G.D. Hill. 1978. Composition, protein quality and toxins of seed of the grain legumes, *Glycine max*, *Lupinus* spp., *Phaseolus* spp., *Pisum sativum* and *Vicia faba*. *New Zealand Journal of Agricultural Research* 21: 457-462.
- Ingalls, J.R. and J.A. McKirdy. 1974. Faba beans as a substitute for soyabean meal or rapeseed meal in rations for lactating cows. *Canadian Journal of Animal Sciences* 54: 87-89.
- Ingalls, J.R., J.A. McKirdy, and H.R. Sharma. 1980. Nutritive value of faba beans in the diets of young Holstein calves and lactating dairy cows. *Canadian Journal of Animal Science* 60: 689-698.
- Jutz, T.C. and R. Leitgeb 1989. The use of horse beans (*Vicia faba* L.) and peas (*Pisum sativum*) in feeding of cows. *Nutrition Abstracts and Reviews (B)* 61: 4010.
- Kadirvel, R. and D.R. Clandinin. 1974. The effect of faba beans (*Vicia faba* L.) on the performance of turkey poults and broiler chicks from 0-4 weeks of age. *Poultry Science* 53: 1810-1816.
- Korol, W., A. Burczynska-Niedzialek and S. Matyka. 1986. Anti-trypsin activity of locally available legume seeds. *Nutrition Abstracts and Reviews (B)* 56: 6497.
- Lacassagne, L., M. Francesch, B. Carre and J.B. Melchion. 1988. Utilization of tannin containing and tannin free faba beans *Vicia faba* by young chicks. Effects of pelleting feeds on energy, protein and starch digestibilities. *Animal Feed Science and Technology* 20: 59-68.
- Leitgeb, R. 1988. The use of faba beans (*Vicia faba* L.) in growing bulls. *Biological Abstracts* 85: 85717.
- Lockerman, R.H., T.J. Kisha, J.R. Sims and A.S. Abdel-Ghaffar. 1983. The effect of soil salinity on dinitrogen fixation and yield of faba bean (*Vicia faba* L.). *FABIS Newsletter* 7: 24-25.
- MacLeod, N.A., A. MacDermid and M. Kay. 1972. A note on the use of field beans (*Vicia faba*) for growing cattle. *Animal Production* 14: 111-113.
- Marquardt, R.R. 1983. Antimetabolites in faba beans: their metabolic significance. *FABIS Newsletter* 7: 1-4.
- Marquardt, R.R., J.A. McKirdy and T.A. Ward and L.D. Campbell. 1975. Amino acid, haemagglutinin and trypsin inhibitor levels and a proximate analysis of faba beans (*Vicia faba*) and faba bean factors. *Canadian Journal of Animal Science* 55: 421-429.
- Marquardt, R.R., L.D. Campbell and A.T. Ward. 1976. Studies with chicks on growth depression factors in faba beans (*Vicia faba* L.). *Journal of Nutrition* 106: 275-284.
- Marquardt, R.R., J.A. McKirdy and A.T. Ward. 1978a. Comparative cell wall constituent levels of tannin free and tannin containing cultivars of field beans (*Vicia faba* L.). *Canadian Journal of Animal Science* 58: 775-781.
- Marquardt, R.R., A.T. Ward and L.E. Evans. 1978b. Comparative properties of tannin-free and tannin containing cultivars of faba beans (*Vicia faba* L.). *Canadian Journal of Plant Science* 58: 753-760.
- McMeniman, N.P. and D.G. Armstrong. 1979. The flow of amino acids into the small intestine of cattle when fed heated or unheated beans (*Vicia faba*). *Journal of Agricultural Science (Cambridge)* 93: 181-188.
- McNab, J.M. and B.J. Wilson. 1977. Nutritive value of field beans (*Vicia faba* L.) for poultry. Scottish Horticultural Research Institute Association. Bulletin No. 15. (Pages 63-72.)
- Miller, E.L. 1980. Protein value of feedstuffs for ruminants. Pages 17-27 in *Vicia faba: Feeding Value, Processing and Viruses* (Bond, ed.). Martinus Nijhoff, The Hague.
- Newton, S.D. and G.D. Hill. 1983. The composition and nutritive value of field beans. *Nutrition Abstracts and Reviews (B)* 53: 99-115.
- Palmer, R. and R. Thompson. 1975. A comparison of the protein nutritive value and composition of four cultivars of faba beans (*Vicia faba* L.) grown and harvested under

- controlled conditions. *Journal of the Science of Food and Agriculture* 26: 1577-1583.
- Pichler, W.A. 1990. Investigations on the use of field beans (*Vicia faba*) in fattening young bulls. *Nutrition Abstracts and Reviews (B)* 60: 815.
- Pisulewski, P. and R. Rys. 1975. Effect of formaldehyde treatment of horse beans (*Vicia faba*) on the nutritional value for sheep. *Nutrition Abstracts and Reviews* 46: 10514.
- Reddy, N.R., M.D. Pierson, S.K. Sathe and D.K. Salunkhe. 1985. Dry bean tannins: A review of nutritional implications. *Journal of the American Oil and Chemical Society* 62: 541-549.
- Ringdorfer, F. 1990. Field beans in sheep fattening. Influence on fattening and slaughter performance. *Nutrition Abstracts and Reviews (B)* 16: 4037.
- Sammour, R.H. 1987. Chemical constituents and electrophoresis of seed proteins of some species of *Vicia*. *FABIS Newsletter* 18: 30-32.
- Sharma, H.R. and J.W.G. Nicholson. 1975. Effect of treating faba beans with formaldehyde on volatile fatty acids and the performance of dairy calves and fistulated sheep. *Canadian Journal of Animal Science* 55: 705-713.
- Singh, P. 1990. Forage production systems for different agro-ecological zone of India. Pages 395-415 in *Proceedings of the First National Symposium on Natural Resources Management for a Sustainable Agriculture*, vol 1.
- Sjodin, J., P. Martensson and T. Magyarosi. 1981. Selection for antinutritional substances in field bean (*Vicia faba* L.). *Zeitschrift für Pflanzenzuchtung* 86: 231-247.
- Sosulski, F.W. and K.J. Dabrowski. 1984. Composition of free and hydrolyzable phenolic acids in the flours and hulls of ten legume species. *Journal of Agriculture and Food Chemistry* 32: 131-133.
- Szelenyinc, G.M., G. Jecsei, B. Juhasz and L. Bodis. 1984. Comparative chemical and biological analysis of horse and soyabean grown in Hungary. *Nutrition Abstracts and Reviews (B)* 56: 609.
- Tewatia, B.S., A.S. Virk, P.C. Gupta, Z.S. Rana, V.K. Khatta and V. Sagar. 1993. Milk yield and its composition in goats fed faba bean. *FABIS Newsletter* 33: 37-40.
- Tewatia, B.S., V.K. Khatta, A.S. Virk and P.C. Gupta. 1995. Effect of formaldehyde treated faba beans (*Vicia faba* L.) on performance of lactating goats. *Small Ruminant Research* 16: 107-111.
- Valentine, S.C. and B.D. Bartsch. 1987. Fermentation of hammer milled barley, lupin, pea and faba bean grain in the rumen of dairy cows. *Animal Feed Science and Technology* 16: 261-271.
- van der Poel, A.F.B., S. Gravendeel and H. Boer. 1991. Effect of different processing methods on tannin content and *in vitro* protein digestibility of faba bean. *Animal Feed Science and Technology* 33: 49-58.
- Virk, A.S., V.K. Khatta, P.C. Gupta and V. Sagar. 1991. Effect of feeding faba beans (*Vicia faba* L.) on nutrient utilization and growth in crossbred goats. *Indian Journal of Animal Nutrition* 8: 149-152.
- Virk, A.S., B.S. Tewatia, V.K. Khatta and P.C. Gupta. 1993. Annual Report. Department of Animal Nutrition, CCS HAU, Hisar, Haryana.
- Virk, A.S., V.K. Khatta, B.S. Tewatia and P.C. Gupta. 1994. Effect of formaldehyde treated faba beans (*Vicia faba* L.) on nutrient utilization and growth performance of goat kids. *Small Ruminant Research* 14: 19-23.
- Wilson, B.J., J.M. McNab and H. Bentley. 1972. Trypsin inhibitor activity in the field bean (*Vicia faba* L.). *Journal of the Science of Food and Agriculture* 23: 679-684.

Research Articles

Breeding and Genetics

القدرة التوافقية في الفول

Combining Ability in Faba Bean

D.K. Kaul and K.L. Vaid

S.K. University of Agriculture Science & Technology Camp Office, Railway Road, Jammu (J&K), INDIA

المخلص

أجريت دراسات على القدرة التوافقية باستخدام تهجينات ثنائية الأليل بين ثمانية أصناف من الفول (*Vicia faba* L.) وكانت القدرة التوافقية العامة (GCA) والخاصة (SCA) بالغتي الأهمية، مما أظهر وجود تأثيرات مهيمنة وذات أثر متجمع على غلة كل نبتة ومحتوى البذور من البروتين.

Abstract

Studies were made on combining ability using diallel crosses among eight cultivars of faba bean. Both general (GCA) and specific combining abilities (SCA) were highly significant, showing the existence of both additive and dominance effects on yield per plant and seed protein content.

Key words: *Vicia faba*; faba beans; combining ability; seeds; yields; protein content; India.

Introduction

Faba bean (*Vicia faba* L.) is the fourth most important food legume in the world after dry bean, dry pea and chickpea (Hawtin and Stewart 1979). Faba bean contains 18.6–37.8% protein (El-Sayed et al. 1982). Kambal (1969) reports no significant differences in yield components. Habetinek (1985) reports the importance of general combining ability in the inheritance of yield. Waly and Abd El-Aal (1986) report that both general and specific combining ability were highly significant in diallel crosses among five cultivars of faba bean, showing the existence of both additive and dominance effects on protein and cellulose content. Picard (1979) showed that seed protein content can be improved without affecting yield.

Therefore, we studied the genetic system controlling yield per plant and seed protein content using a number of faba bean cultivars in a diallel crossing system in an attempt to improve protein content and yield per plant.

Material and Methods

The crop was sown in a randomized block design in three replications at the Pulses Substation, Habbak, Division of Plant Breeding and Genetics, Sher-I-Kashmir University of Agriculture Science and Technology, Srinagar, Kashmir,

India, during *rabi* (winter) 1987/88 and 1988/89. The observations were recorded on 10 plants for each parent and 15 for each F₁. Plants were selected and tagged randomly.

Crosses were made using half-diallel from eight inbred faba bean parents – VH-131 (1), VH-130 (2), FLIP-65 FB (3), FLIP-54 FB (4), Jordan-260 (5), Jordan-261 (6), Local-1 (7) and Local-2 (8) – to study yield per plant and seed protein content.

The genetic analysis was based on the method proposed by Griffing (1956).

Protein content was determined in the dry seeds by the Kjeldahl method. Correlation between yield and protein was carried out following Dewey and Lu (1959).

Results and Discussion

The analysis of combining ability for protein content and yield per plant are shown in Table 1. The partitioning of genotype variance into general (GCA) and specific combining ability (SCA) effects for the traits studied suggests that both additive and non-additive gene effects were controlling the inheritance of these traits.

The significant GCA and SCA effects for each trait indicated that estimates of individual effects for parents and parental combinations could be calculated.

The GCA effects for the two traits and eight parents are given in Table 2 and the SCA effects in Table 3.

Protein content

Parents 1, 3 and 5 had the highest positive GCA effect for protein content, while parents 2, 7 and 8 had the highest negative effect.

Table 1. Analysis of variance of data on protein content and yield per plant in an 8x8 diallel cross of faba bean cultivars.

	d.f.	Mean square	F ratio
Protein content			
Genotypes	35	15.56	59.48 **
GCA	7	14.79	169.64 **
SCA	28	2.78	31.98 **
Yield per plant			
Genotypes	35	201.96	82.18 **
GCA	7	319.02	380.41 **
SCA	28	6.86	8.18 **

** Significant at $P = 0.01$.

Table 2. Estimates of GCA effects for protein content and yield per plant.

Array	Protein content	Yield per plant
P1	1.97 **	-5.38 **
P2	-1.4 **	-3.65 **
P3	1.23 **	3.15 **
P4	-0.22	10.06 **
P5	0.67 **	3.33 **
P6	-0.07	-4.29 **
P7	-0.84 **	2.58 **
P8	-1.33 **	-5.79 **
SE (±)	0.09	0.27

** Significant at $P = 0.01$.

In the estimate of the SCA effect, crosses 1×3 , 1×4 , 1×5 , 1×6 , 3×6 and 4×7 , had the highest positive effects for protein content (Table 3).

Eleven crosses had significant negative effects for protein content.

Table 3. Estimates of SCA effects for protein content and yield per plant.

Cross	Protein content	Yield per plant
1×2	0.23	2.58 **
1×3	2.19 **	-1.82 **
1×4	1.93 **	13.41 **
1×5	2.46 **	7.67 **
1×6	1.16 **	-0.92
1×7	0.50 **	7.08 **
1×8	0.46 *	-1.99 **
2×3	-1.07 **	6.89 **
2×4	-1.27 **	14.29 **
2×5	-4.14 **	5.32 **
2×6	-0.37	-1.64 **
2×7	-1.81 **	5.98 **
2×8	-1.47 **	-0.98
3×4	-4.35 **	10.08 **
3×5	-0.25	3.81 **
3×6	1.71 **	3.98 **
3×7	-0.02	3.21 **
3×8	-0.03	-6.09 **
4×5	-1.62 **	12.05 **
4×6	0.71 **	2.75
4×7	1.05 **	9.08 **
4×8	-0.89 **	1.45 *
5×6	-2.27 **	7.77 **
5×7	0.96 **	12.74 **
5×8	-0.72 **	5.04 **
6×7	-0.15	-5.49 **
6×8	-0.14	-2.41 **
7×8	-1.93 **	5.51 **
SE (±)	0.23	0.66

*,** Significant at $P = 0.05$ and $P = 1.01$, respectively.

Yield per plant

Parents 3, 4, 5 and 7 showed a significant positive GCA effect for higher yield content, while parents 1, 2, 6 and 8 had a significant negative GCA for this trait. For the SCA effect, the crosses 1×2 , 1×4 , 1×5 , 1×7 , 2×3 , 2×4 , $2 \times$

5, 2 × 7, 3 × 4, 3 × 5, 3 × 6, 3 × 7, 4 × 5, 4 × 7, 4 × 8, 5 × 6, 5 × 7, 5 × 8 and 7 × 8 had significant positive effects for yield content, and six crosses had significant negative effects (Table 3).

From these results, parent 1 (VH-131) was superior for GCA and produced high positive SCA effects in most crosses for protein content. For yield per plant, parents 1 (VH-131), 2 (VH-130), 3 (FLIP-65 FB) and 4 (FLIP-54 FB) appeared to be the best parents to produce crosses with high yield potential. This indicates that genetic variation is present and available for plant breeders to improve the protein content and yield of faba bean. Thus, progress can be made in breeding for both protein and yield quality.

Phenotypic and genotypic correlations

There was no correlation between yield and protein content (genotypic $r = 0.098$, phenotypic $r = 0.262$). Similar results are reported by Katiyar and Singh (1990).

References

Dewey, D.R. and K.H. Lu. 1959. A correlation and path coefficient analysis of components of crested wheatgrass

- seed production. *Agronomy Journal* 51: 515–518.
- El-Sayed, F., H. Nakkoul and P. Williams. 1982. Distribution of protein content in the world collection of faba bean (*Vicia faba* L.). *FABIS Newsletter* 5: 37.
- Griffing, B. 1956. Concept of general and specific combining ability in relation to diallel crossing systems. *Australian Journal of Biological Sciences* 9: 463–493.
- Habetinek, J. 1985. [Evaluation of five white-flowered lines of broad bean (*Faba vulgaris* Moench) by means of diallel analysis.] (In Czech.) *Sbornik Vysoke Skoly Zemedelske v Praze, Fakulta Agronomicka A* 42: 91–102.
- Hawtin, G. and R. Stewart. 1979. The development, production and problems of faba bean (*Vicia faba* L.) in West Asia and North Africa. *FABIS Newsletter* 1: 7–9.
- Kambal, A.E. 1969. Components of yield in field beans (*Vicia faba* L.). *Journal of Agricultural Science (Cambridge)* 72(3): 359–363.
- Katiyar, R.P. and A.K. Singh. 1990. Path coefficient studies for yield and yield components in faba bean (*Vicia faba* L.). *FABIS Newsletter* 26: 3–5.
- Picard, J. 1979. *Vicia faba* L. breeding in France. *FABIS Newsletter* 1: 14.
- Waly, E.A. and S.A. Abd El-Aal. 1986. Combining ability for protein and cellulose content in a five-parent diallel of *Vicia faba* L. *FABIS Newsletter* 14: 4–6.

Genetic Principal Components and Classification for Quantitative Characters in Faba Bean

Yuan Mingyi, Shen Haining, Zhang Peilan and Liu Yang

Crop Research Institute
Qinghai Academy of Agriculture and Forestry Sciences
Xining, CHINA

Abstract

Principal component analysis was performed on seven quantitative characters of 50 faba bean lines to evaluate the tested lines. Of these, 18 lines were selected as parents for future hybridization. The distances were calculated among all possible pairs of lines, to classify the 50 lines into 2 populations, 3 types and 9 groups. The classification showed that there was some connection between geographical distribution and divergence, but that most of the lines within the same cluster were close in Mahalanobis (D^2) distance but varied in geographic sites.

المكونات المورثة الرئيسية وتصنيفها لتحديد الخصائص الكمية في الفول

الملخص

أجري تحليل المكونات الرئيسية على سبع خصائص كمية في 50 سلالة من الفول لتقييم السلالات المختبرة، التي تم انتخاب 18 سلالة منها على أنها أبوية. وتم حساب المسافات بين جميع السلالات المزدوجة المحتملة، لتصنيف السلالات الخمسين في عشرين وثلاثة أنماط و9 مجموعات. وقد أظهر التصنيف أنه كان ثمة علاقة بين التوزيع الجغرافي والتباين الوراثي، إلا أن معظم السلالات ضمن المجموعة نفسها كانت قريبة في البعد الوراثي ومتباينة في المواقع الجغرافية.

Key words: *Vicia faba*; faba beans; quantitative genetics; divergence; geographical distribution; China.

Introduction

Cluster analysis is a useful method for plant breeders. Since the mid-1970s many studies on genetic distance and cluster analysis have been carried out on germplasm of various crops. Distance can express genetic differences among parents and could be used as a reliable index for selecting parents (Bhatt 1981). Cross-combinations of rice which exhibited strong heretosis in production had large divergence between their parents (Xu and Waing 1981). There was no direct association between geographical distribution of lines and their divergence (Gan and Wang 1985; Yu and Guo 1983). Similar findings in faba (*Vicia faba* L.) bean are reported by Huang and Li (1984), and Mao and Liu (1979).

The purpose of this research was to evaluate yield and yield components of faba bean germplasm by principal component analysis, to measure differences among them

using Euclidian distance, and to classify them on divergence in order to provide a basis for selecting parents.

Material and Methods

Fifty lines (Table 1) were used, comprising 33 domestic and 17 exotic genotypes, which varied in agronomic characters. The study was conducted at the Crop Research Institute of the Qinghai Academy of Agriculture and Forestry Sciences (QAAF), Xining, Qinghai in 1994. The experiment, laid out in a randomized complete block design with three replications, was grown in 2-row plots, each 2.5 m long and 40 cm apart. Seed yield was recorded for each plot, and seven characters (plant height, number of nodes on the main stem, number of pods/plant, number of seeds/plant, seed weight/plant, 100-seed weight and number of branches/plant) were determined from five randomly selected plants in each plot.

Table 1. Faba bean lines used in experiment and their codes.

Code	Name	Origin	Code	Name	Origin
1	Baiqihong	Gansu, China	26	621	Tunisia
2	72-45	Qinghai, China	27	174	England
3	Sheng lidou	Qinghai, China	28	3009	Bulgaria
4	Yong chan dabei	Qinghai, China	29	175	England
5	Tudouzhai	Sichuan, China	30	Nongl 7	Qinghai, China
6	Xiaohudou	FuJian, China	31	Qinghai 3	Qinghai, China
7	133	Mexico	32	370	Algeria
8	7834	Yunnan, China	33	70-29	Qinghai, China
9	Inch bean	Japan	34	372	Beijing, China
10	580	India	35	Neimon dadou	NeiMoncol, China
11	Lingxia 292	Gansu, China	36	Sudan 3	Sudan
12	3010	Bulgaria	37	Lhasa 1	Tibet, China
13	70-47	Qinghai, China	38	74-34	Qinghai, China
14	71-7	Beijing, China	39	Feng Yidou	Yunnan, China
15	Xinpindou	Yunnan, China	40	Chan Jiao	Qinghai, China
16	8010	Yunnan, China	41	Qunong baipi	Qinghai, China
17	Banhua	FuJian, China	42	Maya	Qinghai, China
18	707	Canada	43	DaJinbai	Sichuan, China
19	Gadadou	Qinghai, China	44	29	Iraq
20	124	Tibet, China	45	669	Ethiopia
21	Badadou	Sichuan, China	46	559	Spain
22	589	Egypt	47	Qinkechan	Hecbei, China
23	14-2	FuJian, China	48	NiuJiao	Qinghai, China
24	552	Turkey	49	Qidong 1	Jiangsu, China
25	579	Lebanon	50	Dakezhichan	Anhai, China

Principal component analysis and Euclidean distance were computed as suggested by Liu (1979).

Results and Discussion

The analysis of variance revealed highly significant differences among all lines for the seven characters. The seven by seven matrix of correlation coefficients was obtained, from which eigenvalues and their eigenvectors were obtained. The four largest eigenvalues were selected; their cumulative contribution reached over 86% (Table 2).

In the first principal component, the maximum loading (coefficient) was plant height based, the second-highest loading was from nodes/main stem, so this was called the height factor. In the second component, the largest loading (coefficient) was from pods and seeds/plant, called pod and seed factor. Seed weight/plant increased as number of pods or seeds increased; however, 100-seed weight decreased as number of pods and seeds increased. The third principal component was mainly offered by branches, called branch

factor; the value of plant height and nodes/main stem was negative suggesting that they were reduced as number of branches increased. The fourth principal component was called the seed weight factor, for the largest positive loading was from 100-seed weight, in which pods and seeds/plant decreased as 100-seed weight increased.

The tested lines were evaluated by principal component analysis using the following criteria. It would be better that the value of the first principal component was larger for taller plants and more nodes, which could hold more pods. The value of fourth principal component should not be too large so that more pods and seeds with higher yield may be obtained. The value of the third component should be medium so as to get a proper population. The value of the second component should be large as more pods and seeds could produce higher yield.

The standardized values of the selected principal components for each line, g_1, g_2, g_3, g_4 , were calculated. According to the above evaluating criterion on principal component, 18 lines were selected (Table 3).

Table 2. Eigenvalue and eigenvectors selected.

Eigenvalue	λ_1	λ_2	λ_3	λ_4
	2.900	1.725	1.231	0.936
Percentage of cumulative contribution	36.7	58.6	74.2	86.3
Plant height	0.4810	0.2127	-0.1005	-0.3016
No. branches/plant	-0.1784	0.3012	0.6134	-0.0425
No. nodes/main stem	0.4261	0.0341	-0.2201	0.2301
No. pods/plant	0.2754	0.5824	0.0637	-0.4258
No. seeds/plant	0.0634	0.5401	0.0721	-0.3801
Seed weight/plant	0.3317	0.3084	0.2904	0.0121
100-seed weight	0.3074	-0.3249	0.3126	0.4018
Principal componet	Height factor	Pod & seed factor	Branch factor	Seed-weight factor

Table 3. Values of four principal components of 18 lines selected.

Code	g_1	g_2	g_3	g_4	Code	g_1	g_2	g_3	g_4
1	2.86	1.73	0.13	3.03	33	2.36	2.17	0.43	2.51
2	3.68	2.06	0.41	2.41	34	2.62	1.68	-0.06	3.62
10	1.97	1.86	1.06	1.76	37	2.47	1.77	0.52	3.11
12	3.45	1.94	0.36	4.62	38	2.43	1.91	-0.19	3.82
14	2.22	2.62	0.40	1.97	40	2.06	2.01	0.28	1.66
19	2.97	2.75	-0.22	1.02	41	4.21	1.56	0.31	4.07
28	2.75	1.85	0.64	2.73	42	2.33	2.42	0.71	2.57
30	3.16	1.64	0.57	3.41	48	2.04	2.58	0.42	2.16
31	2.95	1.82	0.30	3.03	50	1.17	2.34	0.30	3.46

Based on the standardized values of the first four principal components, the Mahalanobis distances (D^2) among all possible pairs of lines were calculated, and then the cluster analysis was done by single-linkage method of the shortest distance to classify the 50 lines into 2 populations, 3 types and 9 groups (Table 4).

One population was characterized by medium or tall plants, it comprised 2 types and 4 groups: Group 1 had 4 lines, mainly from Qinghai, had high seed yield potential (very large seeds); Group 2 had 10 domestic and exotic lines with high seed yield potential (large seeds); Group 3 comprised 5 domestic lines which had high yield potential (medium seeds and more pods); Group 4 had 6 lines with medium yield potential (large seeds). The other population was characterized by short plants, it consisted of 2 types and 5 groups; Group 5 had 7 domestic and exotic lines of medium yield potential (medium seeds and more pods); Group 6 had 5 lines, mainly exotic, with low seed yield potential (large seeds); Group 7 had 7 domestic and exotic lines with low yield potential (medium seeds and more pods); Group 8 had 5 lines with low yield potential (small

seeds and more pods). Because its traits were significantly different from the others, Dakezhizhe (code 50) was partitioned alone in Group 9, characterized by high seed yield potential and large seeds.

This classification showed that divergence was not entirely independent of geographical distribution. For example, the 4 lines in Group 1 were all from Qinghai, except 3010 (code 12) which was from Bulgaria; however, most of the lines within the same cluster were close in distance and originated from various geographic sites. It could be considered that there was no direct connection, in general, between divergence and geographical distribution.

Based on our breeding experience we thought that both integrated characters and divergence should be important; however, the former would be so more frequently. Hence, for high-yield breeding in faba bean it would be better to select at least one parent with medium or high seed yield potential type, and then to aim at their divergence. In addition, cluster analysis for faba bean germplasm is worth further study.

Table 4. Classification of 50 faba bean lines.

Pop†	Type‡	Group	Lines	Group feature
1	1	1	12, 31, 38, 41	Very large seeds
1	1	2	1, 2, 13, 20, 24, 28, 34, 36, 37, 42	Large seeds
1	1	3	14, 15, 19, 33, 48	Medium seeds
1	2	4	27, 30, 35, 40, 43, 47	Large seeds and more pods
2	2	5	6, 7, 10, 16, 18, 26, 49	Medium seeds and more pods
2	2	6	9, 11, 29, 32, 44	Large seeds
2	3	7	4, 8, 21, 22, 25, 39, 46	Medium seeds and more pods
2	3	8	3, 5, 17, 23, 45	Small seeds and more pods
		9	50	Large seeds and high seed yield potential

† 1 = medium or tall plants; 2 = short plants.

‡ 1 = high seed yield potential; 2 = medium seed yield potential; 3 = low seed yield potential.

References

- Bhatt, G.M. 1981. Multivariety analysis applied to selection of parents for hybridization aiming at yield improvement in self-pollinated Crop. *Australian Journal of Agricultural Research* (21): 1-7.
- Gan Ximin and Wang Zaixu. 1985. Genetic distance of quantitative characters in peanut and its application to breeding. *Scientia Agriculture Sinica* 6: 27-31.
- Huang Wentao and Li Fuquan. 1984. Genetic distance of quantitative characters in faba bean. *Science and Technology of Qinghai Agriculture and Forestry* 4: 21-24.
- Liu Laifu. 1979. Genetic distance of quantitative characters in crop and its estimate. *Acta Genetica Sinica* 6(3): 349-355.
- Mao Shengxian and Liu Laiful. 1979. Genetic differences for quantitative characters in winter wheat and its application to breeding. *Hereditas* 1(5): 26-30.
- Xu Jingwen and Waing Luying. 1981. Heterosis and genetic distance in rice. *Journal of Anhui Agricultural Sciences* (Quantitative Hereditas Special Issue): 65-71.
- Yu Shirong and Guo Aiping. 1983. A preliminary study on cluster analysis of thirty-two wheat germplasm from the lower Yangtse Valley. *Acta Agronomica Sinica* 9(2): 85-91.

Induced Leaf Variations in Faba Bean

التباينات المستحدثة لأوراق الفول

Mohammad Yasin¹

Department of Plant Breeding and Genetics
Jawahar Lal Nehru Krishi Vishva Vidyalaya
Jabalpur 482 004, INDIA

المخلص

تمت دراسة تكرار وطيف كلوروفيل M_2 ، وطفرات ورقية أخرى بعد معاملة البذور بأشعة غاما وسلفونات إثيل ميثان (EMS) والأوكسيد الأزوتي (N_2O) في صنفين من الفول (*Vicia faba* L.) وبشكل عام، كان الصنف JV1 أكثر حساسية وكانت معاملة EMS الأكثر فعالية. وكان تكرار الطفرات من طراز *chlorina* أعلى من طفرات الكلوروفيل من طراز *xantha* و *chlorotica*. وقد لوحظ أعلى تكرار في التباينات في بنية الوريقة ثم تلاه الترتيب والشكل والحجم في كلا الصنفين. وتم بحث استخدام الطفرات الورقية هذه في تشكيل مثال توالد الفول.

Abstract

The frequency and spectrum of M_2 chlorophyll and other leaf mutations after gamma ray, ethyl methane sulfonate (EMS) and nitrous oxide (N_2O) seed treatment in two varieties of faba bean were studied. In general, cv JV1 was more sensitive and EMS treatment was most effective. The frequency of *chlorina*-type mutations was higher than that of *xantha* and *chlorotica* type chlorophyll mutations. The highest frequency of variations was observed in leaflet texture, followed by arrangement, shape and size in both varieties. The use of these leaf mutations in formulating an ideotype of *Vicia faba* L. have been discussed.

Key words: *Vicia faba*; faba beans; induced mutation; chlorophylls; gamma radiation; EMS; nitrous oxide; leaves; India.

Introduction

Physiological traits are useful in constructing ideotypes for crop plants. Variations in leaf shape, size, texture, arrangement and color can play an important role in this regard. The induction of such variations through mutagenesis and the transfer of useful mutations to existing material can help in formulating plant architecture as required by cropping conditions.

Material and Methods

Faba bean (*Vicia faba* L.) cvs JV1 and JV2 were used in mutagenic experiments. The mutagens used were gamma rays (5, 10, 15, 20 krad), ethyl methane sulfonate (EMS, 0.8% for 2 and 4 h, after 6 h presoaking of seeds) and nitrous oxide (N_2O for 24 h at 55° atm. press., after 6 h pre-soaking of seeds and allowing sprouting for 3 days). Two-hundred treated seeds were sown per treatment, keeping 10 cm

between plants and 30 cm between rows. M_1 plants were isolated from cross-pollinators (bees) by using selfing bags. Surviving M_1 plants were then harvested and kept separately. The seeds from each M_1 plant were M_1 progeny. Plants grown from M_1 progeny were M_2 plants. Each M_1 progeny was sown in a separate row, with 20 cm between plants and 30 cm between rows. Chlorophyll mutations were counted in the M_2 at the stage of 4–5 leaves. Chlorophyll mutations were classified into three classes (Blixt 1960, 1961) as *xantha* (yellow leaves, a lethal mutant), *chlorina* (yellow-green leaves, also lethal) and *chlorotica* (yellow-green leaves, but viable). Plants were selected and counted as soon as they were large enough for us to distinguish variations. Frequency of mutations was expressed on progeny (per 100 M_1 progeny) and population (per 100 plants) basis.

Results and Discussion

Treatments of different mutagens and their doses resulted in an irregular pattern of frequencies of chlorophyll mutations and other leaf variations on the basis of progeny and population as recorded in the M_2 . Cultivar JV1 was more sensitive than cv JV2, giving a higher percentage of chlorophyll mutations on the basis of progenies to populations (Table 1). The frequency of *chlorina*-type mutations was highest, followed by *chlorotica* and *xantha* types. Several leaf variations were recorded at higher frequencies in JV1 than in JV2 (Table 2). EMS treatment for 2 h in JV1, and 20 krad gamma irradiation in JV2

¹ Present address: Department of Plant Breeding & Genetics, IGKV, Raipur (M.P.), INDIA.

resulted in highest frequencies of leaf mutations, while 5 krad and 10 krad gamma rays and 24 h N₂O treatments failed to produce any leaf mutations (Table 2). The maximum number of chlorophyll mutations was effected by EMS treatment for 2 h, followed by EMS treatment for 4 h; gamma irradiation and N₂O treatment did not produce more than one chlorophyll mutation each, except 20 krad gamma irradiation (Table 3).

Many leaf variations were recorded which could be useful in crop-improvement programs, as well as in studies of systematic development of the crop. Such leaf mutations were classified according to IBPGR (1985).

Shape: Mutant plants had narrow or rounded leaflets compared with the intermediate leaflets of the control.

Texture: Leaflets had rough, fluffy, leathery surfaces rather than the smooth surface of the parental cultivars. Leaflets also had hairs and dot-like structures.

Size: Both smaller and larger leaflets than normal were observed in mutants.

Arrangement: Mutants showed more diverse leaf attachment to the base than that of the parental cultivars. These changes included erect and drooping leaves with shorter internode and leaflets, leading to a change in leaf arrangement and plant canopy.

Foliage color: Compared with the control, some plants were lighter green, and a few mutants were darker green.

Frequencies of different leaf mutants observed are shown in Table 4. The differences in the frequencies of leaf mutations may be due to the number of genes with pleiotropic effects as has been reported by Sjodin (1971). Rao and Jana (1976) and Filippetti and DePace (1983, 1986) also succeeded in inducing the leaf mutations in *Vicia faba* similar to the present findings. All leaf mutants exhibited a somewhat abnormal development. Several irregularities have been observed in the leaflet, such as thick and fluffy leathery surface, presence of hairs and dot-like structures on the surface, conversion of upper leaflets into tendril-like structures, variation in length of leaflets and internodes. Study of genetic aspects of such variations would be useful in understanding the systematic development of this crop and also in the formulation of various plant types.

Table 1. Frequency of chlorophyll mutations based on progeny and population in M₂ generation in two cultivars of *Vicia faba*.†

Cv	Progeny		Population				
	No. progenies studied	Chlorophyll mutations (%)	No. M ₂ plants studied	Chlorophyll mutations (%)	Relative % of chlorophyll mutations type		
					<i>Xantha</i>	<i>Chlorina</i>	<i>Chlorotica</i>
JV1	376	3.98	4218	0.47	15	50	35
JV2	618	2.26	6841	0.29	30	40	30

Table 2. Percentage of progenies and plants showing leaf mutation in M₂ generation in two cultivars of *Vicia faba*.†

Cv	Progeny		Population	
	No. families studied	Freq. mutation (%)	No. plants studied	Freq. leaf mutation (%)
JV1	376	1.32	4218	0.35
JV2	618	0.81	6841	0.12
Overall	994	1.01	11059	0.23

† Pooled data of all treatments, i.e. all gamma-ray, EMS and N₂O combinations.

Table 3. Frequency of chlorophyll and leaf mutations induced by various doses of mutagens in M₂ generation in two cultivars of *Vicia faba*.

Cv	Gama rays				EMS		N ₂ O	Gamma rays + N ₂ O (5 kr + 24 h)
	5 kr	10 kr	15 kr	20 kr	2 h	4 h	24 h	
Chlorophyll mutations								
JV1	1 (1085)†	1 (1204)	0 (2061)	0 (548)	14 (488)	3 (381)	0 (1079)	1 (400)
JV2	1 (2222)	1 (2503)	0 (1837)	0 (1611)	10 (440)	8 (251)	1 (700)	0 (558)
Leaf mutations								
JV1	0	1	0	1	10	2	0	1
JV2	0	0	0	4	1	2	0	0

† Nos in parentheses refer to no. seeds used in M₂.

Table 4. Relative percentage of different leaf mutants in M₂ generation in two cultivars of *Vicia faba*. (Based on absolute figures given in Table 3.)

Cv	Leaf mutants recorded (%)	Relative freq. (%)				
		Shape	Size	Texture	Arrangement	Color
JV1	100	13.3	13.3	40	20	13.3
JV2	100	14.3	14.3	43.0	28.4	0

Small leaflet coupled with narrow shape and compact arrangement could be utilized to develop dwarf and lodging-resistant plant types which could be grown at higher plant density. Though all leaf mutants could not be of direct applied interest, they could provide a basis for physiological studies in constructing an ideotype of faba bean.

Acknowledgement

We thank V.K. Gour for excellent technical guidance.

References

- Blixt, S. 1960. Quantative studies of induced mutations in peas. IV. Segregation after mutation. *Agri. Hovt. Genet.* 18: 219-237.
- Blixt, S. 1961. Quantative studies of induced mutations in peas. VI. Chlorophyll mutations. *Agri. Hert. Genet.* 19: 402-447.
- Filippetti, A. and C. DePace. 1983. Improvement of grain yield in *Vicia faba* L. by using experimental mutagenesis. I. Frequency and types of mutations induced by gamma radiation. *Genetica Agraria* 37: 53-68.
- Filippetti, A. and C. DePace. 1986. Improvement of seed yield in *Vicia faba* L. by using experimental mutagenesis. II. Comparison of gamma radiation and ethyl methane sulphonate (EMS) in production of morphological mutants. *Euphytica* 35: 49-59.
- IBPGR (International Board for Plant Genetic Resources). 1985. Faba Bean Descriptor List. IBPGR Secretariat, Rome.
- Rao, S.A. and Jana. 1976. Leaf mutations induced in black gram by X rays and EMS. *Environmental and Experimental Botany* 16: 151-154.
- Sjodin, J. 1971. Induced morphological variations in *Vicia faba* L. *Hereditas* 67: 155-180.

Colchicine Induced Tetraploids of *Vicia faba* L.

Alexey Y. Kravchenko

Novosibirsk State Agrarian University
Dobrolyubova street, 160
Novosibirsk, 630039, RUSSIA

Abstract

Faba bean tetraploids were induced by colchicine treatment of dry seeds. The concentration of colchicine 0.0025% was most effective in inducing tetraploids. Morphologically, the tetraploids differed little from the diploids. The chromosome behavior in meiosis was irregular. In metaphase I a high frequency of quadrivalent formation was observed. The fertility of the tetraploids was low.

Key words: *Vicia faba*; faba beans; tetraploidy; diploidy; chromosomes; colchicine; induced mutation; plant anatomy; meiosis; fertility; Russia.

Introduction

Vicia faba L. has not produced natural polyploids. However, attempts have been made to produce artificial polyploids by colchicine treatment (Rybin 1939; Schuman 1960; Dikshit and Mehra 1966; Bourgeois 1980), but all the tetraploids obtained have been sterile. The only fertile *Vicia faba* tetraploid was found in the progeny of PO-1 mutant (Sjodin 1971; Poulsen and Martin 1977); it had novel-shaped pollen and low fertility.

The aim of our work was to obtain *Vicia faba* polyploids using colchicine.

Material and Methods

Five hundred dry seeds of faba bean cv Omskie belye were used in each treatment. Treatments comprised six concentrations of colchicine solution: 0.001, 0.0025, 0.005, 0.01, 0.05 and 0.1%. Seeds were soaked in the solution for 8 hours. After treatment the seeds were washed in water and sown in the field.

The changed shape of pollen grain was used to determine the polyploid plants. The analysis of pollen shape and pollen viability were conducted by staining with 2% acetocarmine. For mitotic analysis the root tips were kept in monobromonaphthalene for 24 hours at 4°C, and then fixed

الفول الرباعي الصبغيات المستحدث بـ Colchicine

الملخص

تم استحداث فول (*Vicia faba* L.) رباعي الصبغيات بواسطة معالجة الكولشيسين للبذور الجافة. وكان تركيز الكولشيسين بنسبة 0.0025% فعالاً جداً في استحداث صبغيات رباعية. ومن الناحية الشكلية، فقد اختلفت الصبغيات الرباعية قليلاً عن الصبغيات الثنائية. وكان سلوك الطاقم الصبغي في الانقسام الاختزالي غير منتظم. ولوحظ تشكل صبغيات رباعية بوتيرة عالية في الطور الاستوائي. وكانت خصوبة الصبغيات الرباعية متدنية.

in a mixture of ethanol and acetic acid (3:1). Then they were hydrolyzed in 5N HCl for 50 min., stained with 2% acetocarmine and squashed in acetic acid (45%).

For meiosis examination the young buds were fixed in ethanol and acetic acid (3:1). Immature anthers were squashed in 2% acetocarmine.

Results and Discussion

The effects of colchicine concentration on germination percentage and number of polyploids are shown in Table 1. At a concentration of 0.0025%, the number of polyploids was largest. Further increase in concentration did not increase polyploid production. From 15 polyploid plants obtained, 9 were sterile, and the other 6 polyploids produced one seed per plant.

The plants of the C₁ generation were grown in the greenhouse. Mitotic analysis showed that all plants were tetraploids (2n=24; Fig. 1). Morphologically the tetraploids were similar to diploids. The best distinguishing character was the pollen grain shape.

Meiotic analyses were conducted on C₂ plants. The chromosomes were arranged mainly in quadrivalent configurations in metaphase I (Fig. 2). Bivalents were the second most common associations (Fig. 3). There were few trivalents and univalents (Table 2). In anaphase I, 21.2% of cells exhibited lagging chromosomes (Fig. 4). Bridges were observed in 3.6% of cells (Fig. 4,5). Micronuclei (Fig. 6) were formed in 13.6% of tetrads.

The pollen grains of tetraploids were triangular, elliptic or circular (Fig. 7). The triangular shape was most common. The pollen of tetraploids was wider than that of diploids (Table 3) and the pollen viability in tetraploids was lower than that of diploid pollen (77.2 vs 99.3%).



Fig. 1. Mitotic metaphase plate with 24 chromosomes in *Vicia faba* tetraploid.



Fig. 5. Anaphase I: bridges.

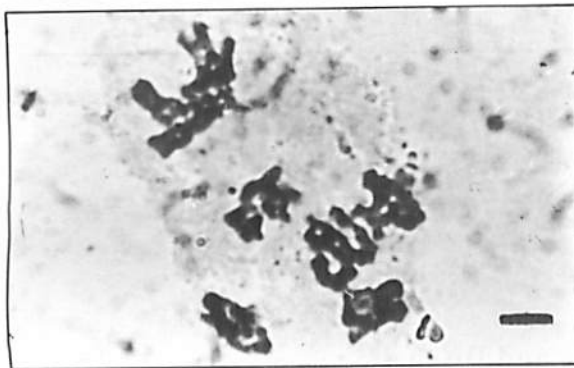


Fig. 2. Metaphase I: 7 quadrivalents.

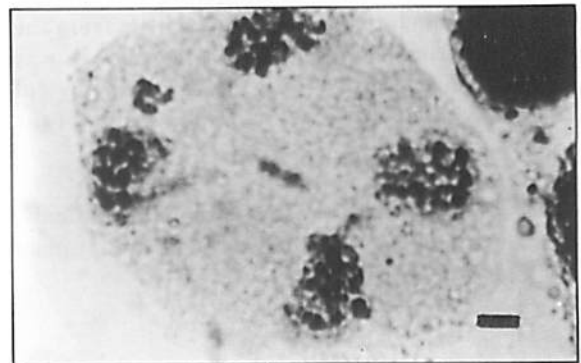


Fig. 6. Micronuclei at tetrad stage. Bars = 5µm.

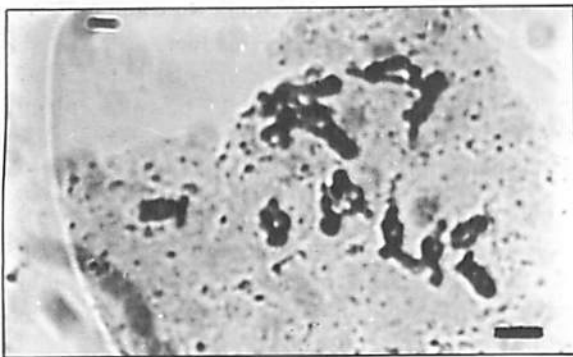


Fig. 3. Metaphase I: 3 quadrivalents and 6 bivalents.



Fig. 7. Pollen grains in *Vicia faba* tetraploid. Bars = 50µm.



Fig. 4. Anaphase I: lagging chromosome and bridge.

Table 1. Germination percentage and number of polyploids after colchicine treatment of 500 seeds of *Vicia faba*.

Colchicine concentration (%)	Germination (%)	No. polyploids	No. fertile polyploids
0.001	84.4	2	1
0.0025	69.0	8	4
0.005	44.4	4	1
0.01	33.0	1	0
0.05	15.4	0	—
0.1	5.4	0	—

The fertility of the tetraploids was low. A sharp decrease in the numbers of pods and seeds per plant was observed (Table 3). The seeds of tetraploids were heavier than those of diploids: the weight of 10 seeds was 6.8 g in tetraploids and 4.7 g in diploids.

The induction of *Vicia faba* polyploids is difficult. Therefore, it is important to determine the most effective treatment(s). Previous attempts to produce fertile polyploids using colchicine failed (Rybin 1939; Schuman 1960; Dikshit

Table 2. Analysis of chromosome behavior in metaphase I of C₂ tetraploids of *Vicia faba*.

Configuration	Frequency	Range
Quadrivalent	4.42	1-6
Trivalent	0.12	0-2
Bivalent	2.90	0-10
Univalent	0.1	0-2

Table 3. Comparison of some characteristics of C₂ tetraploids and diploids of *Vicia Faba*.

Characteristic	Tetraploids		Diploids	
	Mean	Range	Mean	Range
Pollen viability (%)	77.2 ± 2.4	64.4-86.4	99.3 ± 0.1	98.3-100
Pollen length (mm)	49.3 ± 0.3	48.0-52.3	49.0 ± 0.2	48.0-49.8
Pollen width (mm)	42.1 ± 0.2	41.0-43.3	34.7 ± 0.1	34.0-35.3
Pods/plant	2.0 ± 0.7	0-8	12.1 ± 0.8	9-17
Seeds/plant	2.3 ± 0.8	0-10	33.6 ± 2.7	22-51
Seeds/pod	1.1 ± 0.1	1-2	2.8 ± 0.2	1-4
10-seed weight (g)	6.8 ± 0.2	5.8-7.9	4.7 ± 0.1	4.5-5.0

and Mehratra 1966; Bourgeois 1980). However, treatment of seedlings with colchicine solution has been effective in pea (*Pisum sativum* L.) (Kutty and Kumar 1983) and lentil (Gupta and Jagdish 1982), but unsuccessful in chickpea (*Cicer arietinum* L.). Autotetraploids of chickpea were obtained by treatment of dry seeds (Pundir et al. 1983).

In the present study the *Vicia faba* tetraploids were induced by treatment of dry seeds in colchicine solution for 8 hours. The concentration 0.0025% colchicine was most effective for inducing tetraploids.

Morphologically, the tetraploids were similar to diploid plants. The distinguishing characters were a changed pollen grain shape and larger seeds. These data are in agreement with the observations on field bean tetraploids found in the progeny of a pollen mutant (Sjodin 1971; Poulsen and Martin 1977). Similar data have been reported for pea (Errico et al. 1991). The meiotic irregularities of tetraploids induced by colchicine were similar to those in tetraploids of mutant origin (Martin et al. 1986).

Because of meiotic disturbances, the pollen viability in tetraploids was lower than that in diploids. As with many other autotetraploids, those of *Vicia faba* had low fertility.

Along with meiotic anomalies, some physiological and environmental factors may influence fertility of faba bean tetraploids (Martin and Gonzalez-Garcia 1981). The disturbances in conductive tissue were considered to be the main reason of embryo abortion in the tetraploids of red clover (Vavilov et al. 1977). It is likely that further cytoembryological and physiological examination of tetraploids obtained in the present study will make it possible to determine reasons for their low fertility.

Acknowledgments

The experiments were conducted at the Institute of Cytology and Genetics of the Siberian Division of the Russian Academy of Sciences and the Novosibirsk State Agrarian University under general guidance of Prof. Tsilke R.A. I thank Drs Berdnikov V.A. and Gorel F.A. for supporting the investigations, and Drs Kosterin O.E. and Ibragimova S.S. for help with the preparation of this report.

References

- Bourgeois, F. 1980. Tetraploid plants from *Vicia faba* and *Vicia narbonensis* using colchicine treatment. *FABIS Newsletter* 2: 25.

- Dikshit, A.P. and N. Mehra. 1966. Effect of colchicine on morphology and chromosome behaviour of eight varieties of *Vicia faba* Linn. *Science and Culture* 32: 88-89.
- Errico, A., C. Conicella and U. Talierico. 1991. Cytological and morphological characterization of *Pisum sativum* and *Pisum fulvum* tetraploids. *Plant Breeding* 106: 141-148.
- Gupta, P.K. and S. Jagdish. 1982. Induced autotetraploids in lentils. *LENS Newsletter* 9: 15-16.
- Kutty, V.C.M. and H. Kumar. 1983. Studies on induced tetraploids in four diverse cultivars of pea (*Pisum sativum* L.). *Cytologia* 48: 51-58.
- Martin, A. and J.A. Gonzalez-Garsia. 1981. Factors influencing fertility of a tetraploid *Vicia faba* L. Page 359 in *Vicia faba: Physiology and Breeding* (R. Thompson, ed.). Martinus-Nijhoff, The Hague.
- Martin, A., J.A. Gonzalez-Garsia and J.M. Serradilla. 1986. Progress in the diploidisation of a *Vicia faba* tetraploid. *Biologisches Zentralblatt* 105: 85-94.
- Poulsen, M.H. and A. Martin. 1977. A reproductive tetraploid *Vicia faba* L. *Hereditas* 87: 123-126.
- Pundir, R.P.S., N.K. Rao and L.J.G. van der Maesen. 1983. Induced autotetraploidy in chickpea (*Cicer arietinum* L.). *Theoretical and Applied Genetics* 65: 119-122.
- Rybin, V.A. 1939. Tetraploid plants of *Vicia faba* produced by colchicine treatment. *C.R. Acad. Sci. URSS* 24: 483-485.
- Schuman, G. 1960. Eine neue Methode zur Colchicinierung von Gramineen und großkornigen Leguminosen. *Züchter* 30: 118-120.
- Sjodin, J. 1971. Induced morphological variation in *Vicia faba* L. *Hereditas* 67: 155-180.
- Vavilov P.P., V.A. Kabysch, L.I. Putnikov and V.S. Orlova. 1977. Reasons of low seed productivity of red clover and ways of its increase. *Proc. All-Union Acad. Agricult. Sci.* 10: 7-11.

Karyotype Study in *Lathyrus sativus* L. cv P-505

Sushil Kumar and D.K. Dubey

Department of Botany
Janata Mahavidyalaya
Ajitmal (Etawah), U.P. 206 121
INDIA

Abstract

A karyotype study was undertaken on somatic chromosomes in *Lathyrus sativus* cv P-505. Two chromosomes were metacentric, while the remaining five were submetacentric. Chromosome number four had a secondary constriction in its long arm separating a satellite. The total chromatin length was 53.20 μm , with an average chromosome length of 7.60 μm ; total chromatin volume was 138.46 μm^3 . It may be concluded that a single standard karyotype for *L. sativus* does not seem possible.

Key words: *Lathyrus sativus*; karyotypes; chromosomes.

Introduction

Lathyrus sativus L. ($2n = 14$), which is one of the most important proteinaceous pulses (20-28% protein content), is an annual hardy and drought-resistant crop. Karyotypic

دراسة الطراز النووي في الجلبان *Lathyrus sativus* L. صنف P-505

الملخص

أجريت دراسة للطراز النووي على الكروموزومات الجسدية في الجلبان *Lathyrus sativus* L. للصنف P-505. وكانت اثنتان من هذه الكروموزومات وسطية، في حين كانت الخمس المتبقية تحت وسطية.

وكان للكروموزوم رقم أربعة انضغاط ثانوي في ذراعه الطويلة مؤدياً إلى فصل أحد التوابع. وبلغ الطول الإجمالي للكروماتين 53.20 μm بمتوسط طول الكروموزوم 7.60 μm . وبلغ حجم الكروماتين الإجمالي 138.46 μm^3 . ويمكن الاستنتاج بأن طرازاً نووياً معيارياً واحداً من *L. sativus* لا يبدو ممكناً.

studies on different cultivated and naturally occurring genotypes of this species have been carried out by a number of workers (Bhattacharjee 1954; Srivastava and Naithani 1964; Roy and Singh 1967; Fouzdar and Tandon 1975; Das and Prasad 1979; Verma and Ohri 1979; Lavania and Lavania 1983; Yamamoto et al. 1984). These studies revealed wide variations in chromosome morphology and total chromatin length. The karyotype of *Lathyrus sativus* cv P-505 was studied and compared with earlier reports on this species.

Material and Methods

Seeds of *Lathyrus sativus* cv P-505 were germinated in petri-dishes lined with moist filter paper. Root tips of 5–10 mm length were pretreated with 8-hydroxy quinoline for 6 h at 10–15°C, then fixed in acetoalcohol (1:3) and stored in 70% ethyl alcohol at low temperature. Prior to squashing, the root tips were hydrolyzed by heating in NHCl (9:1). Cytological studies were made from temporary acetocarmine (1%) squash preparations. Measurements of the chromosomes were made from well-spread metaphase plates and a karyotype was prepared by arranging the chromosomes in descending order of length. Average values obtained from observations on 10 such metaphase plates were used to draw conclusions regarding the karyotype. Idiograms of the seven chromosomes showing average chromosome length and the position of the centromere and secondary constriction are presented in Figure 1.

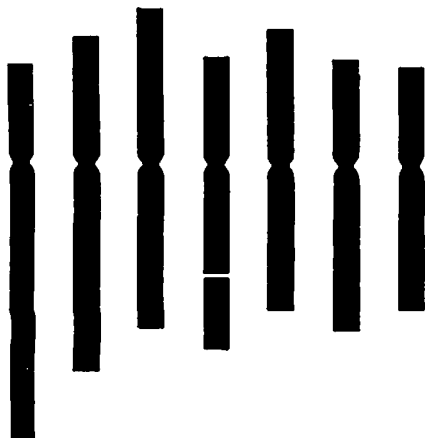


Fig. 1. Idiograms of the chromosomes of *Lathyrus sativus* cv P-505.

The ratio of the length of the short arm to the total chromatin length (TF%) was calculated according to Huziwara (1962), while total chromatin volume was recorded according to Verma and Ohri (1979). The karyotypic system of classification and karyotypic formula is according to Levan et al. (1964), where $A > 8 \mu\text{m}$, $B = 7\text{--}8 \mu\text{m}$ and $C < 7 \mu\text{m}$.

Results and Discussion

The diploid number of chromosomes was 14, which is in agreement with all earlier reports for this species. The morphological characteristics of the seven members of the haploid chromosome complement are presented in Table 1. In the present investigation, two chromosomes were metacentric (m), while the remaining five were submetacentric (sm). However, no strictly median (M) chromosomes were noted. The two metacentric chromosomes could be distinguished from each other on size, while the five pairs of submetacentric chromosomes could be distinguished by their length and arm-length ratio. Chromosome number four had a secondary constriction in its long arm separating a satellite (st) of 1.82 μm length. On the basis of chromosome length and position of centromere or satellite, the karyotypic formula of the cultivar P-505 may be given as follows:

$$2A^{sm} + 2B^m + 1B^{sm(st)} + 2C^{sm}$$

Wide variation in the position of the centromeres and secondary constrictions in chromosomes are reported in this species (Bhattacharjee 1954; Srivastava and Naithani 1964; Roy and Singh 1967; Das and Prasad 1979; Verma and Ohri 1979; Yamamoto et al. 1984). All these workers found only metacentric and submetacentric chromosomes, with the

Table 1. Somatic chromosome morphology in *Lathyrus sativus* cv P-505.

Chromosome pair	Total length (μm)	Arm ratio (L/S)	Relative length (%)	F%	% of TCL	Chromatin volume (μm^3)	Chromosome
1	9.55	2.82	100.00	26.18	19.95	24.85	sm
2	8.41	1.64	88.06	37.81	15.81	21.89	sm
3	7.96	1.06	83.35	48.62	14.96	20.72	m
4	7.27	1.66	76.12	37.55	13.66	18.92	sm (st)
5	7.05	1.07	73.82	48.37	13.25	18.35	m
6	6.82	1.50	71.41	40.03	12.82	17.75	sm
7	6.14	1.46	64.29	40.72	11.54	15.98	sm

TCL = Total chromatin length, i.e. length of all seven chromosomes combined;

F% = Ratio of length of short arm to whole chromosome.

sm = submetacentric; m = metacentric; st = satellite.

number of submetacentric chromosomes varying from 1 to 7. However, Fouzdar and Tandon (1975) found no median or metacentric chromosomes in five cultivars. They also report 1-3 acrocentric chromosomes along with the submetacentric chromosomes. The number of satellited chromosomes varies from 0 to 4 in the genotypes studied so far.

The length of the seven chromosomes in the present study varied from 6.14 to 9.55 μm , with a total chromatin length (TCL) of 53.20 μm and average chromatin length (ACL) of 7.60 μm . Chromosome volume ranged from 15.98 to 24.85 μm^3 with a total chromatin volume (TCV) of 138.46 μm^3 . Like the position of primary and secondary constrictions, large variations in chromosome length, total chromatin length and total chromatin volume are reported for different genotypes of *L. sativus*. Some of these variations could be ascribed to variations in degree of condensation and differing pretreatments applied, but the wide range of variation in chromosome morphology clearly indicates evolutionary alterations in the karyotype of these strains. Thus, it does not seem possible to identify a single standard karyotype for this species.

References

Bhattacharjee, S.K. 1954. Cytogenetics of *Lathyrus sativus* Linn. *Caryologia* 6: 333-337.

- Das, A.K. and A.B. Prasad. 1979. An investigations on the karyotype of six different varieties of *Lathyrus sativus* L. *Journal of Cytology and Genetics* 14: 64-66.
- Fouzdar, A. and S.L. Tandon. 1975. Cytotaxonomic investigations in the genus *Lathyrus*. *The Nucleus* 18: 24-33.
- Huziwar, Y. 1962. Karyotype analysis in some genera of compositae. VIII. Further studies on the chromosomes of *Aster*. *American Journal of Botany* 49: 116-119.
- Lavania, U.C. and S. Lavania. 1983. Karyotype studies in Indian pulses. *Genetica Agraria* 37: 299-308.
- Levan, A., K. Fredga and A.A. Sandberg. 1964. Nomenclature for centromeric position on chromosome. *Hereditas* 52: 201-220.
- Roy, R.P. and M.K. Singh. 1967. Cytological studies in the genus *Lathyrus*. *Journal of Cytology and Genetics* 2: 128-140.
- Srivastava, L.M. and S.P. Naithani. 1964. Cytological studies in certain pulses and beans. *Cytologia* 29: 453-464.
- Verma, S.C. and D. Ohri. 1979. Chromosome and nuclear phenotype in the legume *Lathyrus sativus* L. *Cytologia* 44: 77-90.
- Yamamoto, K., T. Fujiwara and I.D. Blumenreich. 1984. Karyotype and morphological characteristics of some species in the genus *Lathyrus*. *Japanese Journal of Breeding* 34: 273-284.

Variability and Correlation Studies in Grasspea (*Lathyrus sativus* L.)

Sushil Kumar and D.K. Dubey

Department of Botany
Janata Mahavidyalaya
Ajitmal, Etawah (U.P.) 206 121
INDIA

Abstract

Coefficients of variability and correlations between yield and its components were worked out at phenotypic, genotypic and environmental levels in 25 induced mutants of grasspea. Yield per plant, pods per plant and seeds per plant were more variable than the other traits studied. Seed yield showed significant positive correlations with pods/plant, seeds/pod and seeds/plant. Length of main branch, number of primary branches, nodes on main branch and internode length also showed positive correlations with yield, while 100-seed weight

دراسات التباين والارتباط في الجلبان المزروع

الملخص

تمت دراسة معاملات التباين والارتباط بين الغلة ومكوناتها على مستويات الطرز الشكلية والوراثية والبيئية في 25 طافرة مستحدثة من الجلبان المزروع (*Lathyrus sativus* L.). وكانت الغلة/النبته والقرون/النبته والبذور/النبته أكثر تبايناً من الخصائص الأخرى المدروسة. وقد أظهرت غلة البذور ارتباطاً إيجابياً معنوياً في القرون/النبته، البذور/القرن والبذور/النبته. كما أظهر طول الفرع الرئيسي وعدد الفروع الرئيسية والعقد على الفرع الرئيسي وطول السلامة ارتباطات إيجابية معنوياً مع الغلة في حين أظهر وزن المائة حبة ارتباطاً إيجابياً معنوياً بالغلة عند مستوى الطراز الوراثي فقط. وارتبط عدد الأيام حتى الإزهار بالغلة سلباً عند جميع المستويات. وكانت ارتباطات الطراز الوراثي والشكلي بين جميع الصفات في ذات الاتجاه ومتشابهة من حيث الحجم، في حين أظهرت الارتباطات البيئية منحىً مختلفاً. وكانت معظم

showed significant positive correlation with yield at the genotypic level only. Days to flowering was negatively correlated with yield at all levels. Genotypic and phenotypic correlations between all the traits were in the same direction and similar in magnitude, while environmental correlations showed a different trend. Most genotypic correlations were higher than the respective phenotypic and environmental correlations. There were significant positive environmental correlations of yield with pods/plant, seeds/plant and seeds/pod. Various yield-contributing traits also showed significant positive associations among themselves.

Key words: *Lathyrus sativus*; genetic correlation; yield components; fruit; seeds; branches; length; weight; nodes; internodes; phenotypes; genotypes.

Introduction

In any crop-breeding procedure, the potential progress expected in fulfilling the breeder's objectives depends on the magnitude and relationships of genetic variance and covariance for different characters. Grain yield is a complex character and is the multiplicative end product of many factors, termed yield components (Whitehouse et al. 1958; Grafius 1959). To make effective selections for higher yield, a thorough understanding of yield-contributing characters and grain yield is necessary for selecting desirable types. The present study was undertaken to assess the variability in and relationships between grain yield and various yield-contributing traits in the M_3 generation of 25 induced mutants of grasspea (*Lathyrus sativus* L.; also known as *khesari*).

الارتباطات الوراثية أعلى من الارتباطات الشكلية والبيئية. وكانت ثمة ارتباطات بيئية إيجابية هامة للغلة بالقرن/النبتة والبذور/النبتة والبذور/القرن. كما أظهرت خصائص مختلفة تسهم في الغلة ارتباطات إيجابية هامة فيما بينها.

Material and Methods

Twenty-five elite mutants were selected from those isolated in M_2 populations of different treatments involving separate and simultaneous applications of gamma rays, ethyl methyl sulfonate (EMS) and diethyl sulfate (DES) (Kumar 1995). The M_3 populations of these elite mutants were raised in a randomized complete block design with five replications. Days to flowering, length of main branch, number of primary branches, number of nodes on main branch, internode length, number of pods per plant, number of seeds per pod, number of seeds per plant, 100-seed weight and yield per plant were recorded. Coefficients of variation and correlations between these characters were worked out at phenotypic, genotypic and environmental levels.

Results and Discussion

The mean values and coefficients of variability for the traits are presented in Table 1. Yield per plant showed the maximum genotypic variability, followed by seeds/plant, pods/plant and 100-seed weight. Coefficients of genotypic variation for days to flowering, length of main branch, number of primary branches, nodes on main branch, internode length and seeds/pod were smaller.

Table 1. Mean values and coefficients of variation for grain yield and yield components in grasspea.

Character	Mean \pm SE	CV (%)		
		Phenotypic	Genotypic	Environmental
Days of flowering	78.60 \pm 2.07	8.91	8.09	3.73
Length of main branch (cm)	60.03 \pm 4.80	29.34	27.08	11.30
No. of primary branches	7.25 \pm 0.74	31.70	28.24	14.40
Nodes on main branch	23.86 \pm 1.68	14.67	10.76	9.97
Internode length (cm)	2.62 \pm 0.19	24.91	22.58	10.10
Pods/plant	46.23 \pm 11.14	54.06	41.96	34.08
Seeds/pod	2.31 \pm 0.17	24.00	21.47	10.60
Seeds/plant	109.42 \pm 26.34	59.65	48.98	34.05
100-seed wt (g)	8.348 \pm 0.701	40.18	38.39	11.86
Yield/plant (g)	8.639 \pm 2.057	61.05	50.92	33.67

For all traits, phenotypic coefficients of variation were higher than the genotypic coefficients. The difference between genotypic and phenotypic variability for all traits except pods/plant, seeds/plant and yield/plant was small, indicating that these traits may be less influenced by environment. A wide range of variation measured as phenotypic coefficient of variation was observed for yield/plant, seeds/plant, pods/plant and 100-seed weight. Phenotypic variation was smaller for other characters included in the study and it was the least for days to flowering. Coefficients of genotypic and phenotypic variation suggest that there is good scope for yield improvement through selection for yield/plant, seeds/plant and pods/plant. These findings are in agreement with those reported by Shrivastava (1976) who studied variability in 30 lines of grasspea.

The phenotypic, genotypic and environmental coefficients of correlation between the characteristics are summarized in Table 2. At the phenotypic level, grain yield showed strong positive correlations with length of main branch, number of primary branches, nodes on main branch, internode length, pods/plant, seeds/pod and seeds/plant. Days to flowering was the only trait which showed a negative correlation with yield. When correlations between yield-component characters were taken into account, most of the values involving length of main branch, number of primary branches, internode length, pods/plant and seeds/pod were positive and significant. However, days to flowering showed significant negative correlations with all of the other traits, except nodes on main branch and seeds/pod. Correlations of 100-seed weight with pods/plant, seeds/pod and seeds/plant were also negative. Length of main branch, internode length and seeds/plant showed stronger positive correlations with yield. Pods per plant showed a positive correlation with number of primary branches.

At the genotypic level, grain yield showed positive and significant correlations with all the other traits except days to flowering, with which it had a strong negative correlation. Correlations of days to flowering with all the other traits except nodes on main branch and seeds/pod, and that of 100-seed weight with seeds/pod were significantly negative. Seeds per pod appears to contribute substantially to yield at the genotypic level, as the strongest correlation of yield was recorded with this trait.

The strong positive correlations of number of pods and seeds with seed yield recorded in the present study agrees with those of Kaul et al. (1982) in grasspea and Oran et al. (1977) and Katiyar and Katiyar (1994) in chickpea. Akinola and Whiteman (1974) found that pigeon pea yield was influenced by the number of sites available for pod production as was suggested by the strong positive correlation between yield and number of pod-bearing branches, while Kaul et al. (1982) concluded that tall plants with more branches and early maturity will have improved yield potential in grasspea.

The values for the genotypic and phenotypic correlations between the traits were in the same direction and of similar magnitude. The level of significance for these two types was also similar. This leads to the conclusion that most of the phenotypic correlations are due to genotypic causes and not due to extra-genotypic factors. Most genotypic correlations were higher than their respective phenotypic correlations. This is in agreement with the results obtained by Singh and Singh (1969) and Dixit and Dubey (1985) in lentil, Singh et al. (1968) in mung bean, and Vandana (1990) in faba bean.

In the present study, seed yield showed significant positive environmental correlations with pods/plant, seeds/pod and seeds/plant, of which the one with seeds/plant was strongest. More than half of the correlations of days to flowering were negative, of which those with pods/plant, seeds/pod and seeds/plant were significant. Length of main branch showed significant and positive correlations with number of primary branches, nodes on main branch and internode length. The other positive correlations noted were those between number of primary branches and internode length, pods/plant and seeds/plant, and between seeds/pod and seeds/plant. Significant negative correlations occurred between number of nodes on the main branch and internode length, and between pods/plant and 100-seed weight. Significant environmental correlations between character pairs indicate that their associations are subject to environmental fluctuations.

At all three levels, there was negative correlation of seed yield with days to flowering, indicating difficulty to select for high yield and earliness.

Table 2. Correlation coefficients between different characters in induced mutants of grasspea.

Character	Level of correlation†	Length of main branch	No. primary branches	Nodes on main branch	Internode length	Pods/plant	Seeds/pod	Seeds/plant	100-seed wt	Yield/plant
Days to flowering	P	-0.468*	-0.536*	-0.181	-0.473*	-0.377*	-0.182	-0.418*	-0.297*	-0.472*
	G	-0.565*	-0.673*	-0.130	-0.603*	-0.459*	-0.174	-0.337*	-0.320*	-0.554*
	E	0.034	0.042	-0.135	-0.133	-0.201*	-0.220*	-0.265*	-0.163	-0.142
Length of main branch	P		0.891*	0.510*	0.854*	0.430*	0.165	0.320*	0.462*	0.668*
	G		0.599*	0.421*	0.602*	0.380*	0.163	0.526*	0.520*	0.533*
	E		0.542*	0.241*	0.642*	0.126	0.121	0.161	0.034	0.104
No. primary branches	P			0.290*	0.766*	0.354*	0.134	0.273*	0.488*	0.580*
	G			0.434*	0.577*	0.343*	0.149	0.483*	0.563*	0.775*
	E			0.169	0.260*	0.016	-0.005	0.023	0.042	0.016
Nodes on main branch	P				0.015	0.091	-0.125	0.083	0.400*	0.247*
	G				0.192*	0.142	-0.181	0.037	0.352*	0.262*
	E				-0.258*	0.071	-0.014	0.089	-0.006	0.074
Internode length	P					0.270*	0.391*	0.550*	0.185	0.638*
	G					0.605*	0.294*	0.714*	0.332*	0.568*
	E					0.039	0.106	0.054	0.018	0.109
Pods/plant	P						0.210*	0.930*	-0.121	0.498*
	G						0.170	0.562*	-0.181	0.554*
	E						0.123	0.621*	-0.202*	0.879*
Seeds/pod	P							0.343*	-0.331*	0.357*
	G							0.596*	-0.392*	0.368*
	E							0.352*	0.027	0.333*
Seeds/plant	P								-0.241*	0.602*
	G								-0.189	0.815*
	E								-0.129	0.909*
100-seed wt	P									0.154
	G									0.289*
	E									0.067

† P = Phenotypic correlation coefficients; G = Genotypic correlation coefficients; E = Environmental correlation coefficients.

* Significant at $P \leq 0.05$.

References

- Akinola, O. and P.C. Whiteman. 1974. Agronomic studies on pigeonpea. I. Field response to sowing time. *Australian Journal of Agricultural Research* 26: 46–56.
- Dixit, P. and D.K. Dubey. 1985. Correlation studies in lentil (*Lens culinaris* Med.) var. T-36. *Indian Botanical Reporter* 4(2): 97–100.
- Grafius, J.E. 1959. Heterosis in barley. *Agronomy Journal* 51: 551–554.
- Katiyar, P.K. and R.P. Katiyar. 1994. Correlated response for physiological quality and yield attributes in chickpea. *Indian Journal of Pulses Research* 7(2): 119–122.
- Kaul, A.K., M.Q. Islam and K. Begum. 1982. Variability for various agronomic characters and neurotoxin content in some cultivar of khesari (*Lathyrus sativus* L.) in Bangladesh. *Bangladesh Journal of Botany* 11: 158–167.
- Kumar, S. 1995. Studies on macro and micro mutations induced by gamma rays individually and in combinations with ethylmethane sulphonate (EMS) and diethyl sulphate (DES) in *Lathyrus sativus* L. var. P-505. PhD thesis, Kanpur University, Kanpur, India.
- Oran, P., R. Prakash and M.D.F. Haque. 1977. Correlation studies in chickpea (*Cicer arietinum* L.). *Tropical Grain Legume Bulletin* 7: 18–19.
- Shrivastava, Y.C. 1976. Genetical studies in *Lathyrus sativus* L. PhD Thesis, IARI, New Delhi.
- Singh, S.P., H.B. Singh, S.N. Mishra and A.B. Singh. 1968. Genotypic and phenotypic correlations among some quantitative characters in mung bean. *Madras Agricultural Journal* 55: 235–237.
- Singh, T.P. and K.B. Singh. 1969. Interrelationship of quantitative traits with grain yield in field pea. *Indian Journal of Genetics* 29: 483–487.
- Vandana. 1990. Studies on mutagenesis and polygenic variability induced by EMS and DES in faba bean (*Vicia faba* L.). PhD Thesis, Kanpur University, Kanpur, India.
- Whitehouse, R.N.H., J.B. Thompson and N. Riberio. 1958. Studies on the breeding of self pollinating cereals. II. The use of diallele cross analysis in yield prediction. *Euphytica* 7: 147–169.

Inheritance of Seed Weight in Grasspea (*Lathyrus sativus* L.)

توريث وزن البذور في الجلبان المزروع (*Lathyrus sativus* L.)

K.R. Tiwari¹ and C.G. Campbell²

¹ National Grain Legume Research Program, Rampur, Chitawan, NEPAL

² Agriculture and Agri-Food Canada Research Centre, Morden, Manitoba, R6M 1Y5, CANADA

Abstract

A study was undertaken to investigate the mode of inheritance of seed weight, an important agronomic trait in grasspea. One large-seeded line, L720060, was crossed with two small-seeded lines, L900436 and LS82046. The parents, F₁ and F₂ progenies were evaluated under field conditions and the seed weight recorded. The F₁ and F₂ progenies of large-seeded × small-seeded lines had intermediate seed weights. Continuous variation together with normal distribution of the F₂ progenies indicated that this trait is quantitatively inherited. Cytoplasmic influence was not detected. Broad-sense heritability was estimated at 26–31%. Seed weight and seed yield were positively correlated with each other.

Key words: *Lathyrus sativus*; yield components; mitochondrial genetics; genetic correlation; hybridization.

الملخص

أجريت دراسة لبحث أسلوب توريث وزن البذور الذي يعد صفة زراعية هامة في الجلبان (*Lathyrus sativus* L.). وتم تهجين سلالة كبيرة البذرة L720060 بسلاتين صغيرتي البذرة، وهما L900436 وLS82046. وتم تقييم الآباء وأنسال الجيلين الأول والثاني تحت الظروف الحقلية وتم تسجيل وزن البذور. وكان وزن أنسال الجيل الأول والثاني من السلالات الكبيرة البذور × الصغيرة البذور، متوسطاً. وقد أشار التباين المستمر مع التوزيع العادي لأنسال الجيل الثاني إلى أن هذه الصفة قد وُرِثت من الناحية الكمية. ولم يتم الكشف عن التأثير السيتوبلازمي. وقُدِرَت الإمكانية العامة للتوريث بنسبة 26-31%. وارتبط وزن البذور وغلة البذور ببعضها البعض بصورة إيجابية.

Introduction

Grasspea (*Lathyrus sativus* L.), also called chickling vetch or *khesari*, is a hardy legume crop in the tribe Vicicac. Despite its tolerance to drought, it is not affected by excessive rainfall and can be grown on land subject to

flooding (Campbell et al. 1994). It is an annual vine closely resembling field pea (*Pisum sativum* L.) in growth habit, but its leaflets are long and grass-shaped instead of round, hence its common name. Its deep tap-root system and nitrogen-fixing ability make it an ideal choice in sustainable agriculture (Deshpandey and Campbell 1992). Grains are used for human consumption and green plants and dried straw are fed to farm animals. Approximately 150 species of *Lathyrus* are known, most distributed in the Mediterranean region (Smartt et al. 1994). Smaller-seeded types are found in southern and southwest Asia, whereas around the Mediterranean region almost all are highly cultivated forms with large white seeds and flowers (Jackson and Yunus 1984). Seed size is an important agronomic characteristic in grasspea, and is of great interest to plant breeders. There are no reports available on the mode of inheritance of seed weight in grasspea and thus this study was undertaken to investigate the mode of inheritance of seed weight.

Material and Methods

A large-seeded line, L720060, which originated from France, was crossed with two smaller-seeded lines, L900436 and IS82046, which originated from Bangladesh and India, respectively. Seed of parental lines was taken from pure lines which had been grown for several generations in a greenhouse at the Agriculture and Agri-Food Canada Research Centre, Morden, Manitoba. Crosses were made during winter 1991/92 in a greenhouse, and the F₁ grown out. The parents, F₁ and F₂ progenies were evaluated under field conditions in summer 1992. Row and plant spacing was maintained at 1 m and 30 cm, respectively, for parents and F₂ progenies. The F₂ plot size was 8 rows, 12 m long, whereas parents were grown in single rows of 12 m length. The F₁ progenies were space planted with 1 m plant-to-plant spacing and seeded one week earlier than parents and F₂ progenies. The trial was grown in a sandy loam soil. Organic matter content was 5–6%, with soil pH of 7–7.3. Soil test results for macro- and micro-nutrients were in the range of high to very high and thus no fertilizer was applied. Weeds were suppressed with fall application of trifluralin at the rate of 1.5 kg active ingredient/ha. Aphids (*Aphis craccivora* Koch) were controlled by two applications of a mixture of malathion plus Lagon (dimethoate) at 0.2% concentration, two weeks apart. Individual plants were harvested, threshed and dried at 400°C for three days to bring final moisture content to 8%. One hundred seeds were randomly selected, bulked and weighed. The data were subjected to simple statistics and frequency distribution. T test was used to determine the statistical differences between the two means (cross and reciprocal) using the following formula:

$$\text{Test statistic (T')} = \frac{X_1 - X_2}{\sqrt{\left(\frac{S_1^2}{n_1} + \frac{S_2^2}{n_2}\right)}}$$

Where,

X₁= mean of population 1 (cross), X₂= mean of population 2 (reciprocal), s₁ and s₂ = standard deviation of populations 1 and 2, respectively, n₁ and n₂ = number of observations of populations 1 and 2, respectively.

Broad-sense heritability was estimated by comparing the segregating and homogenous populations as follows:

$$H = \left(\frac{V_g}{V_p}\right) \times 100$$

where,

H= broad-sense heritability, V_g= genotypic variance, V_p= phenotypic variance = variance of F₂.

Genotypic variance was estimated by subtracting the environmental variance (V_e) from the phenotypic variance; V_e was estimated as follows:

$$\text{Environmental variance (V}_e\text{)} = \frac{(V_{p1} + V_{p2} + V_{F1} + V_{F1} \text{ (Rec.)})}{4}$$

where,

V_{p1} and V_{p2}= variance of first and second parent, respectively, V_{F1} and V_{F2} = variance of F₁ and F₂ progenies, respectively.

Results and Discussion

The F₁ progenies of the cross of large-seeded L720060 (mean 100-seed weight 13.2 g) with small-seeded L900436 (mean 100-seed weight 8.72 g) and the reciprocal produced mean test weights of 12.79 g and 12.03 g, respectively. The mean test weight of the F₁ and F₂ progenies were intermediate between the parents (Table 1a). The non-significant difference between the cross and the reciprocal indicated that no cytoplasmic factor was involved in the inheritance of seed weight. Broad-sense heritability was estimated at 26%. Frequency distribution for 100-seed weight of the parental lines, F₁ progenies and F₂ progenies is presented in Table 1b. Variability within the parental lines and the F₁ progenies was high, which could be due to the

presence of some shrivelled seeds as the result of a wet and cool experimental year. The F_1 progenies were biased toward the large-seeded parental range. However, F_2 progenies were distributed over the range of both the small-seeded and large-seeded lines, indicating larger variability than in the parental and F_1 progenies. Variation in the segregating F_2 progenies was continuous and it was not possible to separate plants into distinct phenotypic classes of large and small seeded. The population was normally distributed which indicated that test weight was quantitatively inherited.

Table 1a. Simple statistics on 100-seed weight (g) of parents, F_1 and F_2 progenies in grasspea seeds from the cross L720060 × L900436.

Line	N	Mean	SE (±)	SD	CV%
1. L720060	15	13.20	0.51	1.93	15
2. L900436	14	8.72	0.40	1.51	17
F_1 (1×2)	10	12.79	0.53	1.69	13
F_1 (2×1)	10	12.03	0.49	1.56	13
F_2 (1×2)	96	9.80	0.20	1.97	21
F_2 (2×1)	210	10.03	0.13	1.92	19

T tests: F_1 means=ns; F_2 means=ns.

Heritability = 26%.

N=number of plants, SE= standard error, SD= standard deviation, CV= coefficient of variation, ns= non-significant.

Table 1b. Frequency distribution of parents, F_1 and F_2 progenies on 100-seed weight (g) of grasspea seeds from the cross L720060 × L900436.

Line	N	<7	8	9	10	11	12	13	>13
1. L720060	15	0	0	0	0	2	2	5	6
2. L900436	14	1	2	8	3	0	0	0	0
F_1 (1×2)	10	0	0	0	1	1	1	2	5
F_1 (2×1)	10	0	0	0	1	2	2	2	3
F_2 (1×2)	96	4	10	25	25	14	7	8	3
F_2 (2×1)	210	10	24	32	38	41	31	21	13

N= number of plants.

The F_1 and F_2 progenies of the cross of small-seeded LS82046 (mean 100-seed weight of 8.99 g), with L720060 were in the intermediate range (Table 2a). A non-significant difference between the cross and the reciprocal in both the F_1 and F_2 generations indicated that seed weight was under nuclear genetic control. Broad-sense heritability was estimated at 31%. Frequency distribution for 100-seed weight of parents, F_1 and F_2 progenies is presented in Table 2b. Both parents and the F_1 progenies exhibited a high variability which was mainly due to the presence of some shrivelled seeds as a result of a cool and wet experimental year. The F_1 was biased toward the high parental range. The

F_2 progenies overlapped both the parental ranges indicating quantitative control. Variation was continuous together with a normally distributed population in F_2 progenies.

Table 2a. Simple statistics on 100-seed weight (g) of parents, F_1 and F_2 progenies in grasspea seeds from the cross L720060 × LS82046.

Line	N	Mean	SE (±)	SD	CV(%)
1. L720060	15	13.20	0.51	1.93	15
2. LS82046	14	8.99	0.26	0.99	11
F_1 (1×2)	7	11.81	0.67	1.74	14
F_1 (2×1)	10	11.85	0.58	1.83	15
F_2 (1×2)	100	9.22	0.21	2.05	23
F_2 (2×1)	99	9.85	0.19	1.91	19

T tests: F_1 means= ns; F_2 means= ns; Heritability = 31%.

N= number of plants, SE= standard error, SD= standard deviation, CV= coefficient of variation, ns= non-significant.

Table 2b. Frequency distribution of parents, F_1 and F_2 progenies on 100-seed weight (g) of grasspea seeds from the cross L720060 × LS82046.

Cross	N	<7	8	9	10	11	12	13	>13
1. L720060	15	0	0	0	0	2	2	5	6
2. LS82046	14	1	3	6	4	0	0	0	0
F_1 (1×2)	7	0	0	0	0	1	2	3	1
F_1 (2×1)	10	0	0	0	1	2	2	3	2
F_2 (1×2)	100	12	15	25	23	12	10	3	0
F_2 (2×1)	99	8	9	18	17	16	19	7	5

N= number of plants.

The seed weight results of the F_1 progenies biased toward the large-seeded parental range could possibly be due to wider spacings between the plants of the F_1 progenies rather than dominance effect or heterosis. Furthermore, F_1 plants were planted about one week earlier than parental lines and F_2 progenies. These agronomic differences could have led to the benefits of utilizing a greater amount of sunlight, moisture and soil nutrients, which may have had positive effects on seed size. Lamb et al. (1987) report quantitative inheritance of seed weight in sorghum–Sudan grass crosses and no evidence of cytoplasmic effect in the F_2 population, which is in agreement with our findings. Additive genetic effects were found to be more important. Pixley and Frey (1991) also report additive gene action in the inheritance of test weight in oat (*Avena sativa* L.). They estimated heritability for test weight of oat at 63–91%. The relatively low heritability, 26–31%, in the present investigation was mainly due to the higher variability of parental lines and F_1 progenies. Abd El-Moneim and Cocks (1993) report a heritability of 21% and 32% in herbage and seed yield of grasspea, respectively.

Seed weight was positively associated with grain yield per plant ($r = 0.45^{**}$), which indicates that it is quite feasible to breed large-seeded high-yielding varieties of grasspea, or that selection for large seeds will facilitate selection for high yield. Similar to the present investigation, Lamb et al. (1987) and Pixley and Frey (1991) also report a positive correlation between seed yield and test weight. No strong association between neurotoxin content and seed weight was found in the present study.

Acknowledgements

Thanks are due to the International Development Research Centre (IDRC) and Morden Research Station (MRS) for providing Grant and research facilities, respectively. The technical help of JoAnne Rex (MRS) was highly appreciated.

References

Abd El-Moneim, A.M. and P.S. Cocks. 1993. Adaptation and yield stability of selected lines of *Lathyrus* spp. under rainfed conditions in west Asia. *Euphytica* 66: 89–97.

- Campbell, C.G., S.K. Agrawal, Y.Z. Chen, A.M. Abd El Moneim, H.I.T. Khawja, C.R. Yadav, J. Tay and W.A. Araya. 1994. Current status and future strategy in breeding grasspea *Lathyrus sativus* L. *Euphytica* 73: 167–175.
- Deshpande, S.S. and C.G. Campbell. 1992. Genotype variation in BOAA, condensed tannins, phenolics and enzyme inhibitors of grass pea (*Lathyrus sativus*). *Canadian Journal of Plant Science* 72: 1037–1047.
- Jackson, M.T. and A.G. Yunus. 1984. Variation in grass pea and wild species. *Euphytica* 33: 549–559.
- Lamb, J.F.S., F.A. Haskins, H.J. Gorz and K.P. Vogel. 1987. Inheritance of seedling hydrocyanic acid potential and seed weight in sorghum-sudangrass crosses. *Crop Science* 27: 522–525.
- Pixley, K.V. and K.J. Frey. 1991. Inheritance of test weight and its relationship with grain yield of oat. *Crop Science* 31: 36–40.
- Smartt, J., A. Kaul, W.A. Araya, M.M. Rahman and J. Kearney. 1994. Grasspea (*Lathyrus sativus* L.) as a potentially safe legume food crop. Pages 144–155 in *Expanding the Production and Use of Cool Season Food Legumes* (F.J. Muchbauer and W.J. Kaiser, ed.). Kluwer Academic, Netherlands.

Divergence among Induced Mutants of Grasspea (*Lathyrus sativus* L.)

Sushil Kumar and D.K. Dubey

Department of Botany

Janata Mahavidyalaya

Ajitmal, Etawah (U.P.) 206 121

INDIA

Abstract

Mahalanobis D^2 statistic was applied to obtain quantitative estimates of divergence among 25 elite mutants and the parent cultivar P-505 of grasspea. Using Touchers method, the 25 genotypes were grouped into six genetically distinct clusters with wide divergence for yield and its components.

Among the clusters, clusters I and III, each with two genotypes, showed minimum and maximum intra-cluster distances, respectively. The largest cluster, II, contained 10 of the 26 genotypes, and clusters IV, V and VI had 3, 4 and 5 genotypes, respectively, which showed medium intra-cluster distances. Maximum inter-cluster distance was recorded between clusters I and III, while minimum distance occurred between clusters II and V.

التباعد الوراثي بين الطافرات المستحدثة للجلبان المزروع (*Lathyrus sativus* L.)

المخلص

تم تطبيق إحصاء Mahalanobis D^2 للحصول على تقديرات كمية للتباعد الوراثي بين 25 طافرة متفوقة والصنف الأب P-505 من الجلبان المزروع. وباستخدام طريقة Touchers، تم تصنيف 25 طرازاً وراثياً في 6 مجموعات متميزة وراثياً ذات تباعد وراثي واسع للغاية ومكوناتها.

ومن بين المجموعات، أظهرت المجموعتان I و III، التي يحمل كل منهما طرازان وراثيان، حدوداً دنياً وقصوى للمسافات بين المجموعات على التوالي. وقد حوت المجموعة الأكبر، II، على 10 من أصل 26 طرازاً وراثياً، كما حوت المجموعات IV و V و VI و 3 و 4 و 5 طرز وراثية على التوالي مما أظهر مسافات متوسطة بين المجموعات. وسجلت أقصى مسافة بين المجموعات، المسافة بين المجموعتين I و III، في حين لوحظت أدنى مسافة بين المجموعتين II و V.

وبينت المقارنة بين متوسطات المجموعات أن المجموعتين III و V

Comparison of inter-cluster group means revealed that clusters III and V were best in overall performance for the traits studied while cluster VI was worst.

Key words: *Lathyrus sativus*; divergence; mutants; quantitative analysis; yield components.

Introduction

A quantitative estimate of divergence among germplasm may be made using Mahalanobis' 'D² Statistics.' This method provides a measure for group distance based on multiple characters. Several workers have used this method to quantify the degree of divergence based on phenotypic observations (Murty and Arunachalam 1966; Singh and Gupta 1968; Balyan and Singh 1986; Sharma and Luthra 1987; Khare and Singh 1992; Vandana and Dubey 1994). These studies have shown that accessions from the same geographical region may differ genetically as well as morphologically, and also in adaptability. In the present study, this analysis was used to determine the divergence among 25 mutants induced in cv P-505 of grasspea (*Lathyrus sativus* L., also known as *khesari*).

Material and Methods

In a study on induced mutagenesis in cv P-505 of *Lathyrus sativus* using gamma rays, ethyl methane sulfonate (EMS) and diethyl sulfate (DES), a number of macromutants were isolated (Kumar 1995). Of these, 25 elite mutants were selected for the present study. M₃ populations of these mutants were grown along with the parent variety (control) in a randomized block design with five replications. Each plot consisted of three rows, 45 cm apart, with plant spacing of 40 cm. Observations were made of seed yield per plant and nine component characters, namely days to flowering, length of main branch, number of primary branches, number of nodes on main branch, internode length, number of pods per plant, seeds per pod, seeds per plant and 100-seed weight.

كانتا الأفضل من حيث الأداء الإجمالي للصفات المدروسة في حين كانت المجموعة VI الأسوأ.

Estimates of divergence among the 26 genotypes was based on multivariate analysis using Mahalanobis D² statistics. The formation of clusters was done by Touchers method as described by Rao (1952).

Results and Discussion

On the basis of the divergence analysis, the 26 genotypes were grouped into six clusters. Cluster I had two genotypes – mutants AKM 10 and AKM 13. Cluster II was the largest and comprised the parental variety and nine of the mutants, namely AKM 3, AKM 4, AKM 7, AKM 16, AKM 17, AKM 19, AKM 22, AKM 24 and AKM 25. Cluster III comprised two mutant genotypes, AKM 11 and AKM 12. Cluster IV comprised mutants AKM 14, AKM 15 and AKM 23, while cluster V comprised mutants AKM 1, AKM 2, AKM 5 and AKM 9. Cluster VI comprised five mutants, namely AKM 6, AKM 8, AKM 18, AKM 20 and AKM 21. The nine mutants included in cluster II were closer to the parental variety and to each other than to the mutants in the other five clusters. The pattern of cluster formation shows that there is a large divergence for yield and its components in the genotypes isolated from the same parental variety on the basis of macromutations.

Inter- and intra-cluster genetic divergence based on D² values are presented in Table 1. Maximum intra-cluster divergence was 59.72 recorded in cluster III, while it was only 7.14 for cluster I. Inter-cluster divergence was maximum between clusters I and III (167.98), suggesting wide divergence between these two groups. Least divergence occurred between clusters II and V (51.53), indicating a comparatively closer relationship between the genotypes falling in these two groups.

Table 1. Inter- and intra-cluster D² values in grasspea mutants derived from cv P-505.

Cluster	I	II	III	IV	V	VI
I	(7.14)†	68.63	167.98	129.63	52.99	116.16
II		(38.47)	113.92	79.50	51.53	58.75
III			(59.72)	58.77	97.23	161.84
IV				(23.83)	81.83	128.90
V					(32.18)	106.29
VI						(32.24)

† Figures in parenthesis denote intra-cluster values.

Table 2. Cluster means for yield and its components in M₃ generation of grasspea mutants.

Cluster	Days to flowering	Length main branch (cm)	No. primary branches	Nodes on main branch	Internode length (cm)	Pods/plant	Seeds/pod	Seeds/plant	100-seed wt (g)	Yield/plant (g)
I	74.12	68.41	8.75	21.00*	3.42*	45.00	3.57*	160.75	6.62*	10.58
II	77.57	55.42*	6.60	22.42	2.59	55.15	2.27	123.97	7.13*	8.57
III	79.75	87.04*	10.38*	29.12*	3.10	41.62	2.20	93.75	14.84*	13.27
IV	73.83*	64.05	8.67	24.67	2.71	25.42	1.64*	42.92*	13.39*	5.93
V	74.56	79.07*	9.44	26.44	3.11	71.12*	2.57	182.06*	7.60*	14.24*
VI	88.10*	37.44*	4.10*	23.25	1.70*	23.30	2.12	47.85*	6.44*	3.30*
Mean	77.99	65.24	7.99	24.48	2.77	43.60	2.39	108.55	9.34	9.31
C.D. (5%)	4.15	9.60	1.74	3.36	0.39	22.28	0.35	52.69	1.40	4.11

* = Differs significantly from the general mean ($P \leq 0.05$).

A comparison of cluster means for different characters showed considerable differences between the clusters (Table 2). Early maturity was found in cluster IV, while cluster VI showed delayed maturity over the general mean. Significantly longer main branches were recorded in clusters III and V, while shorter main branches were recorded in clusters II and VI. Minimum number of branches, internode length, pods per plant, 100-seed weight and seed yield were recorded in cluster VI. Maximum number of branches, nodes on main branch and 100-seed weight were found in cluster III, while maximum number of pods and seeds per plant and yield per plant were recorded in cluster V. Thus, in overall performance, clusters III and V appeared to be superior as their mean values for most traits were higher for almost all traits (except for the desirable low values for days to flowering) compared with clusters I, II, IV and VI. Cluster VI showed minimum values for almost all the traits and maximum value for days to flowering. Thus, this cluster had the poorest overall performance.

The relative importance of length of main branch, number of primary branches, number of pods and number of seeds, besides seed yield, in contributing towards divergence was established when inter-cluster group means were compared. This study has clearly brought out in quantitative terms the wide divergence induced in the mutants isolated from the same parental genotype through mutagenic treatments.

Dixit (1985) in lentil, and Vandana and Dubey (1994) in faba bean also demonstrate substantial divergence among induced mutants. Singh (1979) and Singh and Gupta (1968) have also obtained quantitative estimates of divergence in their populations of mustard and cotton, respectively.

References

- Balyan, H.S. and S. Singh. 1986. Genetic divergence in lentil. *LENS Newsletter* 13(1): 3-4.
- Dixit, P. 1985. Studies on mutagenesis and polygenic variability induced by separate and synergistic action of a chemical and a physical mutagen in lentil (*Lens culinaris* Med.) var. T-36. PhD Thesis, Kanpur University, Kanpur, India.
- Khare, D. and C.B. Singh. 1992. Divergence analysis in *Vicia faba* L. for nutritional and antinutritional attributes. *Indian Journal of Genetics* 51(1): 58-62.
- Kumar, S. 1995. Studies on macro and micro mutations induced by gamma rays individually and in combinations with ethyl methane sulphonate (EMS) and diethyl sulphate (DES) in *Lathyrus sativus* L. var. P-505. PhD Thesis, Kanpur University, Kanpur, India.

- Murty, B.R. and V.C. Arunachalam. 1966. The nature of genetic divergence in relation to breeding system in crop plants. *Indian Journal of Genetics* 26A: 188–190.
- Rao, C.R. 1952. *Advanced Statistical Methods in Biometric Research*. John Wiley and Sons, New York.
- Sharma, P.C. and S.K. Luthra. 1987. Genetic divergence in lentil (*Lens culinaris* Med.). *Genetika Agraria* 41(4): 349–359.
- Singh, K.N. 1979. Genetic divergence in Indian mustard in acidic soil conditions. *Indian Journal of Genetics* 39: 439–443.
- Singh, R.B. and M.P. Gupta. 1968. Multivariate analysis of divergence in upland cotton. *Indian Journal of Genetics* 28: 51–57.
- Vandana and D.K. Dubey. 1994. Genetic divergence among induced mutants of *Vicia faba* L. *Journal of the Indian Botanical Society* 73: 121–123.

Pests and Diseases

Morphological, Cultural and Pathogenic Variability among Nine Isolates of *Botrytis fabae* from Ethiopia

Dereje Gorfu

Holetta Agricultural Research Center
P.O. Box 2003, Addis Ababa, ETHIOPIA

Abstract

Morphological, cultural and pathogenic variability among nine isolates of *Botrytis fabae*, the cause of chocolate spot of faba bean, from Ethiopia were investigated. Although there was apparent cultural variation, there was no difference in conidia length (L), width (W) and L/W ratio among the isolates. Isolate GB produced confluent sclerotia on malt extract agar (MEA) and potato dextrose agar (PDA) and discrete sclerotia on faba bean dextrose agar (FBDA) and lima bean agar (LBA). The other isolates produced only discrete sclerotia on all the media used. All isolates produced most sclerotia on FBDA and fewest on LBA. Isolate K5 produced the most sclerotia ($10.2/\text{cm}^2$), while isolate S1 produced the fewest ($4.3/\text{cm}^2$).

ANOVA of chocolate spot infection revealed highly significant differences ($P = 0.001$) among isolates, genotypes and isolate \times genotype interaction. The isolates differentially infected the genotypes, i.e. the genotypes responded differently to the nine isolates. Isolate EW was the most virulent.

This study showed that there is a wide variation of pathogenicity among *B. fabae* isolates in Ethiopia. Therefore, breeding and selection programs should use the virulent isolates in greenhouse screening work or consider multi-location screening work for chocolate spot resistance.

Key words: *Vicia faba*; faba beans; *Botrytis fabae*; culture media; cell culture; plant anatomy; pathogens; pathogenicity; Ethiopia.

Introduction

Botrytis fabae Sard. is the only cause of chocolate spot disease in faba bean (*Vicia faba* L.) in Ethiopia (Dereje et al. 1994; Dereje and Tesfaye 1994). It mainly infects the leaf tissue, but in severe cases, petioles, stems and seeds are also infected.

التباين المظهري والزراعي والمرضي بين تسع عزلات من *Botrytis fabae* من إثيوبيا

الملخص

تمت دراسة التباين المظهري والزراعي والمرضي بين تسع عزلات من *Botrytis fabae* Sard. المسببة لمرض التبقع الشوكولاتي على الفول (*Vicia faba* L.) من إثيوبيا. ورغم ظهور تباين زراعي واضح، لم يكن ثمة فرق في طول الأبواغ الكونيدية (L) وعرضها (W) ونسبة L/W بين العزلات. وأعطت العزلة GB جسيمات حجرية مندمجة على هلام مستخلص المولت (MEA) وهلام دكستروز البطاطا (PDA) وجسيمات حجرية منفصلة على هلام دكستروز الفول (FBDA) وهلام فاصولياء ليما (LBA). ولم تنتج العزلات الأخرى سوى جسيمات حجرية منفصلة في كل الأوساط البيئية المستخدمة. وأعطت كل العزلات معظم الجسيمات الحجرية على FBDA وأقلها على LBA. وأعطت العزلة K5 معظم الجسيمات الحجرية ($10.2/\text{cm}^2$)، في حين أعطت العزلة S1 أقلها ($4.3/\text{cm}^2$). وأظهر تحليل التباين (ANOVA) للإصابة بالتبقع الشوكولاتي فروقات معنوية عالية ($P = 0.001$) بين العزلات والأصناف والتفاعل بين العزلة \times الصنف. وقد أصابت العزلات الأصناف بالعدوى بشكل متباين، أي أن الأصناف استجابت بشكل متباين للعزلات التسع، إذ كانت العزلة EW أكثرها فوعة.

أبانت هذه الدراسة وجود تباين واسع في القدرة الإراضية بين عزلات *B. fabae* في إثيوبيا. لذا ينبغي على برامج التربية والانتخاب أن تستخدم العزلات ذات الفوعة في عمليات الغريلة في الدفيئة أو إجراء غريلة متعددة المواقع لمقاومة التبقع الشوكولاتي.

Under severe conditions, chocolate spot caused yield losses of 61% on susceptible genotype PGRC/E 27113 and 34% on tolerant cultivar CS 20 DK (Dereje and Beniwal 1988). However, complete crop loss may occur when there is a prolonged conducive environment for disease development (Dereje et al. 1994).

Surveys and variety testing in Ethiopia have demonstrated differences in severity and incidence of the disease, as well as differences in response of genotypes to infection in different locations (Dereje and Tesfaye 1994). Hence, the presence of variability in *B. fabae* was suspected. Cultural and pathogenic variability, and the existence of races in this pathogen have been demonstrated elsewhere (Hanounik and Maliha 1984, 1986).

This paper reports on the morphological, cultural and pathogenic variability of nine isolates of *Botrytis fabae* collected from different regions of Ethiopia.

Material and Methods

Pathogens

Chocolate spot samples were collected from different faba bean growing regions of Ethiopia. Cultures were purified and single conidium isolation was made, then nine isolates (Table 1) were selected to represent the major growing areas. Cultures were maintained on faba bean dextrose agar and whenever conidia were required they were transferred to chrysanthemum petal culture (CPS) according to Beniwal and Dereje (1987). Inoculum suspension was prepared by suspending the cultures (CPS) in tap water and then blending. This suspension was filtered by passing through a double layer of cheese cloth. The spore concentration was adjusted to 150,000 spores/ml and was used for inoculation test.

Table 1. *Botrytis fabae* isolates collected from faba bean crops at different locations used to study possible differences in virulence and cultural characteristics.

Isolate	Location	Altitude (m)	Cultivar
B1	Bekoji 1	2770	CS 20 DK
B2	Bekoji 2	2770	CS 20 DK
K1	Kulumsa 1	2200	CS 20 DK
K5	Kulumsa 5	2200	CS 20 DK
S1	Sinana	2440	CS 20 DK
GB	Gudoberet	3140	Unknown
HL	Holetta	2390	CS 20 DK
DT	Debretabor	2600	Unknown
EW	Enewari	2300	Unknown

Media tested

Four media, namely faba bean dextrose agar (FBDA), lima bean agar (LBA), malt extract agar (MEA) and potato dextrose agar (PDA), were used to study the cultural characteristics of the isolates. Media were prepared following Hanounik (1986). The experiment was set in a completely randomized design with 10 replications.

Genotypes

The test plants used to differentiate the nine isolates were grown in pots in the greenhouse. They were from different origins (Table 2) with known different reactions to chocolate spot infection (Dereje and Tesfaye 1994; Hanounik and

Maliha 1984). Five plants were raised in each pot and four pots were used for each genotype. Leaves at the 10–14 node position were detached and used for inoculation (experiment 1) or first inoculated and allowed to develop symptoms, then detached for incubation (experiment 2). The adaxial surfaces were turned upwards during inoculation. The experiment was designed as a 9 × 10 factorial experiment in RCBD with four replications.

Table 2. Faba bean genotypes used to study the variation of *Botrytis fabae* isolates from Ethiopia.

Genotype	Origin†
BPL 112-1-1	-
BPL 710-7-1	Colombia
BPL 261-2-1	Greece
BPL 1821-1	Ethiopia
BPL 1179-3-1	Colombia
Coll 30-77-1	Ethiopia
S 83103-8-1	ICARDA
Kuse	Ethiopia
CS 20 DK	Ethiopia
NC 58	Ethiopia

† - = Origin unknown.

Cultural characterization (variation), disease assessment and analysis

Spore length and width for 30 spores/replication were measured under compound microscope for each isolate.

Sclerotia formation was assessed by counting the number of sclerotia per cm², measuring the length, and by describing the patterns of formation (discrete or confluent).

Plants were inoculated at 30 days old with a 14-day-old culture. In experiment 1, leaves were detached and inoculated with spore suspension. Four detached leaves were inoculated with 40 µl of spore suspension/leaf using a micropipette. In experiment 2, potted plants were inoculated with 20 ml spore suspension, left in the greenhouse (for lesions to develop) and leaves were detached after 14 days. The detached inoculated leaves from both experiments were incubated at room temperature (20 ± 2 °C) in aluminum trays with moistened sponge sheet.

Every second day, infection of chocolate spot was assessed according to Hanounik (1986). The data collected were subjected to statistical analysis using MSTAT-C computer statistical package. Means were separated using Least Significance Difference Test.

Results and Discussion

Morphological variation

Overall mean length (L) of conidia was 18.04 μm and overall mean width (W) was 13.12 μm . ANOVA for mean conidia length, width, and length/width ratio of the nine isolates revealed no significance differences, indicating a lack of morphological difference among the isolates studied. Generally, these nine *B. fabae* isolates collected from different regions of Ethiopia were at the short and thin ends of the array of lengths and widths of this fungus given by CAB (1974).

Cultural variation

All nine isolates produced discrete sclerotia on all media, except that isolate GB gave confluent sclerotia on MEA and PDA. However, the size of sclerotia varied greatly within isolates. The sclerotia produced by all the isolates were short (0.5–1.5 mm) on LBA, medium-length (1.6–2.5 mm) on FBDA and MEA, and long (2.6–3.2 mm) on PDA. Since there was great variation in the size of sclerotia, this result only indicates the existence of some cultural variation among *B. fabae* isolates from Ethiopia. This result also agrees with that of Hanounik and Maliha (1984) who report three cultural group isolates collected from different regions.

Sclerotia density was significantly higher ($P = 0.05$) on FBDA than on the other three media (Table 3). This indicates that, among the media used, FBDA is the most suitable for laboratory propagation of all nine isolates. Overall, greatest density of sclerotia (10.3/cm²) was obtained from isolate K5, while the lowest (4.3/cm²) was from isolate S1 (Table 3). There was significant interaction between media and isolates – some isolates developed well in one

media while others did not. This was a differential response of isolates to media indicating media preferences of the different isolates. There was no relation between pathogenicity and cultural characteristics in this study. Berhanu et al. (1992) report a different strain from Sinana, which produced only sclerotia and no conidia. However, in our study all isolates produced only sclerotia and very few conidia on media as expected, since sporulation of *B. fabae* needs a special environmental and nutritional requirements (Beniwal and Dereje 1987; Dereje 1986).

Pathogenic variation

Results of ANOVA revealed that the effects of isolates, genotypes and isolate \times genotype interaction were highly significant (Table 4). This shows that there are genuine differences among isolates and genotypes. There was also differential reaction of genotypes to the different isolates; in other words, isolates differentially infected the genotypes. This suggests differences among isolates for virulence and among genotypes for resistance to isolates of *B. fabae*. The difference obtained on isolate \times genotype interaction indicates pathogenic variation within *B. fabae* and the variability of faba bean lines used in this experiment.

Five virulence groups (Table 5) are postulated using the reaction score of chocolate spot from the detached-leaf tests. Isolates in the same group did not necessarily come from the same location, examples are groups 4 and 5 (Table 5). Isolates collected from Sinana and Debretabor had the same virulence character, while those from Bokoji-1, Kulumsa-5, Gudoberet and Holetta had the same virulence on the 10 genotypes used. However, the 'differentials' used in this study might not show the whole pathogenic variation within *B. fabae* in Ethiopia, since few isolates were used to represent the vast growing regions of the country.

Table 3. Mean sclerotia (number/cm²) for nine isolates of *Botrytis fabae* grown on four media.

Isolate	PDA†	LBA	MEA	FBDA	Isolate mean
B1	6.9	7.0	5.7	7.1	6.7
B2	8.2	9.0	2.8	7.7	6.9
K1	9.8	0.9	3.7	10.7	6.2
K5	2.9	10.4	8.8	16.1	10.3
S1	3.3	2.2	1.9	9.7	4.3
GB	2.6	2.9	8.3	13.0	6.7
HL	5.3	9.2	11.4	8.1	8.5
DT	5.6	2.8	9.2	15.8	8.4
EW	6.6	5.1	6.2	4.7	5.6
Mean of medium	6.0	5.5	6.4	10.3	

LSD_{0.05} for means of media = 1.59, for isolate means = 1.73, for isolate \times medium = 3.098. CV = 26.0%.

† PDA = potato dextrose agar, LBA = lima bean agar, MEA = malt extract agar, FBDA = faba bean dextrose agar.

Table 4. Analysis of variance of infection score for chocolate spot as a result of inoculation of nine isolates on 10 faba bean genotypes.

Experiment†	Source of variation	d.f.	Mean square	Variance ratio	CV (%)
1	Isolate (I)	8	5.17	77.5***	16.6
	Genotype (G)	9	0.90	13.58***	
	I × G	72	0.57	8.54***	
	Residuc	90	0.067		
2	Isolate	8	5.74	68.87***	18.4
	Genotype	9	0.74	8.90***	
	I × G	72	0.64	7.66***	
	Residuc	90	0.083		

*** Significant at $P = 0.001$.

† Experiment 1: detached leaves inoculated and incubated. Experiment 2: Leaves inoculated *in situ*, detached after 14 days, then incubated.

Table 5. Reaction of detached leaves of 10 faba bean genotypes to nine isolates of *Botrytis fabae*.†

Genotype	Isolates‡				
	Group 1	Group 2	Group 3	Group 4	Group 5
KUSE	S	S	S	R	R
S 83103-1-1	S	R	R	S	R
CS 20 DK	R	S	R	R	R
NC 58	S	R	R	R	R
BPL 112-1-1	S	R	R	R	R
BPL 261-2-1	R	S	R	R	R
BPL 1179-3-1	R	R	R	R	R
Coll 30-77-1	R	R	R	R	R
BPL 1821-1	R	R	R	R	R
BPL 710-7-1	R	R	R	R	R

† Reaction: S = susceptible, R = resistant.

‡ Group 1 = EW; Group 2 = K1; Group 3 = B2; Group 4 = S1, DT; Group 5 = B1, K5, GB, HL.

The most virulent isolate was EW from Enewari. The special interaction of isolates EW, K1 and HL with genotypes Kuse and S 83103-1-1 (Table 5) may suggest a gene-for-gene interaction. This needs to be verified with genetic analysis. Four lines, BPL 1179-3-1, Coll 30-77-1, BPL 1821-1 and BPL 710-7-1, were resistant to all the isolates tested. This is in agreement with our previous screening results in that BPL 710, -1179 and -1821 were among the sources of resistance identified under Ethiopian conditions (Dereje and Tesfaye 1994). Hanounik and Maliha (1986) also used these and another three lines to differentiate the groups of *B. fabae* isolates in Syria.

This is the first study of the variability of *Botrytis fabae* in Ethiopia. Released cultivars that were adapted to Ethiopia and lines that were identified as sources of resistance during

past screening work were used in this study. Hence, this evidence on pathogenic variation of this pathogen is of paramount practical importance.

Hanounik and Maliha (1984) studied three isolates of *B. fabae* to show cultural and pathogenic differences in this pathogen. In another study, they (Hanounik and Maliha 1986) used 12 isolates and 6 lines as differentials and suggested four races for the first time.

Generally, there is a wide variability in *B. fabae* isolates in Ethiopia for pathogenic aggressiveness. Therefore, breeding and selection programs should utilize as many virulent isolates as possible from different regions. The most pathogenic isolates, such as EW and K1, should be identified and be used in greenhouse screening, or multi-location

screening should be considered to test material at places like Enewari and Kulumsa.

Acknowledgement

I thank W/O Tsige Mamo for her assistance in the laboratory and greenhouse work.

References

- Beniwal, S.P.S. and Dereje Gorfu. 1987. A simple mass spore production of *Botrytis fabae* the cause of chocolate spot in faba bean. *FABIS Newsletter* 23: 30–32.
- Berhanu Bekele, Y.S. Paul and Dereje Tadesse. 1992. Effects of chocolate spot on faba bean seeds and the isolation of sclerotial strain of *Botrytis fabae* in Sinana. Page 41 in Proceedings of Crop Protection Society of Ethiopia, 5–6 March 1992. Addis Ababa, Ethiopia.
- CAB (Commonwealth Agricultural Bureaux). 1974. *Botrytis fabae*. *CMI Description of Pathogenic Fungi and Bacteria* No. 342. Eastern Press, London, UK.
- Dereje Gorfu. 1986. Sporulation of *Botrytis fabae* as affected by dextrose and faba bean extractes. *FABIS Newsletter* 16: 44–45.
- Dereje Gorfu and S.P.S. Beniwal. 1988. Yield losses caused by chocolate spot in faba bean. *Ethiopian Plant Pathology Newsletter* 13: 16–20.
- Dereje Gorfu and Tesfaye Beshir, 1994. Faba bean diseases in Ethiopia. Pages 328–345 in Cool-season Food Legumes of Ethiopia. Proceedings of the First National Cool-season Legume Review Conference, 16–20 Dec 1993. Addis Ababa, Ethiopia (A. Tilaye, G. Bejiga, M.C. Saxena and M.B. Solh, ed.). ICARDA/IAR, Aleppo, Syria.
- Dereje Gorfu, Mengistu Huluka and Tadesse G/Medhin. 1994. Influence of weather factors on infection rate of chocolate spot of faba bean. Pages 33–34 in Proceedings of Crop Protection Society of Ethiopia, 26–27 April 1994. Addis Ababa, Ethiopia.
- Hanounik, S.B. 1986. Screening Techniques for Disease Resistance in Faba Bean. ICARDA, Aleppo, Syria.
- Hanounik, S.B. and N. Maliha. 1984. Pathogenic and cultural variability in *Botrytis fabae*. *FABIS Newsletter* 10: 21–24.
- Hanounik, S.B. and N. Maliha. 1986. Horizontal and vertical resistance in *Vicia faba* to chocolate spot caused by *Botrytis fabae*. *Plant Disease* 70: 770–773.

Seed Quality and Nutrition

Rapid Spectrophotometric Method for Reduction of Vicine and Convicine in Faba Bean Seed

G. Sixdenier, F. Cassecuelle, L. Guillaumin and G. Duc

INRA, Station de Génétique et d'Amélioration des Plantes
BV 1540, 21034 Dijon Cedex, FRANCE

Abstract

A rapid spectrophotometric method applied on a two-seed sample is recommended for breeding programs aiming at reduction of vicine and convicine content of faba bean seeds.

Key words: *Vicia faba*; faba beans; spectrometry; phytotoxicity; reduction.

Introduction

Faba bean (*Vicia faba* L.) seeds can be a significant source of proteins in food and feeds, but two pyrimidine glucosides (vicine and convicine) found in faba bean have been reported as causing antinutritional effects. In humans carrying a genetic deficiency in glucose-6-phosphate dehydrogenase (G6PD), the aglycones of vicine and convicine produced in the digestive track (divicine and isouramil, respectively) induce a hemolytic disease called favism (Mager et al. 1965). In laying hens, vicine and/or convicine reduce egg weight (Olaboro et al. 1981) and animal performance is improved by removing these products (L. Lacassagne, F. Rudeau, G. Duc and C. Peyronet, personal communication).

The discovery of a spontaneous mutant (*vc*⁻ gene) (Duc et al. 1989) and of induced mutants (Ramsay and Griffiths 1993) with very low vicine and convicine contents in their seed with monogenic inheritance, has made available a genetic way to remove these antinutritional factors. A major limitation in breeding programs is still the cost and the difficulty of the chemical determination of these products for which several methods have been proposed. The more precise but expensive quantification is to use gas (Pitz and Sosulki 1979) or liquid chromatography (Marquardt and Frohlich 1981; Lattanzio et al. 1982; Quemener et al. 1982; Quemener 1988). A rapid qualitative method using paper chromatography was used by Ramsay and Griffith (1992) to

طريقة المطياف الضوئي السريع لانقاص الـ Vicine وConvicine في بذور الفول

الملخص

يُنصح باستخدام طريقة المطياف الضوئي السريع المطبقة على عينة من بذرتين في برامج التربية الهادفة إلى إنقاص محتوى الـ vicine و convicine في بذور الفول (*Vicia faba* L.).

detect mutants with near-zero vicine-convicine. Near infrared reflectance spectroscopy appears not to be useful for determining these products (Duc et al. 1989). Duc et al. (1989) showed that the *vc*⁻ gene causes a simultaneous reduction of vicine and convicine, an additive behavior in heterozygous state and a maternal determinism of the seed phenotype. Consequently, breeders using this gene need a reliable and rapid technique to detect homozygous mother plants from a small seed sample with low vicine and convicine contents in their seeds.

The spectrophotometric technique reported here was devised for such a purpose and in addition appeared to give accurate quantification compared with the HPLC technique.

Material and Methods

Plant material

Five genotypes (synthetic populations) representing the range of variability available for vicine and convicine content from nearly zero (vicine + convicine = 0–0.08% of seed DM, genotypes E and F carrying *vc*⁻ gene) to intermediate (vicine + convicine = 0.2–0.7% of seed DM, genotype A) and rich (vicine + convicine = 0.7–1.2% of seed DM, genotypes B and D) were used in the study. They were grown at Dijon, France in 1995 and more than 300 seed progenies per genotype were sampled and analyzed.

Reference method

The reference method was HPLC adapted from Quemener (1988). Preliminary tests to optimize the wavelength for separation of vicine and convicine established that the best results were achieved at 276 nm. Two whole seeds per progeny were soaked for 3.5 h in 30 ml water in a 90°C water-bath. Normal HCl (100 µl) was mixed with 10 ml of the resulting solution, centrifuged at 13,000 rpm for 15 min and then filtered on Nalgene SFCA filter. The resulting

solution was diluted with water (1:5 v/v) prior to HPLC injection. Chromatographic conditions were Licrospher 125-4, 100 RP-18 (5 µm) column with a precolumn, MILLI-Q water 1 ml/min as eluant, 276 nm wavelength of absorbance measurement. Standards of pure vicine and convicine were obtained from Dr R.R. Marquardt (personal communication).

Rapid method

Preliminary tests to optimize the wavelength for an integrated value of vicine and convicine established that the best results were achieved at 274 nm. Absorbance at 274 nm was read on a spectrophotometer on the extraction solution from a sample of two whole seeds. The extraction procedure of vicine and convicine was the same as previously described without filtration on the Nalgene SFCA filter.

Results and Discussion

Water is an adequate solvent for extracting vicine and convicine (Hegazy and Marquardt 1983). For more than 100 samples, extraction of vicine and convicine from whole seed was 90% of what was extracted from flour with Quemener's method (1988) and therefore appears as a correct procedure for a quantitative assessment of these products.

Figure 1 illustrates the clear HPLC separation of vicine and convicine peaks and the contrasting results from rich and near-zero vicine-convicine genotypes (10-fold reduction in peak area). It also shows the presence of an additional slower-migrating peak (X) - corresponding to an

undetermined substance which caused absorbancy at 274 nm - in genotypes D and F at a frequency lower than 10% of the mother plants. When discarding these plants carrying the X peak, the relationship between HPLC and rapid spectrophotometric method is shown in Figure 2. The wide range of variability available over A, B, D, E and F genotypes is detected by both methods which strongly correlate (Fig. 2; $r = 0.988$, 1498 d.f.). Intra-genotypic variability was uncontrolled and may be explained by factors such as genetic background, environment or position of the seed within the plant.

When including genotypes which expressed the X peak, lower correlations of 0.67 (322 df) and 0.26 (320 df) were obtained between spectrophotometry and HPLC results in genotypes D and F, respectively. From a breeder's point of view, these plants carrying the X peak would be discarded by the rapid method, whereas HPLC would have them selected.

In conclusion, a rapid spectrophotometric method using a two-seed sample is recommended in faba bean breeding programs which incorporate the *vc'* gene to reduce vicine and convicine contents. In a few genotypes, an error may come from an additional unknown substance absorbing at 274 nm which would lead to the elimination of otherwise acceptable plants. This error is small in comparison with the economy realized when changing from HPLC to the spectrophotometric method. It is possible that the unknown substance corresponding to the X peak also has an antinutritional effect and should be eliminated anyway. This progress should hasten the production of new cultivars free of antinutritional factors for human and animal consumption.

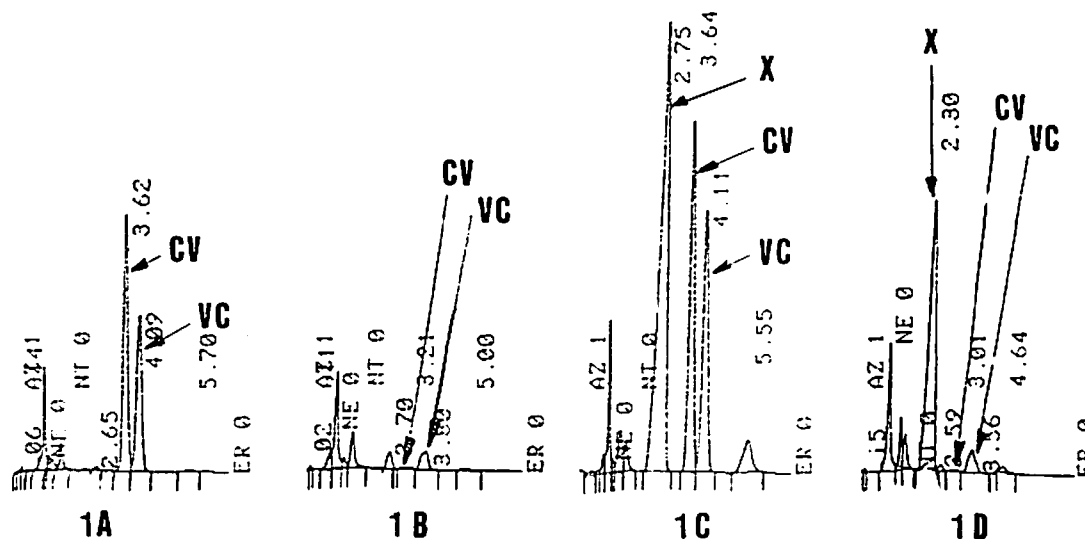


Figure 1. HPLC separation at 274 nm of vicine (VC) and convicine (CV) from (1A) genotype D (vicine + convicine = 1.1 % of seed DM), and (1B) genotype F (vicine + convicine = 0.04% of seed DM). In a few plants, an additional slower-migrating peak (X) was present in genotypes D (1C) and F (1D).

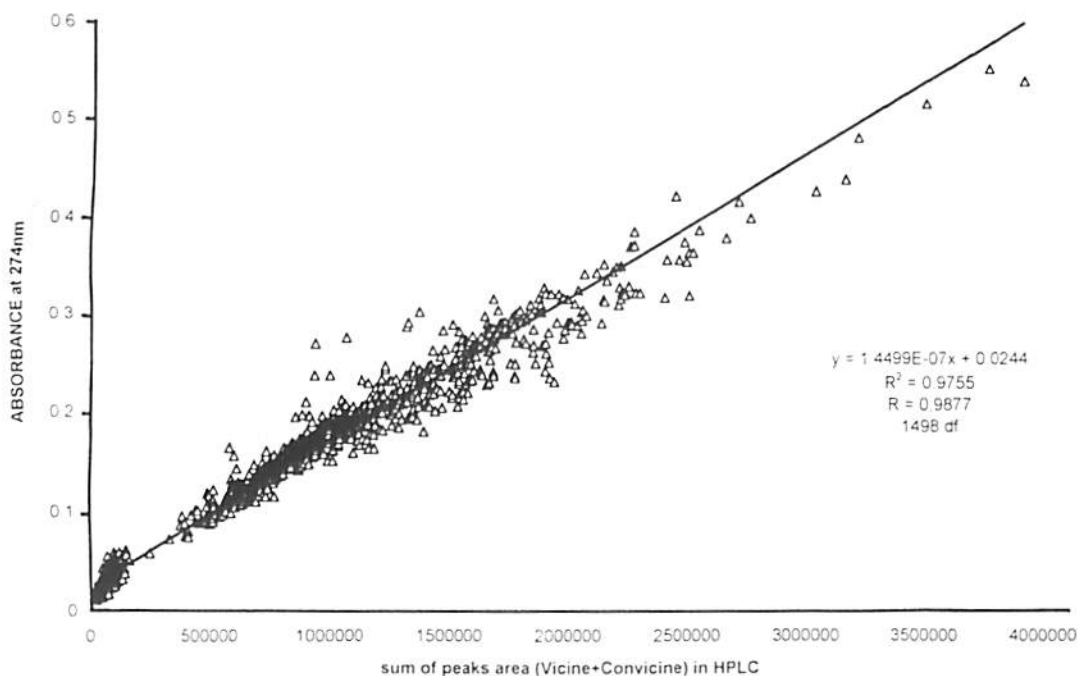


Figure 2. Correlation between spectrophotometry (274 nm) and HPLC determination (276 nm) of vicine + convicine contents on whole-seed extracts.

References

- Duc, G., G. Sixdenier, M. Lila and V. Furtoss. 1989. Search of genetic variability for vicine and convicine content in *Vicia faba* L. A first report of a gene which codes for nearly zero-vicine and zero-convicine contents. Page 305-313 in *Recent Advances of Research in Antinutritional Factors in Legume Seeds* (J. Huisman, A.F.B. van der Poel and I.E. Liener, ed.). Pudoc, Wageningen.
- Hegazy, M.I. and R.R. Marquardt. 1983. Development of a simple procedure for the complete extraction of vicine and convicine from faba beans. *Journal of the Science of Food and Agriculture* 34: 100-108.
- Lattanzio, V., V.V. Bianco and D. Lafiandra. 1982. High performance reversed-phase liquid chromatography (HPLC) of favism-inducing factors in *Vicia faba* L. *Experientia* 38: 789-790.
- Mager, J., G. Glaser, A. Razin, G. Izak, S. Bien and M. Noam. 1965. Metabolic effects of pyrimidines derived from faba bean glycosides on human erythrocytes deficient in glucose-6-phosphate dehydrogenase. *Biochemical and Biophysical Research Communications* 29: 235-240.
- Marquardt, R.R. and A.A. Fröhlich. 1981. Rapid reverse-phase high performance liquid chromatographic method for the quantitation of vicine, convicine and related compounds. *Journal of Chromatography* 208: 373-379.
- Olabro, G., R.R. Marquardt, L.D. Campbell and A.A. Fröhlich. 1981. Isolation of the egg weight depressing factor in faba beans (*Vicia faba* L. *minor*). *Journal of the Science of Food and Agriculture* 32: 1074-1079.
- Pitz, W.J. and F.W. Sosulski. 1979. Determination of vicine and convicine in Faba bean cultivars by gas-liquid chromatography. *Canadian Institute of Food Science and Technology Journal* 12: 93-97.
- Quemener B. 1988. Improvements in the high-pressure liquid chromatographic determination of amino sugars and alpha-galactosides in faba bean, lupine, and pea. *Journal of Agricultural and Food Chemistry* 36: 754-759.
- Quemener, B., J. Gueguen, C. Mercier. 1982. Determination of vicine and convicine by high-pressure liquid chromatography. *Canadian Institute of Food Science Technology Journal* 15: 109-115.
- Ramsay, G. and D.W. Griffiths. 1992. A rapid method for screening green and mature seed of *Vicia faba* for variation in vicine and convicine content. Pages 419-420 in *Proceedings of 1st European Conference on Grain Legumes* (AEP, ed.).
- Ramsey, G. and D.W. Griffiths. 1993. The genetics of vicine and convicine synthesis in faba bean. Pages 397-400 in *Recent Advances of Research in Antinutritional Factors in Legume Seeds* (A.F.B. van der Poel, J. Huisman and H.S. Saini, ed.). Wageningen Pers.

Variety Release Notice

Qinghai 9, A New Spring-sown Faba Bean Cultivar with Large Seeds and High Yield in China

Yuan Mingyi, Shen Haining, Zhang Peilan and Liu Yang

The Crop Research Institute

The Qinghai Academy of Agricultural and Forestry Sciences

Xingning, CHINA

Key words: *Vicia faba*; faba beans; varieties; seed size; yields; protein content; maturity; disease resistance; *Botrytis fabae*; *Ascochyta fabae*; spring; sowing date; planting date; China.

Faba bean (*Vicia faba* L.) cultivar Qinghai 9 was developed by hybridizing Lhasa 1 (female parent) with 176 from Great Britain (male parent) through many generation selections by the Crop Research Institute of the Qinghai Academy of Agricultural and Forestry Sciences (QAAF). Qinghai 9 was examined and approved as an extension cultivar by the Qinghai Crop Cultivar Identification Commission on 29 November 1994.

Qinghai 9 is characterized by high yield, large seed and high protein content compared with the check cultivar Qinghai 3. In 1994, the average seed yield of Qinghai 9 was 4923 kg/ha in the multilocation tests – 24.9% higher than that of Qinghai 3. The maximum yield recorded was 7905 kg/ha at Qiugo, Qinghai in 1995. The average 100-seed weight is 174 g, which is higher than that of Qinghai 3 (162 g). The time from planting until 95% of plants matured was 170 days. The protein content of the seed is 32.7%. Qinghai 9 is resistant to chocolate spot (*Botrytis fabae* Sardina) and ascochyta blight (*Ascochyta fabae* Speg. [= *Didymella fabae* Jellis & Punith.]). Qinghai 9 can be planted in the spring-sowing region in China. Its agronomic traits are shown in Table 1.

غينغاي 9 : صنف فول ربيعي جديد كبير الحبة يتمتع بغلة عالية في الصين

أُستنبط صنف الفول Qinghai 9 بواسطة تهجين 1 Lhasa (أب أنثى) بـ 176 (أب نكر) من بريطانيا العظمى من خلال عدة انتخابات لأجيال عديدة قام بها معهد بحوث المحاصيل في أكاديمية غينغاي للعلوم الزراعية والحراجية (QAAF). وتمت دراسة غينغاي 9 والموافقة عليه من قبل لجنة اعتماد الأصناف في غينغاي كصنف إرشادي في 29 تشرين الثاني/نوفمبر 1994.

ويتميز الصنف غينغاي 9 بارتفاع غلته وبذوره الكبيرة ومحتوى البروتين العالي فيه بالمقارنة مع الصنف الشاهد غينغاي 3. وفي عام 1994، بلغ متوسط غلة بذور الصنف غينغاي 9 ما يعادل 4923 كغ/هـ في الاختبارات المتعددة المواقع – أي أعلى بـ 24.9% من غينغاي 3. وكانت أعلى غلة مسجلة 7905 كغ/هـ في كيوغو، غينغاي في 1995، وبلغ معدل وزن المائة بذرة 174 غ أي أعلى مما هو في غينغاي 3 (162 غ).

وكانت الفترة من الزراعة وحتى نضج النباتات بنسبة 95%، 170 يوماً. وكان محتوى البذور من البروتين 32.7%. ويُعد غينغاي 9 مقاوماً للتبقع البني (*Botrytis fabae* Sardina) والتبقع الاسكوكايتي (*Ascochyta fabae* Speg. [= *Didymella fabae* Jellis & Punith.]). ويمكن زراعة غينغاي 9 في المنطقة التي تزرع فيها المحاصيل في الربيع في الصين.

Table 1. Agronomic traits of Qinghai 9, compared with check cultivar Qinghai 3.

Cultivar	Plant height (cm)	No. pod-bearing branches	Pods/plant	Seeds/plant	100-seed weight (g)	Seed yield		Protein content (%)
						(kg/ha)	(% of check)	
Qinghai 9	155	3.5	18.2	45.6	174.2	4923	124.9	32.2
Qinghai 3	150	3.2	15.7	39.2	162.0	3941.5	100	24.0

News

Editors' Notes

Publication schedule

The publication of *FABIS Newsletter* has been experiencing delays over the past few years. This has been due in part to a lack of material. The Faba Bean Information Service and

ICARDA have, therefore, decided to reduce the production of *FABIS Newsletter* to one issue per year, starting in 1997. We expect that each issue will appear toward the end of the year of publication. Thus, the next issue (No. 40, 1997) is scheduled for publication late in 1997.

Agricultural Libraries Receiving ICARDA Publications

ICARDA publications are deposited in agricultural libraries throughout the world to make them available to other users under normal interlibrary loan and photocopy procedures. These depository libraries are located in the countries listed. Readers requiring information on the library nearest to them should address inquiries to: Library, ICARDA, P.O. Box 5466, Aleppo, Syria.

Algeria	Ghana	Philippines
Bahamas	Guatemala	Saint Lucia
Bahrain	Guyana	Saudi Arabia
Bangladesh	India	Senegal
Benin	Iran	Somalia
Belgium	Italy	Spain
Bhutan	Kenya	Sri Lanka
Botswana	Korea (Republic)	Sudan
Brazil	Lesotho	Swaziland
Canada	Malawi	Syria
Chile	Malaysia	Taiwan
China	Mali	Tanzania
Costa Rica	Mauritania	Thailand
Cyprus	Mexico	Tunisia
Djibouti	Myanmar	United Kingdom
Ecuador	Netherlands	United Arab Emirates
Ethiopia	Nepal	USA
Fiji	Nigeria	Yemen
Finland	Norway	Zambia
France	Papua New Guinea	Zimbabwe

Contributors' Style Guide

FABIS Newsletter publishes the results of recent research on faba bean and other *Vicia* and *Lathyrus* legumes, in English with Arabic abstracts. Articles should be brief, confined to a single subject and be of primary interest to researchers, extension workers, producers, administrators and policy-makers. Articles submitted to FABIS should not be published or submitted to other journals or newsletters.

The views expressed and the results presented in *FABIS Newsletter* are those of the author(s) and not the responsibility of ICARDA. Similarly, the use of trade names does not constitute endorsement of or discrimination against any product by ICARDA.

Manuscript

Contributions should be sent to FABIS/CODIS, ICARDA, P.O. Box 5466, Aleppo, Syria. The name, address, e-mail address (if available), and telex or fax number of the corresponding author should be included in the covering letter. One good-quality original of the text should be submitted, typed double-spaced on one side of the paper only. Alternatively, word-processed files in WordPerfect 5 or 6 or Microsoft Word 6.0 may be sent as e-mail attachment to ICARDA@cnet.com, marked "For FABIS Newsletter." However, there is a size restriction of 128 kb on incoming e-mail to ICARDA – please discuss in advance if you have any doubts. Figures should be original drawings, good-quality laser prints, or black-and-white photographs of good quality. Photographs and figures should be suitable for reduction to a printed size of 8.5 or 17.4 cm wide. Photocopies are not acceptable for publication in *FABIS Newsletter*.

All articles must have an abstract (maximum 250 words) and usually the following sections: Introduction, Material and Methods, Results, Discussion, Conclusions and References. Articles will be edited to maintain uniform style, but substantial editing will be referred to the author(s) for approval. Papers requiring extensive revision will be returned to the author(s) for correction. Authors can refer to a recent issue of *FABIS Newsletter* for format. The following guidelines should be followed:

Include the authority name at the first mention of scientific names.

Present measurements in metric units, e.g. t/ha, kg, g, m, km, ml, L. Where other units are used (e.g. quintal), the metric equivalent should be provided in parentheses.

Define in footnotes or legends any unusual abbreviations or symbols used in the text or figures.

Provide the full name of journals and book titles. Use the following formats for references.

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.

ICARDA Publications and Services

ICARDA Publications

Request a list of all currently available publications from the Communication, Documentation and Information Services (CODIS).

LENS Newsletter

The newsletter of the Lentil Experimental News Service, is produced twice a year at ICARDA in cooperation with the University of Saskatchewan, Canada. Short research articles provide rapid information exchange, and comprehensive reviews are invited regularly on specific areas of lentil research. The newsletter is available free to lentil researchers. An annual supplement to the newsletter contains lentil references, previously issued in *Lentil in AGRIS*. For further information or to subscribe, write to: LENS/CODIS.

Rachis (Barley and Wheat Newsletter)

This publication is aimed at cereal researchers in the Near East and North Africa region and other Mediterranean-type environments. It publishes short scientific papers on the latest research results and news items. *Rachis* seeks to contribute to improved barley and wheat production in the region; to report results, achievements and new ideas; and to discuss research problems. For further information or to subscribe, write to: Rachis/CODIS.

Graduate Research Training Awards, Opportunities for Field Research at ICARDA

The Graduate Research Training Program (GRTP) is intended primarily to assist Master of Science

candidates who are enrolled at national universities within the ICARDA region. Men and women who are selected for the program will have an opportunity to conduct their thesis research work at ICARDA research sites under the co-supervision of university and center scientists. For further information on terms of award, nomination procedure, selection criteria, appointment conditions, the university's responsibilities, and the student's responsibilities, write to: GRT Program, Training Coordination Unit.

Opportunities for Training and Post-Graduate Research at ICARDA

ICARDA has active training courses on the development and improvement of food legumes, cereals and forages with ICARDA's research scientists, trained instructors, and proven programs. For a complete brochure of the training opportunities at ICARDA, write to: Training Coordination Unit.

Library Services

The ICARDA library maintains bibliographic databases for the use of researchers at the center and elsewhere. FABIS and LENS databases contain 6500 and 2800 references, respectively, extracted from AGRIS since 1975 and AGRICOLA since 1970. Literature searches can be conducted by the library staff and results downloaded to diskette or hard copy. Photocopies of up to 5 articles per search can be provided to users, if available. Researchers can request a literature search by letter or telex to: The Manager, LIS.

To obtain further information on these services, please write to the program indicated and state that you saw the advertisement in *FABIS Newsletter*:

International Center for Agricultural Research in the Dry Areas

P.O. Box 5466, Aleppo, Syria

Tel. +963-21-213477, 225112, 235221

Fax +963-21-213490, 225105, 744622

Telex (492) 331208, 331263, 331206 ICARDA SY

E-mail ICARDA@cgnet.com