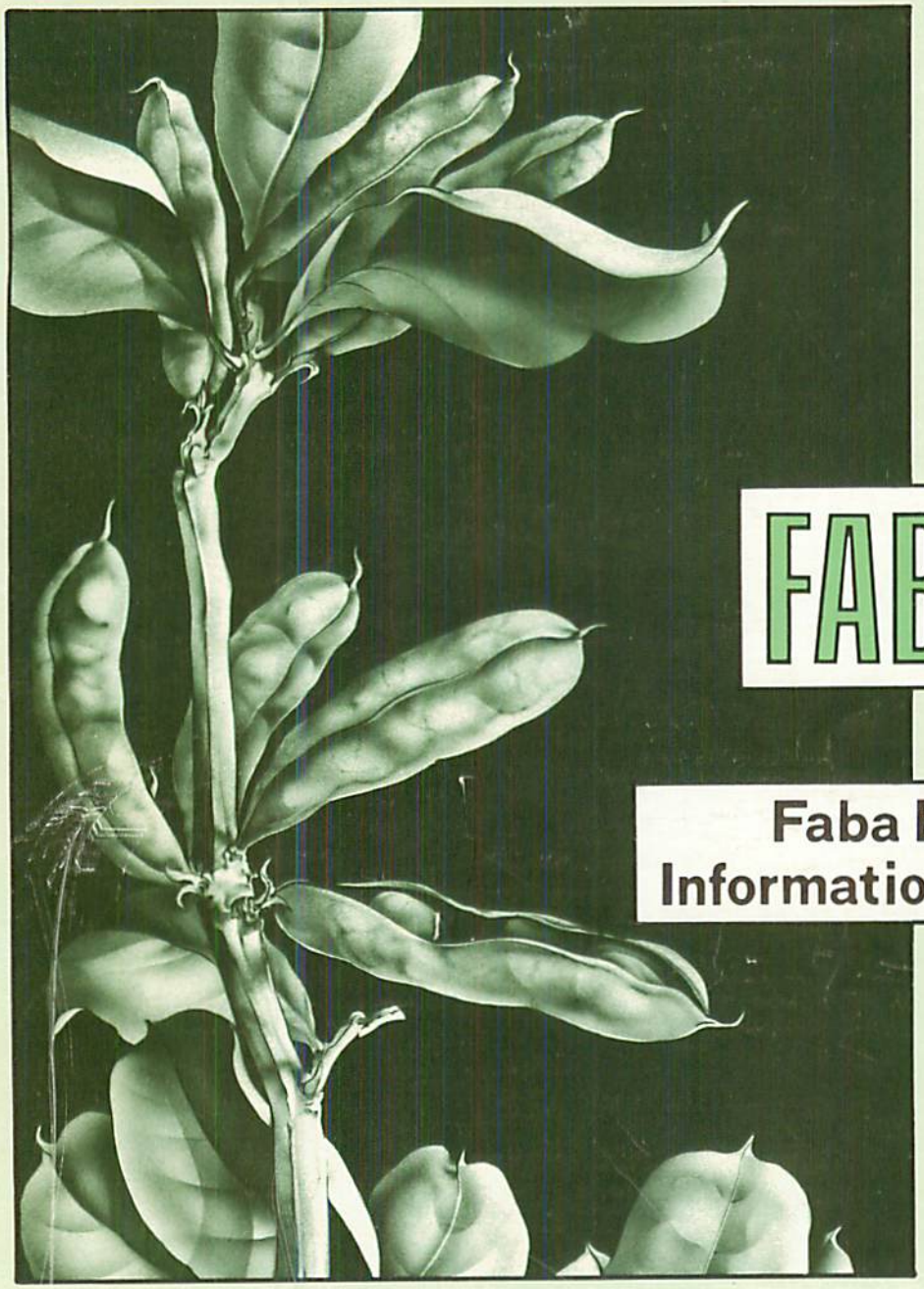

FABIS

Faba Bean Information Service

NEWSLETTER No. 34/35
January—December 1994

INTERNATIONAL CENTER FOR AGRICULTURAL RESEARCH IN THE DRY AREAS

(ICARDA)

ICARDA and CGIAR

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FABIS

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

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ISSN 0255 6448

FABIS

Faba Bean Information Service

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Breeding and Genetics

Need For Standardization in Faba Bean Gene Mapping

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Abstract

Up to 1990, few loci had been mapped of the *Vicia faba* L. genome. Since then, a lack of standardization in approach has led to a series of reports which are not easily comparable. It is proposed that chromosome designation follow convention in that the longest is termed 1 and the shortest 6. Roman numerals should then be used for linkage groups, as is done in other species of Vicieae. Gene symbols for morphological traits have been proposed by ICARDA and should be followed; however, some clarificatory research is required to show which symbols proposed to date are synonyms. Little work has been done on the isozyme loci of *V. faba* and it is proposed that the nomenclatural system of the International Union of Biochemists be followed in future designations. This is the basis used in other pulses and will help in the establishment of inter-generic homologies. Mapping with computer programs needs to be reported with *statistical data* to aid inter-study comparisons.

Key words: *Vicia faba*; faba beans; genetic maps; loci; Croatia; Spain.

Introduction

Prior to 1990 few morphological and isozyme loci had been mapped in the *Vicia faba* genome, and no extended linkage groups had been recognized. More recent studies dealing with faba bean mapping have been carried out by different research teams with the aim of extending the preliminary map of the species. Unfortunately, it has not always been possible to establish comparisons among results from different studies since designation of genes has not been uniform. In an attempt to simplify future

الحاجة إلى توحيد الخرائط الوراثية للفول

الملخص

حتى عام 1990، وضعت خرائط لقليل من المراكز الجينية في المجموعة الجينية للفول. ومنذ ذلك التاريخ، أدى الافتقار إلى أسلوب موحد إلى وجود سلسلة من التقارير التي يصعب تماثلها. ويُقترح اتباع الأسلوب التقليدي في التسميات الكروموزمية بحيث يطلق على أطولها 1 وأقصرها 6. ومن ثم يجب استخدام الأرقام الرومانية لمجموعات الارتباط كما هو الحال في الأنواع الأخرى من هذه العائلة (*Vicieae*). وقد اقترحت إيكاردا طريقة استعمال الرموز الجينية للصفات الشكلية (المورفولوجية) التي يجب اتباعها، غير أنه من المطلوب القيام ببعض الأبحاث التوضيحية لتبيان أي من الرموز المقترحة حتى يومنا هذا تعتبر بحكم المترادفات. كما أُنجزت بحوث متواضعة حول المراكز الجينية المتماثلة الإنزيم في الفول ويقترح أن يتبع نظام المصطلحات الخاص بالاتحاد الدولي للمشتغلين بالكيمياء العضوية في التسميات المستقبلية. وهذا هو الأساس المستخدم في البقوليات الحبية الأخرى وسيساعد في إيجاد تماثل فيما بين الاجناس. ويتطلب وضع خرائط بواسطة برامج حاسوبية تقديم بيانات إحصائية تمكن من إجراء مقارنات فيما بين الدراسات.

comparisons and to establish homologies among the Vicieae, this paper suggests some standard methods of presenting genetic information.

Systems of chromosome designation

Although chromosome identification has been accomplished in *Vicia faba* (Ockay 1957; Michaelis and Rieger 1959; Evans 1961; Sjödin 1971b), the designation of the six chromosomes often differs markedly among publications. The most extensive systems of chromosome designation were originally proposed by Michaelis and Rieger (1959) and Sjödin (1971b). These two systems differ in that chromosomes 4 and 5 are interchanged. Conventionally, chromosomes are numbered according to length, with chromosome 1 being the longest and chromosome 6 the shortest. Following this convention, the Michaelis and Rieger nomenclature would be the most appropriate. Moreover, since most of the recent articles dealing with or related to faba bean mapping (Martin and Baceló 1984; Cabrera and Martin 1989a,b; Cabrera et al. 1989; Torres et al. 1993, in press) have assigned new loci following the Michaelis and Rieger designation, we propose the use of this system in future studies. However, in order to simplify comparisons among the genera in the Vicieae, Arabic numerals (1 to 6) should be use to designate chromosomes and Roman numerals used for linkage groups (before being assigned to specific chromosomes). Since the same conventions have been developed in pea, lentil, chickpea, bean and soybean, it would facilitate the exchange of results within the Vicieae and avoid considerable confusion in the future.

Gene symbols for morphological characters

The choice regarding symbols for genes for morphological characters should be based on the comprehensive reviews published by ICARDA (1981, 1986). Nevertheless, an update of the list of assessed genetic variation in *Vicia faba* is urgently needed for the flower-color genes (*sdp, yf,* w_1, w_2), we recommend the system proposed by Cabrera (1988) that seems to give an improved explanation of the genetic bases of flower-color morphology. The previous nomenclature proposed by Sjödin (1971a) fails to distinguish between all the phenotypes resulting from the combination of these four genes.

Cooperation and coordination among research teams is needed, particularly concerning exchange of plant material – not only parental lines, but crosses or, still better, Recombinant Inbred Lines (RILs). This is the only way to determine whether different plant materials possess different alleles or different genes producing similar phenotypes, and to provide an adequate overlap among different linkage groups established by different laboratories.

Isozyme loci assignation

In comparison with related genera, few isozyme markers have been mapped in the *Vicia faba* genome (Van de Ven et al. 1991; Torres et al. 1993, in press). A more detailed map might be defined by studying additional allozymic variants in wider faba bean crosses. The correct identification of new polymorphic isozymes as well as the use of a standard nomenclature will be required in the future. The isozyme loci should be named following the International Union of Biochemists (IUB 1984) nomenclature as suggested by Weeden (1988). An approach that has proved productive within pulse crops is the comparison of linkage relations among the isozyme loci in each genus. The linkage map developed for pea and lentil has shown two clear parallels with some isozyme systems in *Vicia faba* (Z. Satovic, A.M. Torres, J. Cànovas and J.I. Cubero, unpublished data). This homologous relation among genes could be used in future studies to predict new linkages in *Vicia*, *Lathyrus* and related genera. Nevertheless, in order to establish homologies between isozyme loci and to facilitate future comparisons between species, it would be useful to perform (if possible) subcellular localization studies of different isozymes. Although some authors have tried to generalize the data obtained, neither the degree of polymorphism nor the relative mobility are reliable parameters for determining subcellular location (Weeden and Wendel 1989). However, the method of subcellular localization proposed by Weeden and Gottieb (1980a,b) seems to be simple and does not cause additional effort in the analysis.

RFLP and RAPD markers

Recently developed molecular markers such as RFLPs and RAPDs have excellent potential as tools for gene mapping (Tanksley et al. 1989; Helentjaris 1989). Faba bean has been the focus of relatively little research in this field and few papers dealing with genetic mapping using RFLPs or RAPDs have been published (Van de Ven et al. 1991; Torres et al. 1993).

It is appropriate to underline the importance of probes which encode rRNA gene-repeats localized on the satellite of the chromosome bearing the nucleolus organizer. As

the nucleolus-organizer region in *Vicia faba* is situated on chromosome 1, the above-mentioned probes used as RFLP markers might facilitate the localization of syntenic marker loci as shown by Van de. Ven et al. (1991) and Torres et al. (1993).

Since homologies among RAPD markers studied in different material cannot be unequivocally established, the results of RAPD-marker gene-mapping have restricted value. Nevertheless, the transformation of RAPD markers of interest into SCARs (Sequence Characterized Amplified Regions) can easily resolve this problem: SCARs detect only a single locus and, moreover, their amplification is less sensitive to reaction conditions and they can potentially be converted into codominant markers (Paran and Michelmore 1993).

Statistical methods to detect linkage

The majority of recent linkage studies have been performed by using computer programs such as LINKAGE-1 (Suiter et al. 1983) and MAPMAKER (Lander et al. 1987). While LINKAGE-1 justifities a linkage relationship when the obtained χ^2 of the contingency table proves to be significant, the MAPMAKER linkage decision-making depends on the critical value of LOD established. The use of a critical value for the LOD score cannot be justified in the same manner because it is not connected with a type-I error. The thresholds (LOD_t) chosen by different authors dealing with plant material are normally between the values of 2 and 3 LOD score (Landry and Hubert 1991; Messeguer et al. 1991; Ellis et al. 1992; Devos et al. 1992; Bentiolila et al. 1992). As the LOD is asymptotically distributed as:

$$LOD_r = ½ (\log_e).\chi^2$$

Where χ^2 denotes the χ^2 distribution with 1 d.f. (Morton et al. 1986; Lander and Botstein 1989). An LOD value of 2 roughly corresponds to a χ^2 value of 9.21 with 1 d.f. and LOD 3 corresponds to a χ^2 of 13.81. The use of the value of 2 or 3 LOD score as a threshold is a much more conservative way of detecting linkage than the threshold of χ^2 of 3.84, which with 1 d.f. corresponds to a type-I error, $P<0.05$. Nevertheless, the choice of the LOD threshold (as well as the corresponding type-I error) largely depends on the number of markers screened, the type of material, and the purpose of the study.

If LINKAGE-1 is used, it would be helpful for future comparisons to indicate the value of recombination fraction and the standard error, as well as the amount of information provided by the family analyzed. On the other hand, when using MAPMAKER it would be practical to report the recombination fraction and the LOD score of each pair of markers where the linkage relationship is detected. When more than two loci are in the same linkage group, the recombination fraction before adjustment and a corresponding LOD score between each pair of loci involved would be more informative for future comparisons than the genetic distance in cM obtained by final adjustment using multipoint analysis.

The Kosambi mapping function seems to be prefered over Haldane by many authors (Landry and Hubert 1991; Devos et al. 1992; Timmerman et al. 1993; Menanico-Hautea et al. 1993), as it has given good results in a variety of organisms.

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Outcrossing Rate in Natural Field Varieties of *Vicia faba*

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Abstract

Outcrossing rates were estimated for four varieties of *Vicia faba* L. differing in floral characteristics. Four allozymic systems were assayed. Single locus and multilocus outcrossing rates were estimated for each variety by using the mixed mating model. Multilocus outcrossing estimates ranged from 0.40 to 0.68 among varieties. This variation does not appear to be related to either floral phenology or number of flowers per inflorescence. Sowing date does not seem to play an important role in the outcrossing rates either.

Key words: *Vicia faba*; faba beans; outcrossing; allozymes; loci; Spain.

Introduction

Faba bean is a partially cross-pollinated crop. The range in cross-pollination estimates is from almost complete selfing to very high outcrossing rates (4–89%, Bond and Poulsen 1983). These large differences apparently depend on genetic and environmental factors, as well as on the different methods and markers used for estimation. However, there is no conclusive result about the relative importance of the different varietal traits and environmental factors affecting outcrossing rate. An understanding of the mating system of a species is of fundamental importance to breeding and germplasm-maintenance activities.

The present work was undertaken to study the natural field levels of outcrossing of *Vicia faba* varieties differing in floral characteristics in two sowing dates by the use of isozyme markers and a multilocus model in southern Spain. Given the lack of conclusive studies in this field, our study is exploratory in the sense of studying several variables in order to eliminate, in a second step, the unimportant ones.

معدل التهجين الخلطي في أصناف فول *Vicia faba* مزروعة في حقول طبيعية

الملخص

تم تقدير معدلات التهجين الخلطي في أربعة أصناف من الفول مختلفة في خصائصها الزهرية. اختُبرت أربعة نظم خلطية الأنزيم. وقد حُسبت معدلات التهجين الخلطي الوحيد المركزالجيني والمتعدد المراكز الجينية لكل صنف باستخدام نموذج التزاوج المختلط. تراوحت تقديرات التهجين الخلطي المتعدد المراكز الجينية من 0.40 إلى 0.68 بين الأصناف. وإن هذا التباين - كما يبدو - لاعلاقة له بشكل الأزهار أو بعددها في كل نورة (عنقود زهري). كما يبدو أن موعد الزراعة لم يلعب دوراً هاماً في معدلات التهجين الخلطي.

Materials and Methods

We used four varieties reproduced under open-pollination conditions. Two of them were typical Spanish cultivars ('Alameda' and 'Brocal'); the others, 'Econa' and 'Coci', were chosen because they present different floral characteristics.

Estimates of outcrossing rates were obtained from two sowing dates (early and late) for Alameda and Brocal. However, data from all four cultivars were only obtained from the early sowing date, since Econa and Coci were so late in maturing that there were not enough seeds harvested from the late sowing.

Sixty plants were scored in each variety for three quantitative floral traits (number of days to flowering, time from flowering to maturity, and number of flowers/ inflorescence). Plots of 410 plants/row and 20 rows were sown for each variety. At harvest, plants of two rows from the center of each plot were collected.

Nine seeds/family and about 50 individual plants were assayed and scored for eight allozyme loci. Only three or four loci, depending on the variety, were polymorphic. The mode of inheritance and lack of linkage of the allozyme loci was established by Suso et al. (1993). The loci used were: superoxide dismutase (*Sod-1*, *Sod-2*, *Sod-3*), shikimate dehydrogenase (*Skd*), phosphoglucomutase

(*Pgm-1*), and 6 phosphogluconate dehydrogenase (*6Pgd-2*). The 6PGD has not been described before; it is controlled by two loci, but only one of them (*6Pgd-2*) has been scored in this work because of the very low genetic variability observed in *6Pgd-1*. In cases where there were three alleles, one of them being very infrequent, the data were reduced to two alleles by pooling the least frequent allele with the second. The material and methods used for allozyme assays have already been described (Gates and Boulter 1979; Suso et al. 1993).

Estimates of single and multilocus outcrossing rate were calculated by using MLT (Ritland 1990), a program that is based on the multilocus model of Ritland and Jain (1981). Standard errors of the t estimates were calculated by the bootstrap method. Each estimate of the standard error was based on 100 bootstraps. The goodness of fit of the data to the assumptions of the mixed mating model was evaluated by a Chi-square test. To test for heterogeneity of outcrossing rates for independent loci, we used the Neyman-Pearson likelihood ratio criterium (Rao 1973).

Results and Discussion

Chi-square tests of the goodness of fit of genotypic data were not significant for either the single or multilocus outcrossing estimates, suggesting that the data fit the assumptions of the mixed mating model.

Floral characteristics, sowing date and varietal variation of single, multilocus and average single-locus outcrossing rates and their standard error are given in Table 1. No significant heterogeneity among single-locus estimates was detected within varieties.

Multilocus outcrossing estimates varied from 0.40 to 0.68 between varieties, Coci showing the lowest and Alameda and Brocal the highest. Our results are similar to those of Xanthopoulos et al. (1986) in Greece, lending further evidence that the outcrossing rate in the Mediterranean region is higher than previously thought.

An important feature of the results is the stability of the estimates of both local varieties over the two sowing dates. Thus, the outcrossing rate in *Vicia faba* appears to be a stable intermediate value, in contrast to other studies (Bond and Poulsen 1983) that reported variation in cross-pollination associated with environmental parameters.

Although there are significant differences in all the varieties for all the quantitative traits, there was no evidence of any relationship between either the floral phenology or the number of flowers/inflorescence and the outcrossing rate.

Studies of factors affecting natural crossing rates cover a wide range of topics. It has been suggested (Bond and Poulsen 1983) that factors such as location, plant density and position of inflorescence can influence the amount of crossing. McVetty and Nugent-Rigby (1984) found that the extent of natural cross-pollination differed for varieties, locations and years. Link (1990) showed that autofertility was negatively correlated with cross-fertilization. Stoddard (1986) found that lush foliage, lack of top growth, floral size and color neither enhanced nor hindered bee visitation. Very few studies, however, have examined the interaction among flowering phenology, inflorescence size and outcrossing rate. The question to be considered in this context is whether a shift in the floral phenology or in the size of the inflorescence can modify the outcrossing rate in *Vicia faba*. Our results suggest that it will not; neither floral phenology nor the number of flowers/inflorescence were important in affecting the variation in outcrossing rate. The amount of outcrossing in this species seems to be determined by other factors.

Further studies are needed to elucidate the mechanisms responsible for the faba bean mating system.

Acknowledgement

We are very grateful to Dr Ritland who supplied us with his Fortran program.

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Table 1. Floral characteristics, sowing date and varietal variation of single, multilocus and average single-locus outcrossing rate for *Vicia faba* varieties.

Variety	Days to flowering	Days to maturity	Flowers/ inflorescence	Sowing date†	Locus	t
Coci	56.58	38.90	6.54	1	Sod-1	0.45 (0.08)
	(6.44)‡	(6.14)	(1.09)		Skd	0.54 (0.09)
					Pgm	0.26 (0.011)
					Average	0.39 (0.06)
					Multilocus	0.40 (0.06)
Brocal	35.63	38.38	3.84	1	Sod-1	0.61 (0.06)
	(4.02)	(3.95)	(0.6)		Sod-2	0.66 (0.06)
					Skd	0.78 (0.07)
					Average	0.66 (0.05)
					Multilocus	0.66 (0.05)
Brocal	54.95	32.64	4.55	2	Sod-1	0.60 (0.09)
	(2.30)	(3.08)	(0.6)		Sod-2	0.59 (0.12)
					Skd	0.65 (0.09)
					Average	0.63 (0.07)
					Multilocus	0.67 (0.06)
Alameda	27.59	46.19	4.30	1	Sod-1	0.60 (0.06)
	(3.61)	(4.68)	(0.6)		Sod-3	0.51 (0.19)
					Skd	0.73 (0.08)
					6Pgd-2	0.78 (0.17)
					Average	0.63 (0.04)
					Multilocus	0.65 (0.04)
Alameda	56.08	28.40	4.47	2	Sod-1	0.59 (0.03)
	(2.07)	(3.53)	0.06		Sod-3	0.68 (0.13)
					Skd	0.78 (0.05)
					6Pgd-2	0.87 (0.29)
					Average	0.68 (0.03)
					Multilocus	0.68 (0.03)
Econa	57.25	35.48	7.45	1	Sod-1	0.45 (0.06)
	(6.97)	(6.76)	(1.84)		Sod-2	0.63 (0.21)
					Skd	0.62 (0.11)
					Average	0.50 (0.06)
					Multilocus	0.49 (0.05)

† 1 = early; 2 = late.

‡ Standard errors in parentheses.

Gene Transfer in Faba Bean by Honeybees under Cages

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Abstract

Honeybess (*Apis mellifera* L.) were used to hybridize faba bean (*Vicia faba* L.) under screened cages. Gene transfer from inbred lines and mass-selected populations with resistance to either rust or ascochyta blight into agronomically superior but susceptible cultivars was assessed over four years. Low rates of gene transfer (0 to 40%) were obtained in the first three years. However, more careful management of the bees under moderate temperature and normal precipitation during the flowering period in 1986 increased the rate of gene transfer to 40–67%.

Key words: *Vicia faba*; faba beans; gene transfer; honeybees; hybridization; flowering; Canada.

Introduction

Faba bean is a self-pollinated crop with significant levels of cross-pollination that vary greatly between cultivars and accessions originating from different regions of the world (Picard 1953). Selfing in faba bean is associated with inbreeding depression and a reduction in autofertility (Holden and Bond 1960), while cross-pollination and heterosis are associated with hybrid vigor and improved seed yield (Muehlbauer et al. 1988; Bond 1989). Bond and Poulsen (1983) summarized many studies on outcrossing in faba bean and indicated that the rate of outcrossing ranged from 4 to 84% depending on the genotypes used, the experimental methodology and the number of insect pollinators. Cross-pollination values of 15 and 23% are reported between and within faba bean plots under field conditions in northern Syria (Hawtin and Omar 1980; Omar and Hawtin 1981) and of 0 to 47% under field conditions in northern India (Singh 1984). A cross-fertility rate of 16–46% was obtained when different parental lines with genetic markers were grown close together, and the highest rate was obtained when lines were grown in alternate rows 20 cm apart (Gottschalk 1978). Filippetti and De Pace (1982) present a summary of the range of outcrossing in faba bean under different environmental conditions in southern Italy and report that the rate of outcrossing averaged 18%. However, levels of outcrossing as high as 60 and 70% have been reported (Holden and Bond 1960; Poulsen 1975). McVetty and Nugent-Rigby (1984) report a range of 27–58% cross-pollination with significant differences among cultivars and between years and locations of experiments. More recently, Robertson and Cardona (1986) found that bee activity was reduced in faba bean plots interplanted with plots of either *Brassica* or triticale, but that this did not affect the outcrossing rate, which ranged from 9 to 11%.

The frequency of cross-pollination is influenced mainly by the number of pollinating insects, especially honeybees, present during the flowering period. The use of Saran screen cages to confine bees during the peak flowering period (Nassib et al. 1979) resulted in a cross-pollination rate of 79.9% in Egypt. The efficiency of the bees was increased by 50 to 60% when new broods were introduced into the cages at intervals of 10–15 days in *order to have* workers better adapted to working under confinement. In a recent study, Currie et al. (1990) reported that honeybees are more effective than leafcutter bees in pollinating faba bean in caged plots, and that the bees moved more frequently between rows of different cultivars than between rows of the same cultivar.

انتقال المورثات في الفول المزروع تحت أقفاص بواسطة النحل

الملخص

استخدم نحل العسل (*Apis mellifera* L.) في تهجين الفول (*Vicia Faba* L.) المزروع تحت أقفاص غربلة. تم تقييم انتقال المورثات من السلالات ذاتية التربية والعشائر المنتخبة بالجملة التي تتمتع بمقاومة الصدأ والتبقع الاسكوكيتي، إلى أصناف متفوقة زراعياً ولكنها حساسة وذلك على مدى أربع سنوات. تم الحصول على معدلات متدنية لانتقال المورثات (0 إلى 40٪) في السنوات الثلاث الأولى. غير أن إدارة النحل بصورة أفضل تحت درجات حرارة معتدلة وهطل عادي خلال طور الإزهار في 1986 رفعت من معدلات انتقال المورثات إلى 40–67٪.

Recent studies on genetics of rust (*Uromyces fabae* (Pers.) de Bary) resistance in inbred lines of faba bean identified a total of eight resistance genes (Conner and Bernier 1982; Rashid and Bernier 1986). Resistance to ascochyta blight (*Ascochyta fabae* Speg.) was conditioned by seven resistance genes identified in several inbred lines (Rashid et al. 1991b). Available resistance genes could act as genetic markers to study the efficiency of cross-pollination by bees and the possibility of using this system to generate populations with multiple disease resistance.

The objectives of this study were to assess and improve the efficiency of honeybees in cross-pollinating faba bean under cages using inbred lines and mass-selected populations (MSP) with resistance to individual pathogens as markers, and to transfer the resistance genes to susceptible commercial cultivars in an effort to develop cultivars with multiple disease resistance.

Materials and Methods

Field experiments were conducted at the University of Manitoba Campus Farm, Winnipeg, Manitoba, during the period 1983–86. Two inbred lines with specific genes for resistance (marker genes) were used: ILB938 resistant to two races of rust, and 14434-2 resistant to two isolates of ascochyta blight (Rashid and Bernier 1984, 1986; Rashid et al. 1991a, 1991b). A mass-selected population (MSP), 75-152, with resistance to one race of *A. fabae* was also used. Resistance is a dominant trait in these rust and ascochyta parental lines (Rashid and Bernier 1986; Rashid et al. 1991b). The cultivars Aladin, Pegasus, 80FUM1 and 80FUM3 were used as the agronomically superior but susceptible parents (Table 1). Alternate rows of one cultivar and one inbred line or MSP with resistance genes were planted in small field plots of 1.5 × 2.5 m or 4 × 6 m.

In 1983, the plots were covered shortly before flowering with six small Saran cages (0.5 m wide × 2.5 m long × 1 m high) and two large cages (4 × 6 × 2.5 m). The Saran cages used were of 2.0 mm mesh size. Only large cages were used in subsequent years. A small beehive of approximately 500 honeybees was introduced into each cage at the onset flowering. Prior to introducing the hives into the cages, the bees were allowed to forage on other crops at a distance of at least 10 km from faba bean fields in order not to carry faba bean pollen into the cages. The beehives were replaced by young broods once after two weeks in 1985 and four times at weekly intervals in 1986 in order to provide a high level of bee activity throughout the flowering period. The bees and the cages were removed at the end of flowering to speed up the maturity process. The seed was harvested from each row and the progenies from the susceptible cultivars were tested in the greenhouse with the specific races or isolates of the pathogens mentioned above. Testing was done at the seedling stage as described previously (Rashid and Bernier 1984; Rashid et al. 1991a) and plants were categorized as resistant or susceptible based on their reaction to each pathogen in comparison to the reaction of their resistant parental line which was always included in the testing. Severeal hundred plants were tested from each progeny.

The percentage of gene transfer was calculated as follows:

$$\text{gene transfer (\%)} = \frac{\text{No. resistant plants in progeny of susceptible cultivar} * 100}{\text{Total no. plants tested from progeny of susceptible cultivar}}$$

Every resistant plant from the progeny of the susceptible cultivars must be heterozygous for resistance, one allele having been transferred from the resistant parent as the result of outcrossing to the resistant lines by bee-pollination.

Results and Discussion

In 1983, gene transfer ranged from 0 to 40%. The low level of cross-pollination and gene transfer was attributed to the high rate of bee mortality, and the high aphid populations under the small cages. The use of large cages in subsequent years was less favorable for the build-up of aphid infestations, and more appropriate for the growth of faba bean and for honeybee activity. Notwithstanding these improvements, the use of a single honeybee hive during the flowering period in 1984 did not improve the percentage of gene transfer (26–31%) because of the high rate of bee mortality caused by abnormally high day temperatures (above 30°C) during the flowering period. Replacing the hives once during the flowering period in 1985 did not improve the rate of gene transfer either (29–31%) because of the high precipitation and wet conditions which reduced bee activity during the flowering period (Table 1). The day/night temperature and the amount and duration of precipitation during the growing season in the continental climate of the Canadian Prairies fluctuate greatly between seasons. These environmental conditions clearly affected honeybee activity under the cages during the four-year study and caused variation in the results obtained from individual years.

Table 1. The frequency of cross-pollination and gene transfer among faba bean genotypes exposed to honeybees under cages.

Year	Inbred line/Cultivar combination	Gene transfer (%)
1983	ILB938*/80FUM1§	40
	78-152‡/80FUM3§	0
1984	ILB938*/Aladin§	31
	78-152‡/80FUM3	26
1985	ILB938*/Pegasus§	31
	14434-2†/Aladin	31
	78-152‡/Pegasus	29
1986	ILB938*/Pegasus	52-67
	14434-2†/Aladin	47-52
	78-152‡/Pegasus	40-44

* = Inbred line with resistance gene to rust races 1 and 3.

† = Inbred line with resistance gene to ascochyta races A and Y^1.

‡ = Mass-selected population with resistance to ascochyta race Y^1.

§ = Agronomically superior cultivars.

The combined use (in 1986) of large cages with weekly replacement of the beehives over a four-week period, associated with normal (15–25°C) day/night temperature and light precipitation during the flowering period, resulted in a satisfactory level of honeybee activity with no apparent reduction in bee population. This resulted in 40 to 67% gene transfer, an improvement over the 0–40% gene transfer in previous years. Moreover, the rates of gene transfer were highest (52–67% and 47–52%) in the cages with the rust-resistant inbred lines ILB938 and the ascochyta-resistant inbred line 14434-2 (respectively), in contrast with the low rates (40–44%) in the cage with the ascochyta-resistant MSP 75-152. The high rate of gene transfer from inbred lines can be attributed to the higher degree of homogeneity and the high frequency of resistance genes in the inbred lines in contrast with the MSP.

The results of the 1986 study suggest that honeybees under cages may be useful for gene transfer and hybridization in faba bean, provided that new hives are frequently introduced into the cages to maintain the population of foraging bees at high levels. The use of honeybees to transfer genes of desired traits in this way is less expensive and less time-consuming than manual crossing, especially in large-scale breeding programs and seed production. Honeybees could also be used in faba bean population improvement programs, and in building gene pools for breeding programs for multiple disease resistance. This could also be used in faba bean breeding programs where the interest is in commercial production of composite varieties to maintain the heterogeneity of the gene pool, plant vigor, autofertility, and yield. Moreover, honeybees could be used on cultivars with low levels of autofertility for inbreeding under isolation.

Acknowledgements

We gratefully acknowledge the financial supported of the International Development Research Centre (IDRC) of Ottawa, Canada. The generous supply of beehives by Dr S.C. Jay and the technical assistance of D. Wright of the Department of Entomology, University of Manitoba are also gratefully acknowledged.

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Agronomy and Mechanization

Response of Faba Bean to Phosphorus, Sulfur and Zinc Nutrition in a Black Clay Vertisol

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Abstract

In a field experiment conducted with faba bean (*Vicia faba* L.) on a clay soil, low in available phosphorus, sulfur and zinc, application of increasing levels of P, S and Zn significantly increased the grain yield and the concentration of P, S and N in grain and straw. The concentration of Zn in the plant (grain and straw) decreased with the application of P, while it increased significantly with the application of S and Zn. The combined application of P, S and Zn showed synergistic relationships and the most appropriate treatment combination for faba bean nutrition was 80 kg P, 20 kg S and 20 kg Zn/ha.

Key words: *Vicia faba*; faba beans; clay soil; phosphate fertilizers; sulphur fertilizers; zinc sulphate; plant nutrition; India.

Introduction

In recent years, zinc deficiency has seriously affected about 46% of the soils in India and has become a major constraint in crop production. Spectacular responses in crop yields have been obtained with zinc fertilization (Takkar et al. 1989). It is known that availability and uptake of many plant nutrients is affected by levels of other nutrients present in the growth medium. In particular, P × Zn and P × S interactions in plants and soils have been reported by many workers (Ahmed et al. 1986; Nayak and Dwivedi 1990). The present work was, therefore, conducted to determine the interaction of combined application of P, S and Zn and the subsequent influence on yield and nutrient composition of faba bean.

استجابة الفول للفوسفور والكبريت والزنك في تربة فيرتيزولية غضارية سوداء

الملخص

في تجربة حقلية نفذت على الفول في تربة غضارية فقيرة بمحتواها من الفوسفور والكبريت والزنك، تبين إن إضافة مستويات مرتفعة من تلك العناصر قد أسفرت عن زيادة كبيرة في الغلة الحبية وفي تركيز الفوسفور والكبريت والآزوت في الحب والتبن. وقد تناقص تركيز الزنك في النبات (الحب والتبن) عند إضافة الفوسفور، بينما تزايد بشكل كبير عند إضافة الكبريت والزنك. وأظهرت الإضافة المركبة من الفوسفور والكبريت والزنك العلاقات المتضافرة فيما بينها، كما أن أنسب معاملة لتغذية الفول كانت التوليفة المكونة من 80 كغ فوسفور و20 كغ من الكبريت و20 كغ زنك/هـ

Materials and Methods

A field experiment was carried out on a clay soil (typic chromustert) at the Research Farm of J.N. Krishi Vishwa Vidalaya, Jabalpur, India during *rabi* (winter) 1991/92. The surface soil (0–15 cm depth) had pH 7.4, organic carbon 0.41%, available N (alkaline permanganate) 292 kg/ha, available P (Olsen's) 12.5 kg/ha, available S 14.3 kg/ha and DTPA-extractable Zn 0.43 p.p.m. Faba bean var. JV-2 was the test crop.

Phosphorus at 0, 40 and 80 kg P_2O_5/ha as main-plot treatments, S at 0, 20 and 40 kg/ha as sub-plot treatments and Zn at 0, 10 and 20 kg/ha as sub-sub-plot treatments were applied through diammonium phosphate, gypsum and zinc chloride, respectively. These factors constituted 27 treatment combinations which were tested using split-split-plot design with three replications.

At maturity, plant samples (grain and straw) were analyzed for total P (Koeing and Johnson 1942), S

(Bardsley and Lancaster 1960), N (Piper 1967), and Zn by atomic absorption spectrophotometry.

Results and Discussion

Grain yield

Successive application of P, S and Zn significantly increased the grain yield of faba bean and the beneficial *effect of P* was greater than that of S and Zn (Table 1). The interaction effects of P × S, P × Zn and S × Zn were significant and the best combination doses were $P_{80}S_{20}$, $P_{80}S_{40}$, $P_{80}Zn_{20}$, $S_{20}Zn_{20}$ and $S_{20}Zn_{40}$. Significant interaction was found between P, S and Zn, and the highest grain yield (5.678 t/ha) was recorded in the $P_{80}S_{20}Zn_{20}$ treatment.

Nitrogen content

A significant increase in N content of faba bean straw and grain was observed with the application of P, S and Zn (Table 2). The increase in N content by Zn application may be due to the fact that Zn plays an active role in protein biosynthesis (Praske and Plocke 1971). The interaction effect of P × S was significant for both grain and straw, while the interaction effects of P × Zn and S × Zn were significant only for N content of grain.

Phosphorus content

Application of increasing levels of P, S and Zn showed a significant increase in P content of faba bean straw and grain (Table 3). The interaction between P and S was significant for both straw and grain, and the best combination dose was $P_{80}S_{40}$ (which gave maximum P content). This confirms the synergism between P and S reported by Gupta and Singh (1983).

Sulfur content

Sulfur content of faba bean straw and grain increased significantly with the application of P, S and Zn (Table 4). The significant increase in S concentration by the application of P confirmed the synergistic relationship of P and S which may be attributed to the promotion of root development by P which has been found to induce higher uptake of native and applied S (Kumar and Singh 1980). The interaction of P and S was significant for grain yield, and the best combinations were $P_{80}S_{20}$ and $P_{80}S_{40}$.

Zinc content

There was a decrease in Zn content of faba bean straw and grain by the application of P, while it increased significantly with the application of S and Zn (Table 5). The antagonistic effect of P on Zn content may be due to P slowing Zn-absorption by roots and the subsequent retardation in Zn translocation from roots to shoots (Katyal et al. 1992). For both straw and grain yield, the interaction effect of P × Zn was negative and significant, while a positive and significant interaction was found between S and Zn. The combined effect of P and S was complex: at S_0, increasing levels of P significantly decreased Zn content, while at S_{20}, Zn content increased significantly with increasing levels of P.

Table 1. Effect of phosphorus, sulfur and zinc on grain yield (t/ha) of faba bean.

Phosphorus (kg/ha)	S_0			S_{20}			S_{40}			Mean values		
	Zn_0	Zn_{10}	Zn_{20}	Zn_0	Zn_{10}	Zn_{20}	Zn_0	Zn_{10}	Zn_{20}	P	S	Zn
0	3.455	3.611	3.833	3.989	4.000	4.106	4.125	4.189	4.208	3.946	4.048	4.320
40	4.153	4.184	4.189	4.353	4.611	4.639	4.734	4.847	4.953	4.518	4.571	4.443
80	4.239	4.428	4.428	4.750	5.015	5.678	5.086	5.189	5.300	4.891	4.737	4.593

CD at 5%	P	S	Zn	P × S	P × Zn	S × Zn	P × S × Zn
Grain yield	1.91	1.04	0.57	2.38	1.60	1.10	2.15

Table 2. Effect of phosphorus, sulfur and zinc on N content (%) in faba bean.

Phosphorus (kg/ha)	S_0			S_{20}			S_{40}			Mean values		
	Zn_0	Zn_{10}	Zn_{20}	Zn_0	Zn_{10}	Zn_{20}	Zn_0	Zn_{10}	Zn_{20}	P	S	Zn
						N content in grain						
0	3.84	3.88	3.91	3.94	3.98	4.01	4.06	4.11	4.17	3.99	4.05	4.16
40	4.00	4.04	4.06	4.14	4.20	4.24	4.29	4.32	4.35	4.18	4.20	4.20
80	4.19	4.25	4.30	4.37	4.43	4.49	4.58	4.63	4.68	4.43	4.35	4.24
						N content in straw						
0	1.41	1.43	1.44	1.46	1.49	1.53	1.54	1.57	1.61	1.50	1.56	1.11
40	1.55	1.58	1.59	1.62	1.65	1.70	1.72	1.75	1.79	1.66	1.65	1.65
80	1.63	1.69	1.73	1.76	1.81	1.86	1.89	1.94	1.99	1.81	1.75	1.69

CD at 5%	P	S	Zn	P × S	P × Zn	S × Zn	P × S × Zn
N content in grain	0.04	0.01	0.01	0.03	0.05	0.02	NS
N content in straw	0.01	0.007	0.02	0.02	NS	NS	NS

Table 3. Effect of phosphorus, sulfur and zinc on P content (%) of faba bean.

Phosphorus (kg/ha)	S_0			S_{20}			S_{40}			Mean values		
	Zn_0	Zn_{10}	Zn_{20}	Zn_0	Zn_{10}	Zn_{20}	Zn_0	Zn_{10}	Zn_{20}	P	S	Zn
						P content in grain						
0	0.22	0.23	0.25	0.25	0.26	0.28	0.28	0.29	0.31	0.26	0.29	0.31
40	0.30	0.31	0.31	0.32	0.34	0.36	0.36	0.38	0.41	0.34	0.33	0.33
80	0.31	0.33	0.35	0.36	0.38	0.40	0.40	0.42	0.40	0.37	0.36	0.34
						P content in straw						
0	0.05	0.06	0.07	0.06	0.07	0.07	0.08	0.08	0.09	0.07	0.08	0.08
40	0.07	0.08	0.09	0.09	0.09	0.10	0.10	0.11	0.12	0.09	0.09	0.09
80	0.09	0.10	0.10	0.11	0.12	0.13	0.12	0.13	0.14	0.12	0.11	0.10

CD at 5%	P	S	Zn	P × S	P × Zn	S × Zn	P × S × Zn
P content in grain	0.018	0.019	0.009	0.01	NS	NS	NS
P content in straw	0.01	0.004	0.004	0.009	NS	NS	NS

Table 4. Effect of phosphorus, sulfur and zinc on S content (%) of faba bean.

Phosphorus (kg/ha)	S_0			S_{20}			S_{40}			Mean values		
	Zn_0	Zn_{10}	Zn_{20}	Zn_0	Zn_{10}	Zn_{20}	Zn_0	Zn_{10}	Zn_{20}	P	S	Zn
						S content in grain						
0	0.16	0.18	0.19	0.20	0.22	0.24	0.21	0.23	0.24	0.21	0.20	0.22
40	0.18	0.20	0.21	0.23	0.25	0.27	0.24	0.26	0.28	0.24	0.25	0.24
80	0.20	0.22	0.24	0.26	0.28	0.30	0.27	0.30	0.32	0.26	0.26	0.25
						S content in straw						
0	0.05	0.07	0.08	0.07	0.09	0.11	0.08	0.10	0.11	0.09	0.09	0.08
40	0.07	0.09	0.10	0.09	0.11	0.12	0.09	0.11	0.13	0.10	0.10	0.10
80	0.08	0.10	0.12	0.10	0.12	0.13	0.10	0.12	0.14	0.11	0.11	0.12

CD at 5%	P	S	Zn	P × S	P × Zn	S × Zn	P × S × Zn
S content in grain	0.01	0.006	0.006	0.01	NS	NS	NS
S content in straw	0.006	0.004	0.005	NS	NS	NS	NS

Table 5. Effect of phosphorus, sulfur and zinc on Zn content (%) of faba bean.

Phosphorus (kg/ha)	S_0			S_{20}			S_{40}			Mean values		
	Zn_0	Zn_{10}	Zn_{20}	Zn_0	Zn_{10}	Zn_{20}	Zn_0	Zn_{10}	Zn_{20}	P	S	Zn
						Zn content in grain						
0	55	60	62	60	63	68	61	68	69	63.0	57.3	58.7
40	54	59	60	60	65	70	61	68	67	62.4	65.0	63.0
80	54	55	57	62	66	70	62	66	67	62.0	65.2	65.6
						Zn content in straw						
0	19	23	25	20	25	29	22	29	30	24.7	20.8	21.0
40	18	22	23	23	27	30	23	27	28	24.5	26.2	25.2
80	17	19	21	24	28	30	23	27	29	24.2	26.4	27.2

CD at 5%	P	S	Zn	P × S	P × Zn	S × Zn	P × S × Zn
Zn content in grain	NS	0.15	0.59	0.50	1.03	1.03	NS
Zn content in straw	NS	0.21	0.64	0.42	1.10	1.10	NS

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Physiology and Microbiology

Scanning Electron Microscopic Observations on the Pod Surface of Faba Bean Plants

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Abstract

Observations were made on the morphological changes of stomata and hairs of faba bean (*Vicia faba* L.) pods accompanying the developmental process by means of scanning electron microscopy on indigenous varieties of Finland (A) and Japan (B) and the new "topless" mutant lines of ICARDA (C). Although changes in stomatal density were approximatly equal, they were least on C. The density of pod hairs on C, however, was greater than on the others throughout development. Moreover, the percentage of broken hair in C was stable in contrast to A and B, which increased severely with advancing development. Thus, the behavior of stoma and hair, especially the latter in C, seemed to play a specific supplementary role for dry matter production.

Key words: *Vicia faba*; faba beans; stomata; fruit; plant anatomy; plant developmental stages; Japan.

Introduction

The pod surface of faba beans has a specific role in carbon dioxide exchange (Kogure 1986). There is no apparent photosynthesis on pods in daylight, but there is a unique diurnal change of respiration. At the green-pod stage, the high respiration at night suddenly declines to its lowest value at sunrise. However, this value is maintained for only two or three hours, and then rapidly increases and is maintained at a high value until sunset. The highest value occurs at midnight. We supposed that these phenomena were related with the specific characteristics of gas exchange on pods.

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قراءات لسطح قرون نباتات الفول بواسطة التصوير الطبقي المحوري الإلكتروني

الملخص

أجريت قراءات للتغيرات المظهرية على ثغيرات وشعيرات قرون الفول المترافقة مع عملية تطور النبات بواسطة التصوير الطبقي المحوري الإلكتروني، وذلك لأصناف محلية من فنلندا (أ) واليابان (ب) والسلالات الطافرة الجديدة "Topless" في إيكاردا (جـ). وعلى الرغم من أن التغيرات في كثافة الثغيرات كانت متساوية تقريباً، كانت الأقل في جـ غير أن كثافة شعيرات القرون في جـ كانت أكبر مما هي عليه في الأصناف الأخرى على مدى عملية تطور النبات. وعلاوة على ذلك، كانت النسبة المئوية للشعيرات المكسورة في جـ ثابتة على عكس النسبة في أ وب التي تزايدت بشدة مع تقدم التطور أو النمو، لذا، يبدو سلوك الثغيرات والشعيرات، وخاصة الشعيرات في جـ ذا دور تكميلي محدد في إنتاج المادة الجافة.

Therefore, we studied the morphological changes occurring on pods, especially the developmental process of the stomata and hairs, by means of scanning electron microscopy. Three ecophysiological types of faba bean were used.

Materials and Methods

Two cultivars, 'F-16' (A) from Finland (indeterminate growth habit, short thin pods and small seeds) and 'Nintoku-issun' (B) of Japan (semi-determinate growth habit, fat pods and large seeds), and a new "topless" mutant line FLIP-86-14FB (C) from ICARDA (complete determinate growth habit, medium-sized pods and medium-sized seeds) (ICARDA 1987) were used. The seeds were sown in nursery bed on 8 November 1990, and seedlings transplanted on 27 November 1990 to pots which were filled with course sand. Seedlings were grown with water culture solution in an unheated glass house.

The main shoot and two branches were used for this experiment; other branches were cut off. The start of flowering was on 5 March in A and C, and 15 March in B. Self-pollination of flowers was done five times by hand-tripping at successive nodes, which were then marked. Sampling of pods was done on days 5, 10, 15, 20, 30, 40 and 50 after pollination. Materials were fixed with a solution of formalin, acetic acid and alcohol, and stored at low temperature. Fixed materials were cut into small blocks, dehydrated in alcohol, and then dried by the critical-point method. These materials were mounted on polished brass stubs with silver paste and coated with Pt-ion and examined using a scanning electron microscope, Hitachi S-800.

Results and Discussion

Table 1 shows the developmental process of the pods. Although these changes give similar growth curves among genotypes, the growth of inner seeds seemed to cause the differences of development duration and turning point. Figure 1 shows the pod surface 20 days after pollination of FLIP-86-146FB (C) of ICARDA. It was impossible to count the exact number of stomata and hairs because too many hairs covered the pod surface before day 20.

Stomatal numbers per unit area decreased severely until day 30, and afterward decreased gradually accompanying the development (Table 2). Though the tendency was approximately the same for the three materials, the density of stomata on A and B was higher than on C. Many wrinkles or crumpled lines on both auxiliary cells and epidermal cells in contact with guard cells were found at day 20 after pollination, but were not found on these cell surfaces after day 30. We supposed these phenomena were caused by the enlargement of the cell surface area of both cells together with spreading pod surface area. The auxillary cell behavior was clearer in C than in A or B.

It is well known that plants have hairs of diverse morphology and function. The pod-hairs of faba bean consist of single cells sticking out from the surface of epidermal cells in a conical shape, 50–80 μm long. There were important differences among the three lines for number of hairs per unit area (Table 2). Though the decrease in hair numbers was similar for all three lines, hair density of C was greater than *that of A and* B throughout the developmental process. The percentage of broken hair was highest on C at an early stage (Table 2); however, it increased severely with development of A and B, contrasting with the stable low value of C. Therefore, though the enlargement of epidermal cells seemed directly related to the developmental process of the pods, some peculiarities were recognized in the behavior of hair in the new line C.

The results of this experiment suggest that there were important differences in the physiological behavior of pods between domestic varieties of Japan and Finland (old varieties) and the new line. Differences in photosynthesis and mobility performance of chemical components among these three materials were reported earlier (Kogure et al. 1992a, b). The reports showed that the new line translocated assimilates directly from leaves to seeds, but the shortage of active leaf mass and its duration resulted in low yield. In this experiment, the longevity of pod hair of the new line was clearly different from that of the indigenous varieties. So, this characteristic seems to play a supplementary role in gas exchange and dry matter production of the pod (Kogure 1992b).

Table 1. Changes in length, width, and surface area of pods of F-16 (A), Nintoku-issun (B) and FLIP-86-146FB (C).

Days after pollination	Pod length (cm)			Pod width (cm)			Pod surface area (cm^2)		
	A	B	C	A	B	C	A	B	C
5	1.7	2.2	1.8	0.15	0.25	0.19	0.7	1.1	0.7
10	1.8	2.6	2.4	0.24	0.42	0.29	0.7	2.2	1.1
15	2.8	4.5	3.2	0.43	0.76	0.48	2.2	6.6	2.9
20	6.7	8.2	8.2	1.03	1.33	1.16	13.6	21.8	18.9
30	8.0	12.2	10.4	1.40	2.29	1.74	22.4	55.4	34.1
40	8.3	14.5	13.2	1.53	2.86	2.16	25.5	82.7	56.8
50	8.5	13.7	14.3	1.68	3.07	2.37	27.9	83.6	67.1

Table 2. Changes in stomatal and hair number and percentage of broken hair of cultivars A, B and C.

Days after pollination	No. of stomata (10^4/cm^2)			No. of hairs (10^4/cm^2)			Percentage of broken hair (%)		
	A	B	C	A	B	C	A	B	C
5	-	-	-	-	18.9	31.3	-	-	-
10	-	-	-	20.3	-	28.5	-	-	-
15	-	-	-	10.2	13.8	23.1	-	-	-
20	2.77	3.07	2.51	3.9	6.7	4.8	35.5	27.3	45.0
30	1.95	1.95	1.68	2.2	2.2	3.0	47.2	32.8	41.2
40	1.82	1.82	1.40	1.6	1.6	3.0	61.2	58.1	38.5
50	1.26	1.40	0.84	1.5	1.3	2.5	62.2	55.4	46.7

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Imbibition Damage in Faba Bean Lines Near-isogenic for Flower Color

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Abstract

Twelve seed lots of faba bean (*Vicia faba* L.) lines near-isogenic for flower color and the presence of testa tannins were tested for the rate of water absorption by seeds and subsequent imbibition damage. Leachate conductivity of steep water, laboratory germination, seed coat cracking and hard seeds were also investigated. White-flowered lines had more rapid uptake of water and greater incidence of imbibition damage on cotyledons than lines with colored flowers. Electrical conductivity and seed coat cracking were also higher in white than in colored lines. Higher rates of water uptake were associated with harder seeds, increased seed coat cracking and thicker testae which lead to poor vital staining on cotyledons and higher seed leachate.

Key words: *Vicia faba*; faba beans; seeds; damage; water uptake; cotyledons; flowers; colour; testa; swelling; United Kingdom.

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Introduction

In French beans (*Phaseolus vulgaris* L.) (Powell et al. 1986a), peas (*Pisum sativum* L.) (Powell 1989) and long beans (*Vigna sesquipedalis* (L.) Fruhw.) (Abdullah et al. 1991), white-seeded cultivars often have faster water uptake by seeds than cultivars with colored seeds. Rapid water-uptake results in extensive imbibition damage (seen in the death of cotyledon tissue) and consequently reduced vigor; cultivars with colored seeds have higher vigor with little imbibition damage.

Zero-tannin white-flowered faba beans that have been developed recently to overcome the anti-nutritional drawbacks of high-tannin cultivars with colored flowers, have been reported to suffer poor emergence under certain conditions (van Norel 1985). This work examined imbibition damage in low-tannin lines of faba bean.

Materials and Methods

Imbibition damage due to rapid uptake of water was investigated at the University of Nottingham School of Agriculture, UK, using 12 seed lots of three pairs of faba beans near-isogenic for flower color and the presence of testa tannins.

Near-isogenic lines WCI/WWI (colored flower/white flower) and SCI/SWI were provided by PBI (Plant Breeding International) of Cambridge, UK, and SVPC/SVPW was provided by CPRO-DLD (Centre for Plant Breeding and Reproduction Research), Wageningen, The Netherlands. Germination of seed lots was above 95%.

Twenty individually weighed seeds of each lot were soaked either in 20 ml de-ionized water or in 30% (w/v) solution of polyethylene glycol (PEG) 4000 at 20±2°C. The latter had a water potential of -1.9 MPa and was used to reduce the speed of imbibition of water. Seeds were re-weighed at predetermined intervals and cumulative weight increase as percentage of initial seed weight was calculated. Seeds in the PEG solution were transferred to 20 ml de-ionized water at 9 h until a final weighing at 24 h. The *number of seeds* with at least one crack in the testa and seeds that remained hard to the touch after 24 h of soaking were recorded at the final weighing. Electrical conductivity (EC) of the steeping water after 24 h soaking was measured with a conductivity meter (Jenway 4070) (Powell and Matthews 1978).

The testae of the seeds were then removed by hand and embryos were soaked in a 1% (w/v) solution of 2,3,5-triphenyl tetrazolium chloride for 24 h at 20°C (Grabe 1970). Cotyledons were then scored for the degree of cotyledonary staining (Powell and Matthews 1978). Testa and whole-seed dry weights were determined after drying at 85°C for 48 h.

The weight gain and vital staining of individually weighed seeds of SCI and SWI from which the testa had been chipped, was recorded and compared to intact testae at 20±2°C, with 20 replicates for each treatment both in water and in 30% (w/v) solution of PEG 4000.

Germination of 50 undamaged seeds of near-isogenic lines in four replicates was tested in sand at different moisture contents, dry (15.9±0.31%) and wet (21.6±0.36%) on the basis of dry weight. Seeds were germinated both at 5/20°C (i.e. 7 h at 5°C, then kept at 20°C) and at 20°C in air-tight plastic trays. The percentage of normal and abnormal seedlings produced (ISTA 1985) was counted after 14 days.

ضرر التشُرب في سلالات الفول المتشابهة الصفات الوراثية بالنسبة للون الزهرة.

الملخص

اختبرت 12 مجموعة بذور، مأخوذة من سلالات فول متشابهة الصفات الوراثية من حيث لون الزهرة ووجود التانين في غلاف البذرة الخارجي، للحصول على معدل امتصاص الماء من قبل البذور وأضرار التشُرب اللاحقة. كما تمت دراسة موصلية الترشيح للماء الشديد الانحدار، الإنبات في المختبر، تصدع غلاف البذرة والبذور الصلدة. تبين أن السلالات ذات الأزهار البيضاء تمتص الماء على نحو أسرع وتتعرض فلقاتها للإصابة بأضرار التشُرب على نحو أكبر من السلالات ذات الأزهار الملونة. كما كانت الموصلية الكهربائية وتصدع غلاف البذرة أعلى في البيضاء منها في الملونة. وقد ارتبطت المعدلات الأعلى لامتصاص الماء بالبذور الأصلد وتزايد تصدع غلاف البذرة وسماكة أكبر للغلاف الخارجي مما أدى إلى تصبغ سيء على الفلقات وترشيح أعلى للبذور.

Results and Discussion

White near-isogenic lines had greater weight increase in water than colored lines (Fig. 1 a,b,c). The difference in weight increase was more apparent in the first hour of soaking between SCI and SWI (Fig. 1b) and between WCI and WWI (Fig. 1a); there was a narrow but constant difference between SVPC and SVPW after 5 h (Fig. 1c). Despite the presence of hard seeds in SVPC (Table 1) after 24 h soaking in water, germination in sand was 95%. This indicates that those seeds remaining non-imbibed for 24 h gradually absorbed water and then proceeded to germinate.

The white near-isogenic lines had higher EC of steep water, more cracks in the testa and smaller percentages of cotyledons with complete TTC staining compared with the colored lines (Table 1).

Chipping the seed coat prior to soaking increased leachate conductivity and lowered the incidence of completely stained cotyledons in both lines (Table 2), although weight gain was still smaller in chipped seeds of SCI (colored flower) than in intact seeds of SWI (white flower) (Table 2).

White lines produced more abnormal seedlings than their colored counterparts (Table 1) usually in wet sand, except in SVPW which produced more abnormal seedlings in dry than wet sand irrespective of temperature (Table 1). Temperature had no consistent effect on seedling growth.

The decrease in the percentage of cotyledons with complete vital staining following slow imbibition in PEG solution (Tables 1 and 2) may indicate that the damage which occurred by soaking in de-ionized water was due to rapid imbibition of water. Significant correlations of cumulative weight increase in water with electrical conductivity (Fig. 2a) and vital staining of the cotyledons (Fig. 2b) may indicate the involvement of rapid water-uptake in producing seeds with low vigor. This reaction was more pronounced in white types.

Imbibition damage, as a result of rapid water-uptake, has been observed in several grain legumes. Imbibition damage in peas was associated with an increase in solute leakage (Powell and Matthews 1981), which acted as a nutrient substrate for pathogens (Matthews 1971) and subsequently increased the infection of germinating seeds and seedlings in the soil (Powell 1989). Imbibition damage has reduced the respiration rate and the rate of food reserve transfer from the cotyledons to the growing axis in French beans, which resulted in a slower growth rate (Powell et al. 1986b). Imbibition damage in soybean was exasperated by rapid imbibition of water at low temperatures by seeds of low-moisture content (Hobbs and Obendorf 1972).

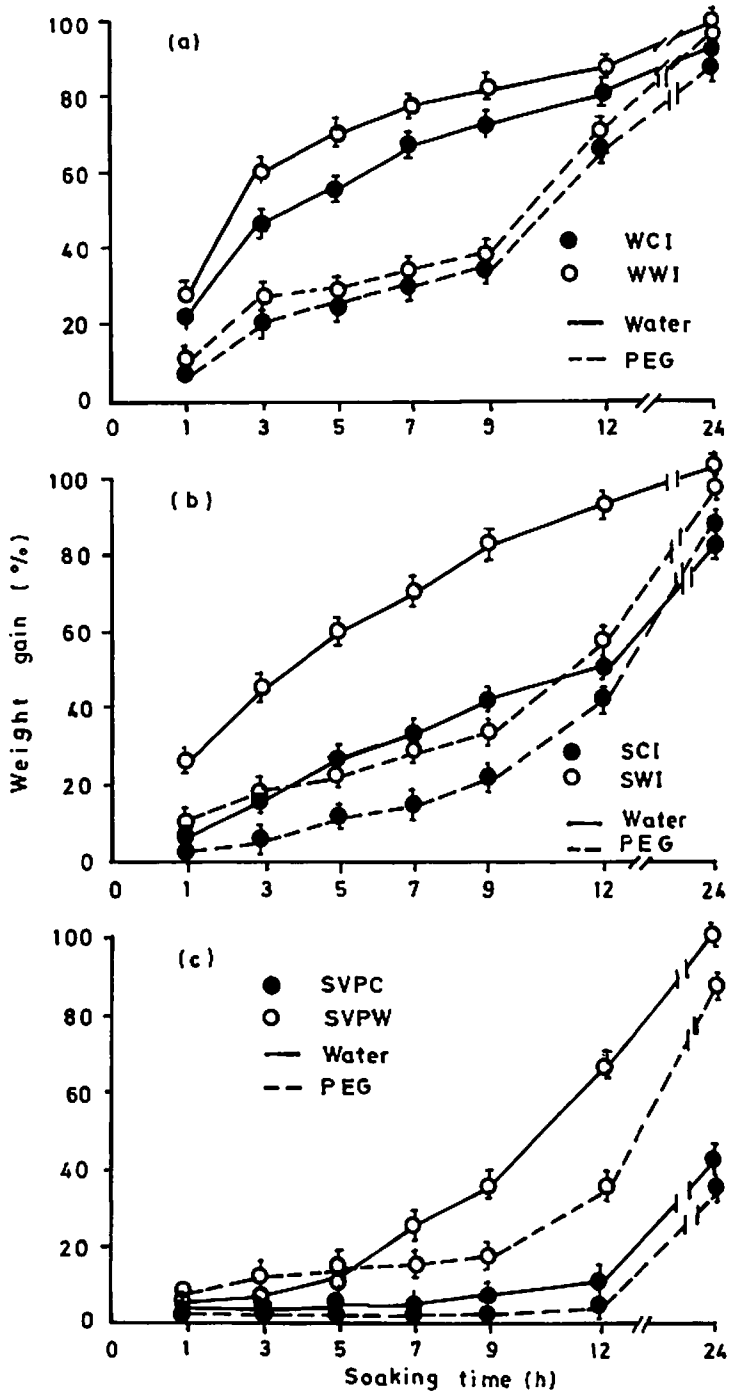

Fig. 1. Cumulative weight grain as a percentage of initial seed weight in water and in 30% PEG 4000 solution of near-isogenic lines at 20±2°C. (Bars represent SE.)

Table 1. Final laboratory germination, electrical conductivity, seeds having at least one crack in the testa and hard seed percentages of near-isogenic faba bean lines.

Line†	Seed color	Electrical conductivity ($\mu S\ cm^{-1}\ g^{-1}$ seed)	Seed coat cracking (%)	Hard seeds after 24 h soaking in water (%)	Cotyledons with complete staining (%) after 24 h soaking in		Abnormal seedlings after germination in sand (%)	
					Water	30% PEG	15.9% MC	21.6% MC
WCI	buff	204	14	0	70	86	5.2	7.3
WWI	black	220	28	0	28	66	9.5	11.0
SCI	buff	150	5	11	79	81	1.0	1.0
SWI	beige	350	30	0	59	80	3.0	11.0
SVPC	green	58	2	52	96	98	3.0	2.0
SYPW	green-gray	182	3	9	78	98	11.2	3.0
SE		4.9	1.7	2.2	–	–	1.41	

† Lines printed in italic are white flowered.

Table 2. Weight grain after soaking in water for 9 h, electrical conductivity of steep water, and cotyledons with complete vital staining of intact and chipped seeds of the near-isogenic lines steeped in water and in PEG 4000 (30% w/v) at 20±2°C.

Line	Testa	Treatment	Weight grain at 9 h (%)	EC ($\mu S\ cm^{-1}\ g^{-1}$ seed)	Cotyledons with complete staining (%)
SCI	intact	water	46.5	140	82
		PEG	22.4	—	89
	chipped	water	71.6	164	48
		PEG	25.9	—	70
SWI	intact	water	80.4	178	42
		PEG	34.7	—	86
	chipped	water	82.6	294	9
		PEG	33.1	—	45
SE			1.89	4.1	—

SWI is white-flowered counterpart of SCI.

Our data showed that white-flowered lines generally sustained greater imbibition damage and subsequently leaked more electrolytes than colored lines, indicating that imbibition damage could cause problems under adverse soil conditions.

Higher rates of water uptake in white types could be as a result of their seed coat cracking as seen in soya beans (Oliveira et al. 1984) and French beans (Powell and Matthews 1981). Significant correlations of cumulative weight increase in water with seed coat cracking (Fig. 3b) and the percentage of hard seeds (Fig. 3c) and increased damage, when the testa was chipped (Table 2), indicate the importance of the physical condition of the seed coat.

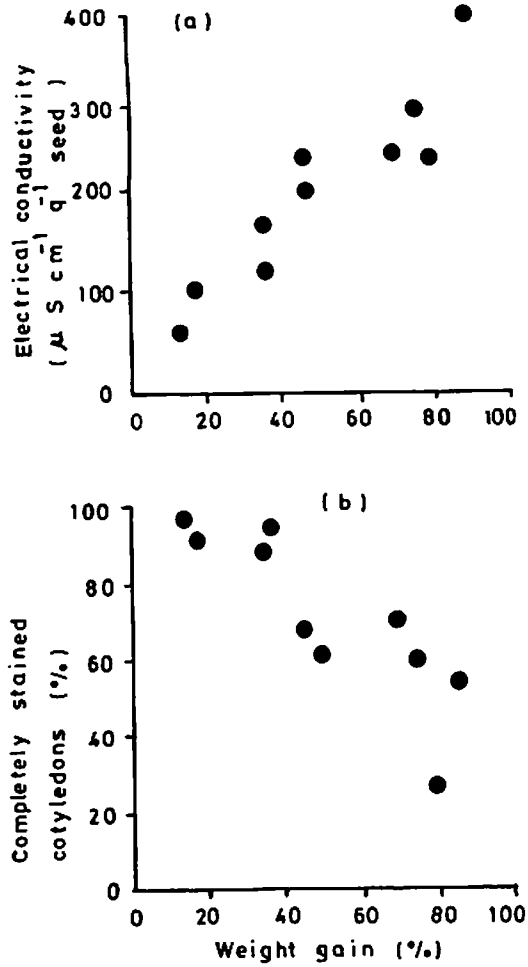

Fig. 2. Relationship between cumulative water uptake and the percentage of cotyledons with (a) complete vital staining ($r=-0.62$, $P<0.001$) and (b) electrical conductivity ($r=0.85$, $P<0.001$).

The impediment to rapid water-uptake in SCI when the testa was chipped indicates tight adherence of the seed coat to the cotyledons compared with its white-flowered counterpart, SWI. In white-seeded peas, rapid uptake of water was associated with loose adherence of the testa to the cotyledons (Powell 1989). Furthermore, cumulative weight increase in water being inversely correlated with the proportion of the testa (Fig. 3a) may suggest that rapid water-uptake can be in part attributed to thinner testae in the white-flowered lines (Hebblethwaite et al. 1991).

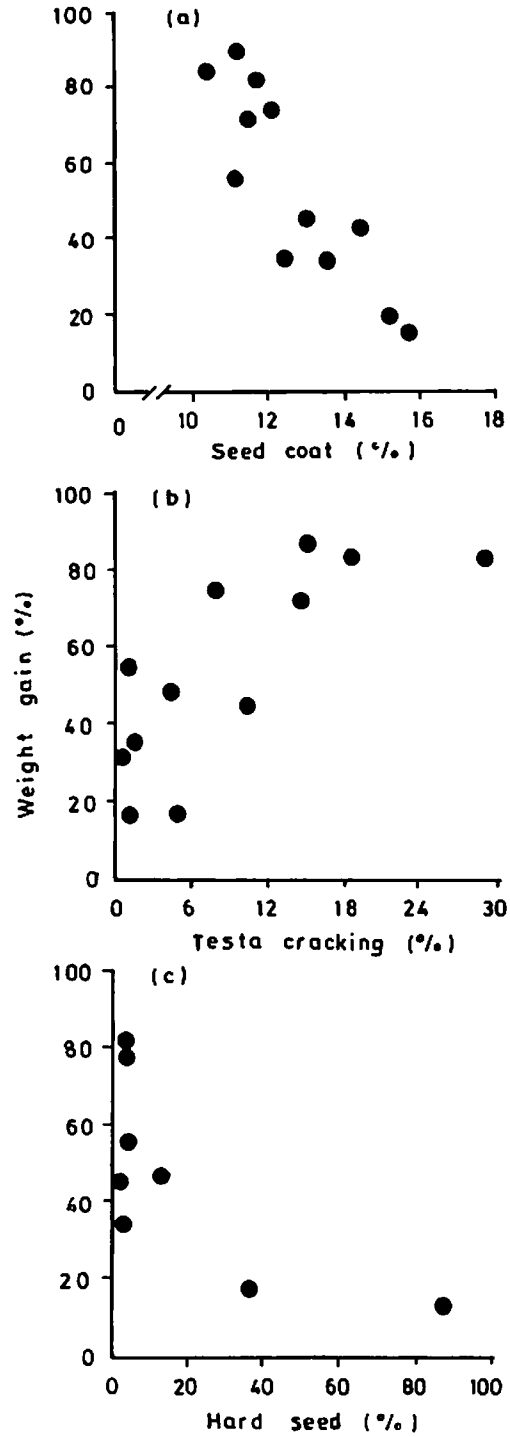

Fig. 3. Relationship between cumulative weight increase during imbibition (as a percentage of initial seed weight) and (a) the proportion of testa to the whole seed weight ($r=-0.85$, $P<0.001$), (b) the percentage seeds with at least one crack in the testa ($r=0.70$, $P<0.01$) and (c) and the percentage of hard seeds ($r=-0.63$, $P<0.05$).

The cotyledons of white lines frequently lost their testae during germination and the decaying testae were accompanied by fungal growth. Perhaps, tannins in colored types protect the testa against decomposition by micro-organisms. Easily decomposed testa could provide a greater potential for infection during the germination of white types.

Hydrophobic compounds in the testa of colored cultivars (perhaps tannins and other phenolic compounds) may give a "delayed permeability action", although variations occurred between the high-tannin lines. The slow water absorption seen in SVPC requires further examination: this trait may help to overcome the poor vigor seen in white types due to rapid uptake of water.

In conclusion, imbibition damage as a result of rapid uptake of water in white-flowered faba beans may result in poor emergence under cold and wet conditions. Therefore, in order to improve seed vigor, cultivars with slow rates of water uptake and consequently less propensity to imbibition damage should be aimed for in breeding programs.

Acknowledgements

We thank Mr J. Craigon for advice on the trial design and statistical analysis, and Mr D. Hodson and Mrs J. Higgins for technical assistance.

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Pest and Diseases

Foot Rot Disease of Faba Bean in Ethiopia

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Abstract

Foot rot of faba bean (*Vicia faba* L.) was studied for several years to establish the causal agent and to analyze the build up of the disease. *Fusarium avenaceum* (Fr.) Sacc. was found to be the pathogen that caused foot rot in faba bean. The incidence of this disease has recently increased to a serious level in some fields at Holetta Agricultural Research Center, Ethiopia. The disease becomes particularly severe when August rainfall is above average and extends after mid-October. It tends to be associated with acidic soils.

Key words: *Vicia faba*; faba beans; root rots; *Fusarium*; acid soils; Ethiopia.

مرض تعفن القدم على الفول في إثيوبيا

الملخص

دُرس مرض تعفن القدم على الفول لسنوات عديدة لتحديد العامل المسبب وتحليل ظروف تفاقمه. وقد وجد أن *Fusarium avenaceum* (Fr.) هو العامل الممرض الذي يسبب تعفن القدم في الفول. وقد تزايدت الإصابة به في الفترة الأخيرة إلى مستويات خطرة في بعض حقول محطة هوليتا للبحوث الزراعية في إثيوبيا. ويتفاقم المرض على نحو خاص عندما تكون أمطار آب/اغسطس فوق المعدل وتستمر إلى مابعد منتصف تشرين الأول/اكتوبر. وينحو هذا المرض للاقتران بالترب الحامضية.

Introduction

Foot rot disease of faba bean was first observed at Holetta Agricultural Research Center in 1973 on a few plants (Yitbarek 1983). The disease also appeared in other experimental sites such as Quiha and Kokate. A disease sample (No. 5054) from Holetta was sent to the Commonwealth Mycological Institute (CMI), UK for confirmation of local identification, *Fusarium avenaceum*, in 1978. CMI subsequently confirmed that the foot rot sample of faba bean was caused by *Fusarium avenaceum* (Fr.) Sacc. (CMI herbarium No. 241885). At that time the disease was at very low incidence.

However, in 1983, the disease was very severe in some trial and production fields at Holetta, killing about 90% of the plant population (Yitbarek 1983). In 1986 and 1990, it was also severe in experimental plots at Holetta. At Welmera, near Holetta, up to 15% infection was found in field trials in 1990.

Although we had some fragmentary notes of this disease, complete information was lacking on the causal agent and disease development. This paper presents the results of some investigations made on the causal agent and its pathogenicity, descriptions of symptoms, and disease development in the different areas and seasons. It also gives the precautions taken to reduce the spread of the pathogen.

Results

Symptoms and symptom development

Dark to brown lesions, often elongated, appeared on the stems of faba bean plants at about ground level in early to mid-September. Initially, these lesions were about 0.5 cm wide and 2.0 cm long. As the season progressed, the lesions grew and eventually destroyed the foot part (from ground level to about 30 cm height). Eventually, an orange fungal growth appeared at the center of the lesions. This orange growth developed on most parts of the lesions and had white margins; the orange centers were a mixture of conidia and mycelia of *Fusarium avenaceum*, while the white margins were pure mycelia. The level of infection was always high, between 5 and 25 cm up the stem above ground level. The lesions were mostly continuous along the stem, with intermittent attenuation at about 5 cm.

These lesions did not circumscribe the stems so that at least some parts of each stem remained without infection. However, infected plants were easily broken at the infection position and hence lodged. The leaves soon died and the stems became dark or dark-brown. When infected plants died and the tissues dried they turned a dark color. Most of the infected plants were without pods.

Microscopic observation and culturing

Observations of the causal organism (*F. avenaceum*) under the microscope revealed that the orange fruiting body on the infected parts were conidia of this fungus, 10.8 to 67.2 µm long and 3.6 to 5.0 µm wide with 3 to 7 septations. The fungus showed variation in color when cultured on media. The mycelium was generally white and the conidia were light-brown to orange both on infected tissues and in cultures. This abundant fungal growth on infected tissue gave rise to white mycelium and orange up-growth which resembled sporodochia. It did not give a true sporodochia. This is a characteristic feature of *F. avenaceum*.

When infected stem pieces were cultured on faba bean dextrose agar (FBDA), potato dextrose agar (PDA) and yeast extract agar (YEA), fast growth and abundant sporulation of *F. avenaceum* was observed only on PDA, while there was only some sporulation on FBDA and YEA. These cultures of *F. avenaceum* were kept at 20 to 24°C, which was optimum for its growth.

Pathogenicity test

Pathogenicity of *F. avenaceum* was studied at Holetta by inoculation of glasshouse plants. Inoculum, spore and mycelial suspension, was placed on the basal portion of the plants using cotton and the plants were immediately covered with cellophane to reduce evaporation. The plants showed wilting symptoms after two days. Black lesions, which resembled water-soaked tissue, started to develop on the inoculated parts. Infected plants died in four days, unlike the control which was healthy throughout the crop life. Isolation of the fungus from the wilted plants gave a pure culture of *F. avenaceum* which was similar to the source inoculum.

Foot rot occurrence and some precautions undertaken to limit spread

Foot rot disease of faba bean has been building up from season to season. Since 1983, it has increased at Holetta Research Center. Incidence was noticeable, severe or even very severe in some seasons, i.e. 1983, 1985, 1986, 1988, 1990 and 1992. In these years, some weather conditions prevailed which were different from the seasons free from foot rot. Rainfall was prolonged by 10 days (20 October vs 10 October). In the years with foot rot, August rainfall was more than average by a mean of 14.1 mm and the amount of rainfall for 11–20 August was very high compared with years when no foot rot occurred.

At Holetta, the soils in which foot rot occurred were Nitosol (red clay soil) with low pH. This disease has never been observed in black soil (Vertisol), which has higher pH. The other root disease which was dominant in Vertisols of Ethiopia is black root rot which is caused by *F. solani* (Mart.) Sacc. em. Snyder & Hansen p.p. According to several observations, foot rot seems to occur in more acidic soils.

Two production fields with total area of 5 ha were severely damaged at Holetta Research Center in the 1992 cropping season. Almost all plants (97.3%) were infected and about half (48.6%) of the plants in these fields were without pods. The infection was associated with heavy sporulation of *F. avenaceum*. Since this pathogen was mainly disseminated through crop residues, infected seeds or even air-borne spores, immediate precautions were thought important. Therefore, to reduce the dissemination of this pathogen and the probability of its spread, restrictive measures were immediately taken. These restrictive measures included: (1) burning of the whole crop in the field, mainly to reduce the spore on crop residues and seeds; (2) immediately plowing after burning of the fields to destroy the remaining inocula on the soil surface; and (3) flaming of farm implements (passing the flame of a gas-jet burner over the plow) after plowing of these infested fields and before the next plowing, which may reduce the dispersal of the pathogen by the implements.

Cultural control measures consisting of crop rotation and sanitation, especially deep plowing to bury crop residues after harvesting, are recommended for this pathogen. This is because the main disseminating mechanism is through spore dispersal from stubble or crop residues. A host-range study is in progress to establish a crop-rotation scheme for soils infested by this pathogen.

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Survey of Fungi Observable on Seeds of Faba Bean Germinating on Blotter

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Abstract

In the course of a seed pathological study conducted at the Research Centre for Agricultural Botany, Hungary, 442 samples of faba bean (*Vicia faba* L.) seeds were tested for fungi. The seeds were tested by incubation on moistened blotter with and without surface sterilization. The fungi were identified from both seed surface and representative pure cultures. Sixty-nine taxa of fungi were identified; 33 were observed on unsterilized seeds, while 24 were identified from sterilized ones. The predominant potentially seed-pathologic fungi were *Alternaria* spp., *Fusarium* spp. and *Trichotecium roseum*. *Ascochyta fabae*, *A. pinodes*, *Botrytis cinerea* and *B. fabae* were also observed on several seed samples. Rates of occurrence were correlated with total infection, except for *Ascochyta*. Some storage fungi were also observed.

Key words: *Vicia faba*; faba beans; fungi; seeds; Hungary.

Introduction

Faba bean is an important source of protein, but the crop can be heavily affected by different fungal diseases (Gaunt 1983; Salt 1983). Several fungi including phytopathogenic ones have been reported from faba bean seeds (Simay 1992a). Some were also recorded from Hungary (Simay 1986, 1987a, b, 1990, 1992b). This paper presents a summary of the results of identification of fungi observed during seed pathological tests on faba bean.

Material and Methods

Seeds of faba bean were harvested from an experimental field at the Research Centre for Agricultural Botany, Tápiószele, Hungary. The pathological investigations were made on 442 samples during 1985–89. Twenty-five seeds were surface-sterilized with 10% NaOCl solution and rinsed with sterile distilled water before germinating on moistened blotter. A further 25 seeds were germinated without surface-sterilization.

Fungal infection was determined mostly after one week, but seeds infected with rapidly spreading fungi (e.g. *Rhizopus*) were handled earlier, and seeds which remained uninfected and ungerminated were incubated for a further week. Identifications were carried out from sporulation developed on the seed surface, although some representative cultures were made for further investigation and to confirm identification. The cultures were grown on 2% malt extract agar, Leonian's malted agar, potato dextrose agar and oat meal agar. Minimum media were also used in some cases (Simay 1992b). Identifications were based on Arx (1981), Bánhegyi et al. (1985), Domsch et al. (1980) and Ellis (1971).

Some freshly harvested seeds (211 samples) were stored at room temperature for two years to test for storage fungi such as *Penicillium*. These seeds were tested in the same way as fresh ones (see above).

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حصر للفطور الملاحظة على بذور الفول النابتة على ورق نشاف

الملخص

في سياق دراسة عن أمراض البذور، نُفذت في مركز بحوث علم النبات الزراعي بهنغاريا، تم اختبار 442 عينة من بذور الفول بحثاً عن الفطور. وذلك بحضنها على ورق نشاف رطب بتعقيم سطحها وبدون تعقيم. حُددت الفطور من كلتا الفئتين المعقمة وغير المعقمة، ومن مستنبتات نقية نموذجية. وجد أن هناك 69 نوعاً من الفطور : 33 منها لوحظت على البذور غير المعقمة، في حين تم تحديد 24 نوعاً على البذور المعقمة. وكانت أكثر الفطور المرضة للبذور قدرة وانتشاراً هي : *Fusarium* spp., *Alternaria* spp. و *Trichotecium roseum*. *Ascochyta fabea*، *A. pinodes*، *Botrytis cinerea* و *B. fabae*. كما لوحظت على عدة عينات من البذور. وقد ارتبطت معدلات الحدوث بمجمل الإصابات إلا في حالة *Ascochyta*. كما لوحظت أيضاً بعض فطور التخزين.

Data of occurrence were recorded and rate of infection was calculated. Correlations between of total infection and rate of occurrence of known pathogens *Alternaria*, *Ascochyta*, *Botrytis*, *Fusarium*, *Penicillium* and *Trichotecium* were also calculated.

Results and Discussion

During the tests, 69 taxa of microscopic fungi were identified (Table 1), including saprophytic and known pathogenic species. Gaunt (1983) and Salt (1983) mention *Alternaria*, *Ascochyta*, *Botrytis* and *Fusarium* as economically important pathogens. Lenti (1990) states that *Trichotecium roseum* is an important seedling pathogen in Hungary. The mean rate of occurrence of these species was calculated for all of the 442 samples at first, but the high standard deviation (Table 2a) suggested very heterogenic data. Thus, corrected means were calculated in a second step using only samples which were infected with the organisms (Table 2b).

Table 1. Fungi identified from faba bean seed samples.

Fungus	Occurrence on samples	
	Without surface sterilization	With surface sterilization
Acremonium Link ex Fries sp.	+†	+
A. kiliense Grütz	+	-
A. strictum W. Gams	+	+
Alternaria Nees ex Fries sp.	+	+
A. alternata (Fries) Keissler	+	+
A. tenuissima (Kunze ex Person) Wilts.	+	+
Arthrinium Kunze ex Fries sp.	+	+
Ascochyta fabae Speg.	+	+
A. pinodes Jones	+	-
Aspergillus Mich. ex Fries sp.	-	+
A. flavus Link ex Gray	+	+
A. niger van Tieghem	+	-
Aureobasidium pullulans (de Bary) Arnaud	+	+
Bipolaris sorokiana (Saccardo) Shoemaker	+	-
B. spicifera (Bain.) Subramanian	+	-
Botrytis cinerea Persoon ex France	+	+
B. fabae Sardina	+	+
Chaetomium globosum Kunze ex Steudel	+	-
Chrysosporium Corda sp.	+	-
Cladosporium Link ex Fries sp.	+	+
C. cladosporioides (Fres.) de Vries	+	+
C. herbarum (Persoon) Link ex Gray	+	-
Colletotrichum dematium (Fr.) Grove f.sp. *truncata* (Schwabe) Arx	+	-
Epicoccum purpurascens Ehrenb. ex Schlecht	+	+
Fusarium Link ex Fries sp.	+	+
F. avenaceum (Fr.) Saccardo	+	+
F. culmorum (W.G. Sm.) Saccardo	+	+
F. equiseti (Corda) Saccardo	+	+
F. graminearum Schwabe	+	+
F. oxysporum Schlecht. en. Snyder & Hansen	+	+
F. pallidoroseum (Cooke) Saccardo	+	+
F. solani (Mart.) Sacc. em. Snyder & Hansen *p.p.*	+	+
F. tricinctum (Corda) Saccardo	-	+
F. verticillioides (Sacc.) Nirenberg	+	-

Table 1. Fungi identified from faba bean seed samples (cont.).

Fungus	Occurrence on samples	
	Without surface sterilization	With surface sterilization
Gliocladium Corda sp.	+	+
G. catenulatum Gilman & Abbott	+	+
G. roseum Bain.	+	-
Gonatobotrys simplex Corda	+	-
Harzia acremonioides (Harz) Cost.	+	+
H. verrucosa (Togn.) Hol.-Jech.	-	+
Macrophomina phaseolina (Maubl.) Ash.	+	-
Microascus Zuchal sp.	+	-
Mucor Mich. ex St.-Am. sp.	+	-
M. hiemalis Wehmer	+	-
M. racemosus Fres.	-	+
Papulaspora Preuss sp.	+	-
P. sepedonioides Preuss	+	+
Penicillium Link ex Fries sp.	+	+
Phoma Saccardo sp.	+	+
P. eupyrena Saccardo	+	+
P. glomerata (Corda) Wollenweber & Hochapfel	+	-
P. herbarum Westend.	+	-
P. pinodella (L.K. Jones) Morgan-Jones & Burch	-	+
Phomopsis phaseoli (Desm.) Saccardo	-	+
Pithomyces chartarum (Berk. & Curt.) M.B. Ellis	+	-
Pythium Pringsheim sp.	+	+
Rhizoctonia de Candolle ex Fries sp.	-	+
R. solani Kühn	-	+
Rhizopus Ehrenberg sp.	+	+
R. stolonifer (Ehrenb. ex Link) Lind	+	+
Sclerotinia trifoliorum Eriksson	+	-
Scopulariopsis Bain sp.	+	+
S. brevicaulis (Sacc.) Bain	+	-
Stachybotrys chartarum (Ehrenb. ex Link) Hughes	-	+
Stemphylium botryosum Wallroth	+	+
Trichoderma Persoon ex Fries sp.	+	+
Trichotecium roseum (Pers.) Links ex Gray	+	+
Torula herbarum Persoon ex Gray	+	+
Ulocladium Preuss sp.	+	+

† + fungus present, - fungus not observed.

Alternaria, *Fusarium* and *Trichotecium* predominated on unsterilized seeds, and *Alternaria* and *Fusarium* were abundant on sterilized seeds (Table 2b). The rates of occurrence of fungi were higher on seeds tested without sterilization, but this difference was not significant for *Ascochyta* and *Botrytis*.

Correlations between the occurrence of specific fungi and the total infection were also calculated (Table 3). There were significant correlations for *Alternaria*, *Botrytis*, *Fusarium* and *Trichotecium*, but not for *Ascochyta*.

Table 2a. Mean rate of occurrence (± SD) of some pathogenic fungi on faba bean seeds.

Fungus	Occurrence (%)	
	without surface sterilization	with surface sterilization
Alternaria spp.	8.72 ± 8.95	5.19 ± 5.31
Ascochyta spp.	0.71 ± 1.75	0.73 ± 1.68
Botrytis spp.	0.66 ± 1.93	0.58 ± 1.89
Fusarium spp.	2.15 ± 3.85	1.73 ± 3.03
Trichotecium spp.	2.79 ± 5.31	0.85 ± 2.56
Seed-infection in 442 samples	23.15 ± 18.84	12.81 ± 12.67

Table 2b. Number of infected samples and mean infection rates (± SD) of some seed-pathogenic fungi on faba bean.

Fungus	No. of samples		Occurrence (%)	
	without surface sterilization	with surface sterilization	without surface sterilization	with surface sterilization
Alternaria spp.	393	345	9.80 ± 8.91	6.64 ± 5.14
Ascochyta spp.	71	76	4.42 ± 1.62	4.26 ± 1.15
Botrytis spp.	56	46	5.21 ± 2.37	5.52 ± 2.67
Fusarium spp.	140	140	6.79 ± 3.91	5.45 ± 2.94
Trichotecium spp.	153	61	8.06 ± 6.25	6.18 ± 3.81

Table 3. Correlations of occurrence of some pathogenic fungi with seed-infection rate in 442 samples tested.

Fungus	Correlations (r) for samples	
	without surface sterilization†	with surface sterilization
Alternaria spp.	0.805***	0.818***
Ascochyta spp.	-0.014	0.035
Botrytis spp.	0.297**	0.397***
Fusarium spp.	0.692***	0.622***
Trichotecium spp.	0.546***	0.613***

† Correlation was r=0.73*** between infection rates registred at samples without and with surface sterilization.

** P=0.01, *** P=0.001.

Most of the other fungi are already known to occur on faba bean seeds (Simay 1992a). However, some were observed on this host for the first time in Hungary. *Colletotrichum dematium* was first observed causing seed rot of *V. faba* in Hungary by Simay (1990). Other species recorded for the first time on faba bean in Hungary were *Bipolaris sorokiana* (Fig. 1), *Gliocladium catenulatum* (Simay 1988), *Phoma pinodella* (Simay 1992b) and *Phomopsis phaseoli* (Fig. 2). *Macrophomina phaseolina* and *Rhizoctonia solani* were not observed on faba bean seeds in Hungary previously, but were found in fields (Simay 1987c, 1993). These rather minor pathogens occurred sporadically, but they might have an important economic role if the varieties tolerant or resistant to the present important pathogens like *Ascochyta fabae* (Fig. 3) and *Fusarium oxysporum* (Fig. 4) become widespread.

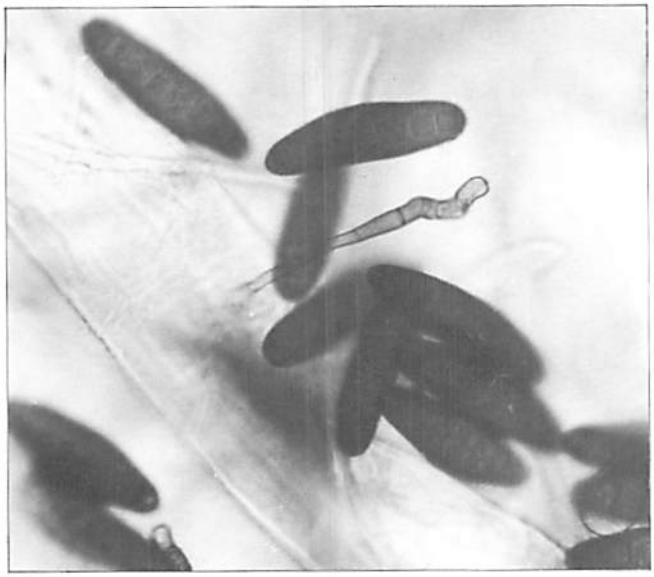

Figure 1. Conidia of *Bipolaris sorokiana*.

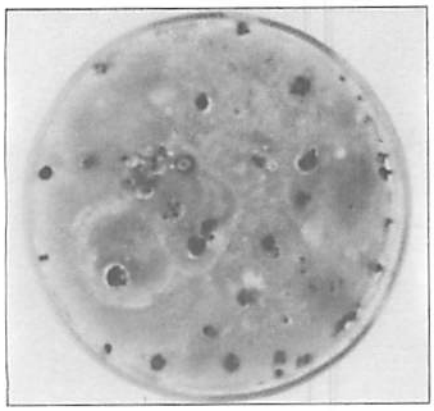

Figure 2. Culture of *Phomopsis phaseoli* with stromatic pycnidial bodies.

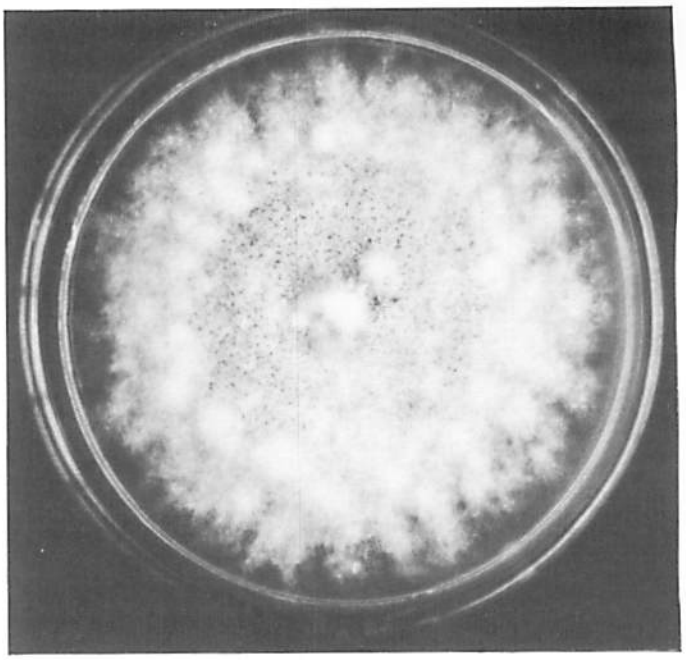

Figure 3. Culture of *Ascochyta fabae*.

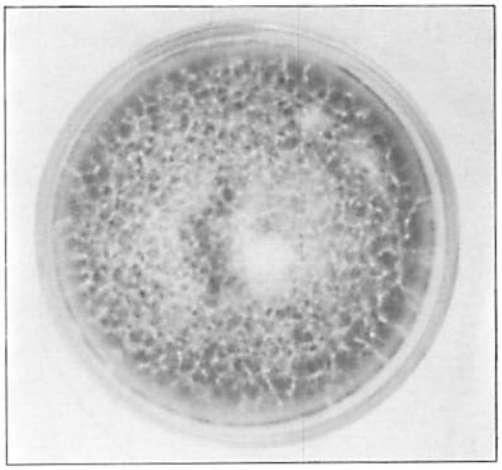

Figure 4. Culture of *Fusarium oxysporum*.

Saprophytic fungi were more abundant on seeds germinated without sterilization (Table 1). Some of them might be weakly parasitic, such as *Cladosporium cladosporioides* (Devi and Singh 1991) and *Papulaspora sepedonioides* (Weresub 1974) (Fig. 5), while others are potentially hyperparasites on or antagonists to parasites like *Acremonium* spp. (Gams 1971) (Fig. 6), *Chaetomium globosum* (Chang and Kommedahl 1968) (Fig. 7) and *Gliocladium catenulatum* (Simay 1988).

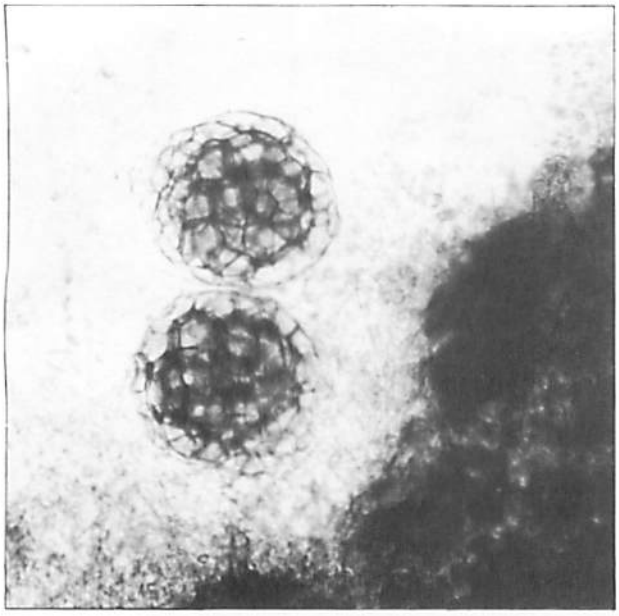

Figure 5. Papulaspores of *Papulaspora sepedonioides*.

Figure 6. ***Acremonium*** **grown between hyphae of *Botrytis*.**

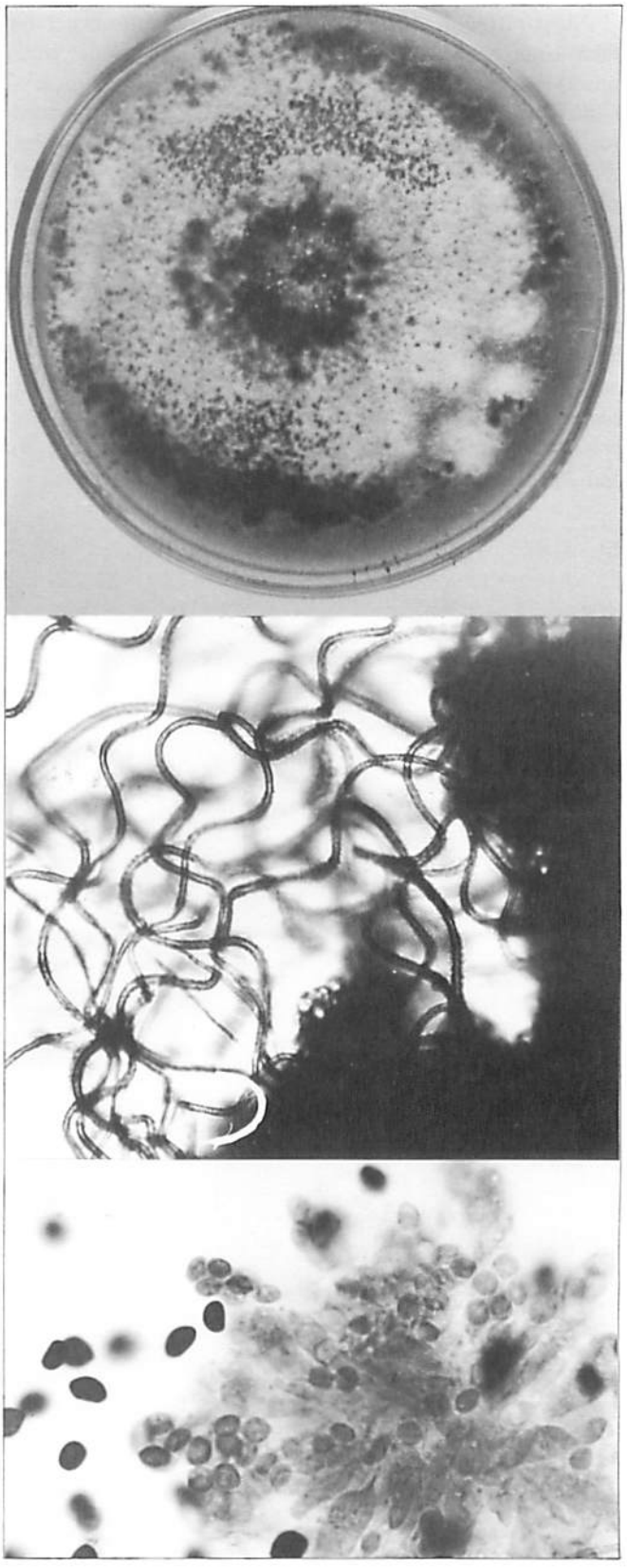

Figure 7. *Chaetomium globosum* (a) culture, (b) terminal hairs of perithecium, (c) asci with spores.

Table 4. Mean rate of occurrence of *Penicillium* (± SD) on stored seeds.

	No. of samples infected		Occurrence (%)	
	without sterilization	with sterilization	without surface sterilization	with surface sterilization
Before storage	193	132	12.54 ± 7.59	7.09 ± 6.74
After storage	209	140	17.58 ± 10.89	7.46 ± 5.60
Total infections				
Before storage			34.68 ± 20.63	19.74 ± 14.97
After storage			28.02 ± 17.99	13.88 ± 10.01

Storage fungi, *Aspergillus* spp. and *Penicillium* sp. (Christensen 1972), were also observed and the *Penicillium* was predominant on seed samples tested after two years of storage. It contaminated 193 and 209 samples tested before and after storage at room temperature and germinated without sterilization. However, *Penicillium* was also abundant on 211 stored samples investigated with sterilization (Table 4).

The correlations of storage *Alternaria*, *Ascochyta*, *Botrytis*, *Fusarium* and *Trichotecium* and total infections were similar to the data for fresh samples, and the *Penicillium* rates also correlated significantly both before and after storage (Table 5).

Table 5. Correlations of occurrence of *Penicillium* with seed infection rate on 211 stored seed samples.

	Correlations (r) for samples	
	without surface sterilization	with surface sterilization
Before storage	0.596***	0.291**
After storage	0.659***	0.618***

This and the significantly higher rate of occurrence on unsterilized seeds, despite the moderately lower total infection, suggest that *Penicillium* replaces field fungi during storage rather than being a primary infection agent. However, this needs further investigation.

Acknowledgements

I thank Mr István Karsai (genebank specialist of faba bean at the Research Centre for Agricultural Botany, Tápiószele) for seeds, and Mrs A. Boros and Mrs M. Jambrits for excellent technical assistance.

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Comparative Study on the Use of Alkaline Phosphatase and Penicillinase Based Direct Antigen Coating ELISA for the Detection of a Potyvirus from Faba Bean

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Abstract

The alkaline phosphatase (ALP) and penicillinase (PNC) based direct antigen coating indirect ELISA systems have been used to detect a potyvirus causing mosaic disease of faba bean (*Vicia faba* L.). The systems were comparable for their suitability for assaying the virus, which was detectable at a dilution of 1:10,000 for antigen and antiserum. The two systems produced ELISA color reaction when the conjugate was used at a dilution of 1:5000 with *p*-nitrophenyl phosphate and bromothymol blue mixture as substrates for the ALP and PNC systems, respectively. Incubation periods of 90 minutes at 27°C were necessary for each step except for the final substrate incubation (60 minutes at room temperature) to obtain detectable reaction. Of the different ELISA plates tested, Titertek brand (polystyrene) was the most sensitive for detecting the virus. The microtitre plates could be kept dry for seven days after coating or moist for 14 days and still give detectable results. Considering the cost

مقارنة طريقتي اختبار إليزا غير المباشر المستندتين إلى إنزيم الفوسفتاز القلوي وإنزيم البنسيليناز للكشف عن مجموعات فيروسات بوتي على الفول

الملخص

تمت مقارنة طريقتي اختبار إليزا غير المباشر المستندتين إلى إنزيم الفوسفتاز القلوي (ALP) وإنزيم البنسيليناز (PNC)، للكشف عن مجموعة فيروسات بوتي المسببة لمرض موزاييك الفول. وكانت الطريقتان متماثلتين في ملاءمتهما لاختبار الفيروس الذي تم اكتشافه عند تخفيف 1: 10،000 للمستخلص النباتي والمصل المضاد. وقد أظهرت الطريقتان رد فعل لوني خاص بإليزا عندما استُخدمت الأجسام المضادة المرتبطة بالإنزيم عند تخفيف 1 : 5000 مع فوسفات النيتروفينول ومزيج البروموثيمول الأزرق كمواد تفاعل في طريقتي إنزيم الفوسفتاز القلوي وإنزيم البنسيليناز على التوالي. وكانت فترة حضانة مدتها 90 دقيقة عند درجة حرارة 27م ضرورية لكل خطوة للحصول على رد فعل يمكن رصده، باستثناء الحضانة الأخيرة لمادة التفاعل (60 دقيقة عند درجة حرارة الغرفة). ومن أطباق إليزا المختلفة التي اختُبرت كانت ماركة Titertek (بوليسترين) أكثر الأطباق حساسية للكشف عن الفيروس، وأمكن حفظ أطباق إليزا جافة لمدة سبعة أيام بعد تغطيتها بالأجسام المضادة أو لمدة 14

and availability of the substrate, the PNC-based ELISA appears to be a better system for routine diagnosis of plant viruses especially in the developing countries.

Key words: *Vicia faba*; faba beans; alkaline phosphatase; potyviruses; ELISA; plant viruses; India.

يوماً رطبة حيث أعطت في كلتا الحالتين نتائج أمكن رصدها. فإذا وضعنا نصب أعيننا الكلفة وتوافر مادة التفاعل، تبين أن اختبار إليزا المستند إلى إنزيم البنسيليناز أفضل طريقة للقيام بتشخيص روتيني للفيروسات النباتية ولاسيما في البلدان النامية.

Introduction

A mosaic disease of faba bean caused by a potyvirus with an incidence of 0–100% was reported by Bhardwaj et al. (1990). However, information on detection of virus through ELISA (enzyme-linked immunosorbent assay) was lacking.

ELISA is now well established as a method for detecting plant viruses, being particularly suited for use in mass indexing programs, epidemiological investigations and other situations involving a large number of samples. Two enzyme systems widely used in ELISA are alkaline phosphatase (ALP) and horseradish peroxidase (HRP). Another enzyme, penicillinase (PNC), has recently been used (Sudarshana and Reddy 1989). This system is especially suited for ELISA testing in developing countries since it is readily available and less expensive. This communication describes to use of ALP- and PNC-based ELISA systems for the detection of a potyvirus strain infecting faba bean from India.

Since early field detection is an important step in disease control, the delay between coating and testing was investigated.

Materials and Methods

The antigen was extracted in coating buffer (Clark and Adams 1977) from infected faba bean plants maintained under insect-proof glasshouse in the Department of Mycology and Plant Pathology, Dr Y.S. Parmar University of Horticulture and Forestry, Solan, India. The direct antigen coating (DAC) procedure described by Hobbs et al. (1987) was used. The plates were coated with 200 µl per well of plant extract prepared in carbonate buffer and washed with washing buffer using procedures followed by Clark and Adams (1977). Antiserum against peanut mottle virus (PMV), a potyvirus, obtained from ICRISAT, Patancheru, India, was used. Rabbit F_c-specific antibodies produced in goats were conjugated to ALP and highly purified PNC by the single-step glutaraldehyde method (Clark and Adams 1977). For the PNC system, the last washing of the plate (just before the addition of the substrate) was done with distilled water containing 0.05% Tween-20.

Four dilutions of the extracted sap and antiserum were used: 1:100, 1:1000, 1:10,000 and 1:100,000. The enzyme-labelled rabbit F_c-specific antibodies were used at three dilutions, 1:1000, 1:5000 and 1:10,000.

After adding antigen, antiserum conjugate and substrate, the plates were incubated for 60 min., 90 min. and 120 min. at 27°C.

In the ALP system, the substrate was prepared fresh every time by dissolving a tablet of *p*-nitrophenyl phosphate (PNPP) in 20 ml of distilled water.

In the PNC system, bromothymol blue (BTB) served as an indicator and was prepared by dissolving 20 mg BTB in 0.2 *M* NaOH and neutralizing with HCl. The volume was made up to 100 ml. Penicillin (0.5 mg/ml) was incorporated as substrate and pH adjusted to 7.2.

Three brands of microtitre plates—polystyrene (Titertek and Laxbro brands) and polyvinyl chloride (Chinese make)—were compared for their relative sensitivity for detecting the virus. All the reactants were used at the same concentrations for coating the plates. The time required for color development was noted.

Results

Both systems (ALP and PNC) worked well and their sensitivities were comparable. Optimization of the concentrations of antigen, antiserum and enzyme-labelled immunoglobins, and minimum incubation period required were worked out for both systems.

Experiments on optimization of the concentration of reactants (antigen and antiserum) showed that the virus was detectable at a dilution of 1:10,000 for both reactants in both systems.

The maximum dilution of enzyme-labelled immunoglobins giving positive reaction was 1:5000 in both cases. Minimum incubation period of 90 min. at 27°C was required for all incubations, except for the substrate incubation which required 60 min. at room temperature to give positive results in both systems.

In positive reactions, the ALP system substrate turned yellow, and the PNC system substrate changed from blue to greenish to deep yellow.

The Titertek brand of polystyrene plate was more sensitive than the Laxbro brand. The time taken to exhibit positive results was 20, 30 and 50 minutes for Titertek, Laxbro and Chinese plates, respectively. Thus, microtitre plates made of polystyrene were more sensitive than the polyvinyl chloride microtitre plates for detecting the present virus.

Storage of coated plates revealed that the microtitre plates could be kept dry for seven days after coating or moist for 14 days after coating without affecting the result.

Discussion

Direct antigen coating indirect ELISA system is capable of detecting viruses at a much higher dilution than the double antibody sandwich ELISA system (Hobbs et al. 1987). In the present investigations both enzyme systems worked well and their sensitivity was comparable; however, the PNC system using BTB indicator was more convenient for visual scoring of the results which is in agreement with the findings of Sudarshana and Reddy (1989), Handa and Bhardwaj (1991) and Bhardwaj et al. (1991).

The maximum dilution of the reactants obtained in this study were similar to those obtained by Jafarpour et al. (1979) who detected bean common mosaic virus from faba bean at the same dilution (1:10,000).

PNC was already known as a desirable enzyme label for ELISA because of its high turnover rate and its commercial availability (Joshi et al. 1978). These features have led to its use for the detection of microbial antigens (Yolken et al. 1984) in addition to several plant viruses (Hobbs et al. 1987; Sudarshana and Reddy 1989). Also, ready-to-use PNC substrate mixture can be stored for longer intervals (though pH needs to be readjusted each time for BTB) than those for the ALP system (Handa and Bhardwaj 1991). PNC can be used with different indicators (Ross and O'Callaghan 1975). For example, the SIC method was used by Joshi et al. (1978), whereas Premier et al. (1985) used BTB. In the present studies, the BTB was more useful than PNPP (ALP system) for visual scoring of the results.

Acknowledgements

We sincerely thank the Legume Virology Unit of ICRISAT for providing antiserum against the virus and conjugation facilities.

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Seed Quality and Nutrition

Chemical Composition of Seeds of 11 Field-grown Faba Bean Cultivars in Two Cultivation Periods

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Abstract

The chemical composition (major constituents, tannins, macro- and micro-nutrients) of ten winter- and one spring-type field-grown faba bean (*Vicia faba* L.) cultivars was studied over two cultivation periods. With the exception of crude protein and iron concentrations, the seasons affected chemical composition significantly. The variation among genotypes was always highly significant and most cultivars exhibited a consistent rank in protein concentration in both seasons. A consistent rank was also observed in the N-free extract, although the values were systematically higher by 7–19% in the second season. This was attributed to the more favorable conditions which prevailed during seed-filling in that season. Protein and N-free extract were inversely correlated, in accordance with previous work. No consistency in the rank among cultivars was observed for either lipid and fiber content; lipids were higher while fiber was lower in the second season. The latter was explained in terms of the higher values of the N-free extract in that period. Tannin concentration varied between 1.35 and 2.3% and exhibited a highly significant positive correlation with total ash and negative correlations with N-free extract and seed yield. Total ash concentration was higher in the first season with little variation among cultivars. Potassium and calcium concentrations were substantially higher than those cited in other works with large variation among cultivars, in contrast with phosphorus and magnesium. No consistent rank among the cultivars in the two years was observed for most minerals, except iron. Significant negative correlations were detected between ash and lipids, ash and N-free extract, iron and average seed weight; whereas positive ones were found between ash and fiber, potassium and lipids, potassium and magnesium, potassium and copper, manganese and fiber, and manganese and magnesium.

التركيب الكيميائي لبذور 11 صنفاً من الفول المزروع في الحقول في فترتين مختلفتين

الملخص

تمت دراسة التركيب الكيميائي (المكونات الرئيسية، التانين، المغذيات الكبرى والصغرى) لعشرة طرز شتوية من الفول وطراز ربيعي واحد مزروعة في الحقول في فترتين مختلفتين. وباستثناء البروتين الخام وتراكيز الحديد، أثر الموسمان في التركيب الكيميائي بصورة كبيرة، وكان التباين بين الطرز الوراثية، وعلى نحو دائم، كبيراً جداً. وأظهرت معظم الأصناف قيمة ثابتة في تركيز البروتين في كلا الموسمين. كما لوحظت قيمة ثابتة في المستخلص الخالي من الآزوت، رغم أن القيم كانت وعلى نحو منتظم، أعلى بنسبة 7–19٪ في الفصل الثاني. ويُعزى هذا إلى الظروف المواتية أكثر التي سادت خلال فترة إمتلاء البذور في ذلك الموسم. وقد ارتبط البروتين والمستخلص الخالي من الآزوت عكسياً وفق بحوث سابقة. ولم يلاحظ تماثل أو توافق في أي من قيم المواد الدسمة ومحتوى الألياف بين الأصناف. ففي حين كانت المواد الدسمة أعلى كانت الألياف أدنى في الموسم الثاني. وتُفسر الأخيرة بالقيم الأعلى للمستخلص الخالي من الآزوت في تلك الفترة. وقد تراوحت نسبة تركيز التانين بين 1.35 و 2.3٪، وكشفت عن ارتباط إيجابي معنوي بدرجة كبيرة بمجموع الرماد، وارتباط سلبي بالمستخلص الخالي من الآزوت والغلة البذرية. وكان مجمل تركيز الرماد أعلى في الموسم الأول مع وجود تباين بسيط بين الأصناف. وكانت تراكيز البوتاس والكالسيوم أعلى بكثير من تلك التي نُوه عنها في دراسات أخرى مع وجود تباين كبير بين الأصناف، وذلك على النقيض من تراكيز الفوسفور والمغنيسيوم. ولم يلاحظ وجود نسبة موحدة لمعظم المعادن بين الأصناف خلال العامين باستثناء الحديد. ولقد تم رصد ارتباطات سلبية معنوية بين الرماد والمواد الدسمة، بين الرماد والمستخلص الخالي من الآزوت، الحديد ومتوسط وزن البذرة، في حين وجدت ارتباطات إيجابية بين الرماد والألياف، البوتاسيوم والمواد الدسمة، البوتاسيوم والمغنيسيوم، البوتاسيوم والنحاس، المنغنيز والألياف، والمنغنيز والمغنيسيوم.

Key words: *Vicia faba*; faba beans; chemical composition; seeds; planting date; genotypes; proteins; ash; lipids; tannins; potassium; phosphorus; magnesium; iron; calcium; Greece.

Introduction

Faba bean is a valuable food crop rich in both proteins and carbohydrates. It is used as animal feed as well as for human consumption. Its nutritional quality has been repeatedly evaluated for humans and different animal species (e.g. Ingals and McKirdy 1974; NcNab and Wilson 1977; Fowler 1980; Ali et al. 1981; Papadopoulos et al. 1991). According to Simpson (1983), feeding value differs among faba bean genotypes reflecting different chemical composition. Thus, available carbohydrate concentration and crude fiber are higher in winter cultivars (Eden 1968; Pritchard et al. 1973), whereas crude and true protein concentrations are higher in spring cultivars (NIAB 1967; Eden 1968; Ford and Hewitt 1980). In addition, considerable variation among cultivars has been detected in other important chemical constituents such as soluble sugars, uncombined amino acids and tannins (Pritchard et al. 1973; Martin-Tanguy et al. 1977; Barratt 1982).

Most of the work mentioned above focused on spring-type cultivars which are almost exclusively cultivated in the higher latitudes of Europe and Canada. In the present study, special attention was paid to the chemical composition of winter cultivars in view of their importance for southern Europe and the Mediterranean basin. The results were taken from an EEC comparative trial of winter-type faba bean cultivars carried out for two cultivating periods. Thus, variations in the chemical composition caused by both genotype and growing season were assessed. Special attention was paid to mineral elements, for which little information was available.

Materials and Methods

The field experiments, part of the European Economic Community Joint Faba Bean Trials with winter-type cultivars, were established at the Athens Agricultural University Farm in Kopaida (100 km north of Athens) during 1985/86 and 1986/87. The soil was an organic silty clay loam. Twelve cultivars were sown in 1985/86 and 13 in 1986/87; full results for both years were obtained from 11 cultivars (Table 1). All of them were of winter-type, except the spring cultivar Troy, which was used as control.

The experiments were set up in a randomized block design with three replicates. The distance between rows was 40 cm, while that between plants within a row varied from 9–10 cm for the small-seeded to 18 cm for the large-seeded cultivars. The crops were sown on 6 December 1985 and 5 December 1986; they were harvested when the pods became dark—on 19 June 1986 and 2 July 1987, respectively. All necessary measures (i.e. seed treatment, chemical and mechanical weed control, and pest control) were taken to ensure satisfactory crop growth.

Different weather conditions prevailed in the two seasons. The second season was considerably colder during early March when the plants were at the 3- to 4-leaf stage. However, it was slightly warmer than the first season during April and June.

Seed yields and 1000-seed weights were determined for each cultivar by harvesting the central 5 m^2 of each plot.

Seed samples from each replicate and cultivar were analyzed for chemical composition. The samples were ground in a laboratory mill over a 1-mm sieve. The analytical procedure of Henneberg and Stohman, as applied by the American Chemical Association (AOAC 1984), was followed. Thus, moisture content was determined by oven-drying at 105°C and total ash by burning at 550°C; crude protein by the Kjeldahl procedure, multiplying the total N by 6.25; total lipids by petroleum ether extraction according to the Soxhlet technique; crude fiber by boiling for 30 min. first in 0.128 *M* H_2SO_4 solution and then in 0.233 *M* KOH solution, using the Fibertec System apparatus (Tecator models 1010 and 1021) with glass filters of 40–90 μm porosity; nitrogen-free extract by subtracting the sum of moisture, ash, nitrogenous compounds, lipids and fiber contents from 100. Phosphorus concentration was determined according to the photometric procedure of the AOAC (1984) using molybdanovanadic ammonium and reading in a spectrophotometer at a wavelength of 400 μm. To determine the concentrations of calcium, magnesium, potassium, iron, copper, zinc and manganese, 1 g of the dry sample was subjected to liquid digestion in a mixture of acids (5 ml HCl + 15 ml HNO_3 + 10 ml $HClO_4$). The concentrate was diluted to a volume of 100 ml with distilled water and read at the appropriate wavelength in an atomic absorption apparatus (AA Perkin Elmer 380 Spectrophotometer). Tannins were determined only for one year (1985/86) according to the method of Burns (1971) as modified by Maxson and Rooney (1972).

Table 1. The origin and classification (according to Higgins et al. 1981) of the faba bean cultivars tested in this work. (The numbers in brackets are the codes used in Tables 5–7.)

Cultivar		Origin	Variety
Brocal	(1)	Superior Technical School of Agronomy, Cordoba, Spain	*equina*
Gemini	(2)	University of Palermo, Italy	*major*
312	(3)	Laboratory of Plant Breeding, University of Thessaloniki, Greece	*equina*
R-29-T	(4)	INRA, Rennes, France	*minor*
Vt-1	(5)	University of Tuscia, Viterbo, Italy	*major*
Troy	(6)	University of Hohenheim, Germany	*minor*
Chiaro TL	(7)	University of Napoli, Italy	*minor*
Palacio	(8)	Superior Technical School of Agronomy, Cordoba, Spain	*equina*
PAM-1	(9)	University of Palermo, Italy	*major*
Alto	(10)	University of Dijon, France	*minor*
ILB 1814	(11)	ICARDA, Aleppo, Syria	*major*

Results and Discussion

The chemical composition of the seeds and its variation among years and cultivars is shown in Table 2.

With the exception of crude protein content and iron concentration, the cultivation periods significantly affected chemical composition, though differently in the constituents examined. Thus, ash, fiber, phosphorus, magnesium and manganese concentrations were higher in the first season, whereas the opposite was true for lipids, N-free extract, potassium, calcium, copper and zinc.

The variation among cultivars was always highly significant, indicating a decisive effect of the genotype on the composition of the seeds. The interaction between cultivars and seasons was always highly significant, too.

A more detailed analysis of the results for the different cultivars and seasons leads to the following conclusions.

Crude protein (Table 3)

The results fell within the range already established for winter cultivars (Eden 1968).

There were some cultivars with systematically high values (27.1–29.7%) in both seasons (e.g. R-29T, PAM-1, Alto, Gemini), while others (Palacio, Troy, 312, ILB-1814) exhibited lower values (between 24.3 and 26.6%). The low values for Troy in both seasons (25.3–25.5%) are unusual for a spring-type cultivar. In agreement with Abdalla and Gunzel (1979), no correlation of proteins with seed size or final yield was observed. Furthermore, no difference between the two seasons was observed. Thus, crude protein appeared to be affected mainly by genotype.

Nitrogen-free extract (Table 3)

The range of the values for N-free extract also fell within that quoted by Eden (1968) for winter cultivars.

As with protein, some cultivars (Brocal, Palacio, 312) exhibited high (57.68–61.47%) and others (R-29T, Chiaro-TL, PAM-1) systematically low (54.55–58.35%) values in both seasons. In agreement with the findings of other investigators (Pritchard et al. 1973; Bhatty 1974; Barratt 1982), a negative correlation was found between protein and N-free extract in the two seasons ($r=-0.58$, $P<0.01$). Seeds were systematically heavier in the second season, possibly reflecting more favorable conditions for seed-filling: with the exception of the large-seeded ILB-1814 and Vt-1, all cultivars produced from 7.1 to 19% heavier seeds in the second season. Given that the amount of rainfall during seed-filling was roughly the same in the two years, the higher temperatures prevailing in the second season (20.3 vs 17.2°C in the first season) could be responsible for the heavier seeds. A broomrape (*Orobanche*) attack during seed-filling in the first season (Karamanos and Avgoulas 1988) may also have contributed to this difference.

In any case, no significant correlation was detected between the average seed weight and the N-free extract.

Table 2. Chemical composition of faba bean seeds of 11 cultivars in two cultivation periods.

	Major constituents (% DW)					Macronutrients (g/kg DW)				Micronutrients (mg/kg DW)				Tannins† (% DW)
	Ash	Prot.	Lipids	Fiber	N-free extr.	K	P	Ca	Mg	Cu	Zn	Mn	Fe	
Total average	4.15	26.8	1.27	9.62	58.2	29.3	8.43	4.52	1.03	8.24	44.6	16.3	172.0	
Season														
1985/86	4.79	26.8	1.05	10.20	57.2	24.0	9.83	3.68	1.40	6.51	38.3	21.0	172.2	1.71
1986/87	3.15	26.9	1.48	9.04	59.1	34.7	7.03	5.35	0.66	9.98	50.8	11.6	171.7	
Sign. level	***	ns	***	***	***	***	***	***	***	***	**	***	ns	
Cultivar														
Brocal	4.02	26.4	0.79	8.34	60.4	26.1	8.11	4.95	1.14	8.02	48.5	14.5	196.9	1.63
Gemini	4.07	27.5	1.34	8.44	58.7	13.3	8.55	5.61	1.14	6.80	39.0	8.5	165.0	1.35
312	4.11	26.5	1.35	9.59	58.4	20.6	7.85	3.69	1.06	5.96	42.0	15.1	123.2	1.71
R-29-T	4.34	29.1	1.29	9.87	55.3	34.0	7.45	4.82	1.07	7.22	34.6	14.2	203.9	1.87
VT-1	3.93	27.0	1.45	9.86	57.8	39.3	8.42	4.01	1.02	5.61	54.4	15.7	115.8	1.62
Troy	5.00	25.3	0.63	10.53	58.5	28.0	8.60	4.60	0.95	7.70	46.2	13.6	192.1	2.26
Chiaro TL	3.89	27.2	1.72	10.42	56.8	34.6	8.85	5.03	1.11	6.03	57.7	15.7	177.4	1.73
Palacio	4.08	24.7	1.13	9.84	60.3	26.8	8.20	4.42	0.96	11.52	55.6	22.1	235.2	1.56
PAM-1	3.98	27.5	1.23	10.02	57.3	35.8	9.31	4.49	0.94	11.32	40.8	18.1	106.4	1.42
Alto	3.97	27.3	1.51	9.42	57.8	36.5	9.00	4.74	1.00	8.13	28.1	23.7	255.6	1.87
ILB 1814	4.29	26.4	1.47	9.45	58.4	27.8	8.39	3.31	0.95	12.37	43.4	17.8	120.3	1.82
Sign. level	***	***	***	***	***	***	**	**	**	**	*	***	***	
Cvs. × seasons	***	***	***	**	***	*	***	***	**	**	*	***	***	

† Data are available only for the first season.
*, **, *** Significant at P = 0.05, 0.01 and 0.001, respectively.

Table 3. The average values of the major constituents (% dry weight) of seeds from 11 faba bean cultivars in two cultivation periods.

Total protein			
Cv.	Season 1	Cv.	Season 2
4	28.6 a	4	29.7 a
1	27.5 ab	2	27.8 ab
10	27.4 ab	9	27.8 ab
9	27.3 ab	7	27.6 b
2	27.1 abc	10	27.2 bc
5	27.1 abc	5	26.9 bcd
7	26.8 abc	3	26.6 bcde
11	26.7 bc	11	26.2 bcde
3	26.5 bc	1	25.4 cde
6	25.5 cd	6	25.3 de
8	24.3 d	8	25.2 e

N-free extract			
Cv.	Season 1	Cv.	Season 2
1	59.3 a	1	61.5 a
2	59.2 a	8	61.4 a
8	59.1 ab	6	60.6 ab
11	57.9 abc	10	59.4 bc
3	57.7 abc	3	59.2 bc
5	56.7 bcd	5	58.9 bc
6	56.4 cd	11	58.8 bc
10	56.3 cd	9	58.4 c
9	56.2 cd	2	58.2 cd
7	55.8 cd	7	57.8 cd
4	54.6 d	4	56.1 d

Total lipids			
Cv.	Season 1	Cv.	Season 2
10	1.63 a	11	2.13 a
7	1.53 ab	4	1.95 ab
5	1.31 abc	7	1.92 ab
8	1.25 abc	2	1.62 ab
3	1.20 abcd	5	1.57 abc
2	1.07 bcde	3	1.50 bc
9	1.02 cde	9	1.45 bc
11	0.82 cdef	10	1.38 bcd
1	0.70 def	8	1.02 cde
4	0.63 ef	1	0.88 de
6	0.42 f	6	0.85 e

Total fiber			
Cv.	Season 1	Cv.	Season 2
7	11.43 a	6	9.95 a
9	11.23 a	7	9.40 b
6	11.12 a	3	9.18 bc
5	10.78 a	4	9.03 bc
8	10.75 a	11	8.97 bc
4	10.72 a	5	8.93 bc
10	10.08 a	8	8.93 bc
3	10.02 a	9	8.80 bc
11	9.93 a	1	8.78 c
2	8.13 b	2	8.75 c
1	7.90 b	10	8.75 c

Ash			
Cv.	Season 1	Cv.	Season 2
6	6.62 a	11	3.88 a
4	5.47 b	5	3.72 ab
11	4.70 bc	2	3.67 abc
10	4.65 bc	9	3.67 abc
8	4.65 bc	3	3.63 abcd
1	4.65 bc	8	3.52 bcde
3	4.60 c	1	3.40 cdef
2	4.48 c	6	3.38 cdef
7	4.43 c	7	3.35 def
9	4.30 c	10	3.28 ef
5	4.15 c	4	3.22 f

Tannins	
Cv.	Season 1
6	2.27 a
4	1.87 ab
10	1.87 ab
11	1.82 bc
7	1.74 bc
3	1.71 bc
1	1.63 bc
5	1.62 bc
8	1.56bc
9	1.42 c
2	1.35 c

Figures in the same column followed by the same letters are not significantly different (P>0.05, Duncan's multiple range test).

Total lipids (Table 3)

Apart from a few cultivars which exhibited systematically high (Chiaro-TL and Vt-1) or low (Troy, Brocal) values in both seasons, no consistent rank among cultivars was observed for total lipids. Nevertheless, as for N-free extract, the values were on average about 43% higher in the second season for all cultivars. The wide range of the values (0.4 to 1.6% in the first and 0.9 to 2.1% in the second season) disagrees with the minor intervarietal variation reported by Eden (1968).

Crude fiber (Table 3)

Apart from the small-seeded Chiaro-TL and Troy which showed consistently high and the medium-seeded Brocal and Gemini which showed low values in both seasons, no consistent rank among the cultivars was observed in the two years for crude fiber. A significant negative correlation ($r=-0.57$, $P<0.01$) was observed between the N-free extract and fiber content, in accordance with the negative correlations observed between storage and structural carbohydrates in many crop plants. In our case, the more favorable conditions for seed-filling may well have been responsible for the about 13% lower values in the second season.

Tannins (Table 3)

The results for one season (1985/86) showed that Troy had the highest (2.27%), while PAM-1 and Gemini had the lowest (1.42 and 1.35%, respectively) tannin content. Tannin content exhibited negative correlations with the N-free extract ($r=-0.60$, $P<0.05$) and final seed yield ($r=-0.79$, $P<0.01$). A high positive correlation ($r=0.83$, $P<0.01$) was also detected between tannin content and total ash.

Total ash (Table 3)

Although little variation among cultivars was observed within each season, significantly lower total ash values (about 36%) were observed in the second season. In fact, the range of values found here for the first season (4.2 to 6.6%) extends well beyond that quoted by Eden (1968) for winter-type cultivars.

With the exception of ILB-1814 which showed high values in both seasons, the rank of cultivars appeared to reverse in the two seasons. The ash content exhibited significant negative correlations with total lipids ($r=-0.59$, $P<0.01$), N-free extract ($r=-0.52$, $P<0.05$) and a positive one with fiber ($r=0.53$, $P<0.05$). A more detailed analysis for the various minerals follows below.

Phosphorus (Table 4). No consistent rank was observed among the cultivars for phosphorus content in the two season. Only PAM-1 tended to exhibit systematically high and 312 systematically low values. In the second season, the values were consistently lower by about 28%. In general, the values were close to those found in other works (White 1966; Eden 1968).

Potassium (Table 4). The values for K content were substantially higher than those cited in other works (White 1966; Eden 1968). The cultivars Vt-1 and Alto showed the highest while Gemini and 312 the lowest values in both seasons. Little consistency in the ranks was observed. The values were 46% higher in the second season. Considerable variation among cultivars occurred, ranging to threefold between the maximum and minimum values. A significant positive correlation with total lipids ($r=0.46$, $P<0.05$) was detected.

Calcium (Table 4). As with K, the values of Ca were considerably higher than those quoted in the works of White (1966) and Eden (1968). This could be ascribed to the high concentration of calcium in the soil; however, no consistent rank pattern was obvious in the two seasons. The values were on average higher in the second season. A remarkable variation among the cultivars of up to fourfold between the two extremes was observed in the first season.

Magnesium (Table 4). In contrast to Ca and K, the values of Mg were comparable to those found in other works (White 1966; Eden 1968). With the exception of a few cultivars (Gemini and Brocal) which showed high values in both seasons, no consistent rank pattern was observed. In the second season, the values were significantly lower than in the first and the variation among cultivars was very small. A high positive correlation ($r=0.65$, $P<0.01$) between Ca and Mg was detected.

Iron (Table 5). The rank of cultivars showed many similarities in the two seasons. Thus, Alto and Palacio showed consistently high (188 to 295 p.p.m.) while Vt-1 and PAM-1 consistently low values (63 to 150 p.p.m.) in both periods. No difference between the seasons was found. A strong negative correlation ($r=-0.62$, $P<0.01$) between Fe-concentration and average seed weight was detected with no obvious interpretation.

Manganese (Table 5). The rank of cultivars for Mn showed some similarities in the two seasons: Alto, ILB-1914 and Palacio had consistently high, while Gemini and Troy had consistently low values. In the second season the values were about 45% higher than in the first. Mn was positively correlated with fiber ($r=0.58$, $P<0.01$) and Mg-concentration ($r=0.58$, $P<0.01$).

Table 4. Average values of the macronutrients (g/kg dry weight) of seeds from 11 faba bean cultivars in two cultivation periods.

Potassium			
Cv.	Season 1	Cv.	Season 2
5	36.9 a	10	43.3 a
7	33.0 ab	9	43.1 a
10	29.7 abc	5	41.7 ab
4	59.5 abc	11	40.9 ab
9	28.4 abc	4	38.5 ab
6	22.3 abc	7	36.2 ab
8	22.3 abc	6	33.6 ab
1	19.2 bc	1	33.1 ab
3	14.9 c	8	31.3 ab
11	14.8 c	3	26.4 bc
2	13.0 c	2	13.6 c

Phosphorus			
Cv.	Season 1	Cv.	Season 2
9	11.0 a	2	8.4 a
7	10.7 a	1	8.0 ab
10	10.7 ab	9	7.7 ab
5	10.3 abc	11	7.4 ab
4	10.0 abcd	6	7.3 ab
6	9.9 abcd	10	7.3 ab
8	9.9 abcd	7	7.0 abc
11	9.4 bcde	5	6.5 abc
3	9.2 cde	8	6.5 abc
2	8.7 de	3	6.5 bc
1	8.3 e	4	4.9 c

Calcium			
Cv.	Season 1	Cv.	Season 2
2	6.1 a	8	6.1 a
7	5.1 ab	6	6.0 a
4	4.8 ab	9	5.9 a
1	4.5 ab	5	5.8 ab
10	4.2 abc	1	5.4 ab
6	3.3 abc	10	5.3 ab
9	3.1 abc	2	5.1 ab
3	2.9 bc	11	5.0 ab
8	2.8 bc	7	5.0 ab
5	2.2 bc	4	4.9 ab
11	1.6 c	3	4.5 b

Magnesium			
Cv.	Season 1	Cv.	Season 2
1	1.6 a	7	0.8 a
2	1.5 a	2	0.8 a
4	1.5 ab	10	0.7 ab
3	1.5 ab	11	0.7 ab
7	1.5 abc	1	0.7 ab
6	1.4 abcd	5	0.7 ab
5	1.4 abcd	9	0.6 ab
10	1.3 bcd	8	0.6 ab
8	1.3 bcd	4	0.6 ab
9	1.3 cd	3	0.6 ab
11	1.2 d	6	0.5 b

Zinc (Table 5). No rank consistency was aparent for Zn concentration in the two years. The values were higher, though not significantly, in the second season.

Copper (Table 5). Apart from some cultivars (ILB-1814, PAM-1, Palacio) which showed systematically higher values in both seasons, no obvious rank consistency was observed for Cu concentration. The values were significantly higher in the second year. A positive correlation was found between Cu and K ($r=0.45$, $P<0.05$).

Table 5. Average values of macronutrients (g/kg dry weight) of seeds from 11 faba bean cultivars in two cultivation periods.

Iron			
Cv.	Season 1	Cv.	Season 2
10	295 a	8	234 a
1	240 ab	10	217 ab
8	236 ab	7	188 abc
6	233 ab	4	188 abc
4	220 abc	2	163 bc
2	167 bcd	1	154 c
7	167 bcd	11	153 c
3	102 cd	6	151 c
11	88 d	9	150 c
5	84 d	5	148 c
9	63 d	3	144 c

Manganese			
Cv.	Season 1	Cv.	Season 2
10	34.6 a	11	15.1 a
8	31.6 a	5	13.8 a
9	25.6 ab	10	12.8 ab
11	20.5 bc	8	12.6 ab
7	19.1 bc	3	12.4 ab
4	18.2 bc	7	12.2 ab
1	18.1 bc	1	11.0 b
3	17.8 bc	9	10.7 b
5	17.6 bc	4	10.2 b
6	17.2 bc	6	9.9 bc
2	10.6 c	2	6.5 c

Zinc			
Cv.	Season 1	Cv.	Season 2
8	52.5 a	7	76.1 a
9	48.9 a	5	74.7 ab
6	48.3 a	8	58.6 abc
11	44.0 ab	3	57.7 abc
1	42.2 ab	1	54.9 abc
7	39.4 ab	2	51.8 abc
5	34.2 ab	6	44.0 abc
10	33.0 ab	11	42.8 abc
4	26.8 b	4	42.4 abc
3	26.3 b	9	32.8 bc
2	26.2 b	10	23.2 c

Copper			
Cv.	Season 1	Cv.	Season 2
10	10.2 a	11	15.9 a
11	8.9 ab	8	15.2 ab
9	8.7 ab	9	13.9 ab
8	7.9 abc	1	11.7 abc
7	6.4 bcd	6	9.6 abc
6	5.8 bcd	4	9.0 abc
4	5.4 cd	2	8.4 bc
2	5.2 cd	3	8.1 bc
5	4.9 cd	5	6.3 c
1	4.4 d	10	6.1 c
3	3.8 d	7	5.6 c

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Protein Content and Trypsin Inhibitor in Raw Faba Bean Seeds

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Abstract

To measure variability in protein and trypsin inhibitor content, 113 lines/varieties of faba bean (*Vicia faba* L.), originating from several countries, were grown at Valenzano (Bari, Italy) in the 1992/93 season. The experimental design was a randomized complete block with two replications. A broad range of variability was observed for both characters, especially for trypsin inhibitor content (CV = 22%). There was no correlation between trypsin inhibitor content and protein content (r = –0.19). Numerous lines/varieties were selected for high protein content (>30%) and low trypsin inhibitor levels (<2000 TIU/g DM).

Key words: *Vicia faba*; faba beans; seeds; protein content; trypsin; Italy.

Introduction

High protein content and quality are important characteristics of pulses. To increase the total content and the quality of seed protein are often important objectives for faba bean breeders. Unfortunately, the nutritive value of legume seeds is limited by the presence of toxic substances that are either indigestible or antagonistic to digestion; examples are alkaloids, phytohemagglutinins, saponins, tannins, cyanogenic factors and trypsin inhibitors (Liener 1982; Gupta 1987; Della Gatta et al. 1989; Hussein 1982). These toxic factors combine with trypsin to form an inactive complex, thereby reducing protein digestion (Feeny et al. 1969; Prabhu et al. 1984). Thus, the content and type of trypsin inhibitors can be used as an important parameter in evaluating the quality of faba bean seeds. In this study, the amounts of seed proteins and trypsin inhibitors in faba bean lines obtained form the Plant Breeding Institute, University of Bari, Italy, were determined.

Materials and Methods

The study was carried out on 113 lines/varieties of faba bean, from different geographical origins, assembled by the Plant Breeding Institute, Bari, Italy (Table 1). The investigations were carried out on material grown in the experimental field at Valenzano (Bari, southern Italy, 41°7'10" N) in 1992/93. The experimental design was randomized complete block with two replications; each plot had 50 plants spaced 80 cm between rows and 20 cm along the row. Observations were made on protein content (percentage on dry matter basis) and trypsin inhibitor content (TIU/g DM). The protein content was determined by Kjeldahl procedure on powdered samples (N × 6.25). For trypsin inhibitor measurement, the raw samples were extracted for 2 h at room temperature with a glycine buffer of pH 11 containing urea and EDTA. The measurement was carried out using a modification of Kakade's method (Della Gatta et al. 1988).

Results and Discussion

Variability analysis

Histograms showing the frequency distributions for protein content and trypsin inhibitor levels are presented in Figures 1 and 2, respectively.

محتوى البروتين والمادة الحائلة لتكون التربسين في بذور فول خام

الملخص

لقياس التفاوت في محتوى البروتين والمادة الحائلة لتكون التربسين في الفول، زرعت 113 سلالة/صنفاً مستقدمة من عدة بلدان، في فالينزانو (باري في إيطاليا) في الموسم الزراعي 93/1992. نفذت التجربة في تصميم للقطع العشوائية الكاملة بمكررين. لوحظ وجود مدى واسعاً من التفاوت بين كلتا الصفتين ولاسيما في محتوى المادة الحائلة لتكون التربسين (CV = 22٪). ولم يكن هناك ارتباط بين محتوى تلك المادة ومحتوى البروتين (r = - 0.19). انتخبت العديد من السلالات/الأصناف لارتفاع محتواها من البروتين (>30٪) ومستوياتها المتدنية من المادة الحائلة لتكون التربسين (<2000 TIU / غ DM).

Table 1. List of the lines and varieties of faba bean analyzed.

83 Lines

L8-4; L8-5; L8-11; L8-12; L8-13; L8-14 violetto; L8-19; L8-23; L8-24; L8-28; L8-29; L8-31; L8-32; L8-33; LA 16/7; LA 22/4; LA 27; LA 32; LA 35/9; LA 34/5; LA-37/10; LA 40/6; LA 43/2 violetto; LA 44/2; LA 44/3; LA 47/10; LA 53/1; LA 56/14; LA 57/1; LA 63/6; LAMA 21; LAMA 72; AD-58/2; AD-58/3; AD-A1/1; AD-A1/3; AD-A1/4; AD-E/1; AD-E/2; AD-E/3; AD-E/4; Dwarf/1; Dwarf/2; Dwarf/3; Dwarf/4; Dwarf/5; 109949; 108951; 108954; 108969; 108969 nero; 109265; 109269; 109270; 109274; 109274 nero; 109281; 109286; 109287; 109292; 113076; 113068; 112925; LA 27 major; AD-A1 major; Dwarf minor; G 13/9A; M.F.Casarano; Sann. 1; Sann. 2; Sann. 3; Sann. 4; Sann. 5; Sann. 6; Sann. 7; Sann. 8; Sann. 9; Sann. 10; Sann. 11; Sann. 12

30 Varieties

Gemini; Aguadulce; Bond; Cannerexpress; Conamore; Felicia; Giza 4; H2OS; Hedosa; Minica; Peleponnes; Rebaya; Rowena; 13/Syrien; 14/Cagnote; 34/Marocco; 91/25; 135/Ethiopen; Vesuvio; Torre Lama Chiaro; Torre Lama Scuro; Simorite; Sikania; Polo; Arbel; Supersimonia

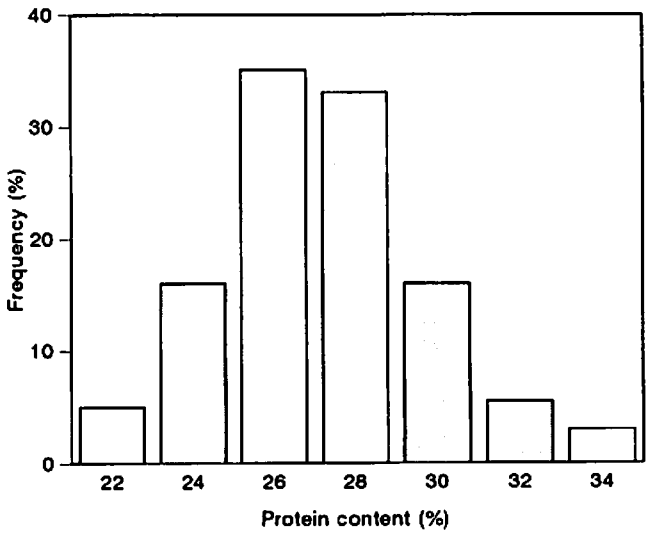

Figure 1. Frequency distribution for protein content in 113 lines/varieties of faba bean.

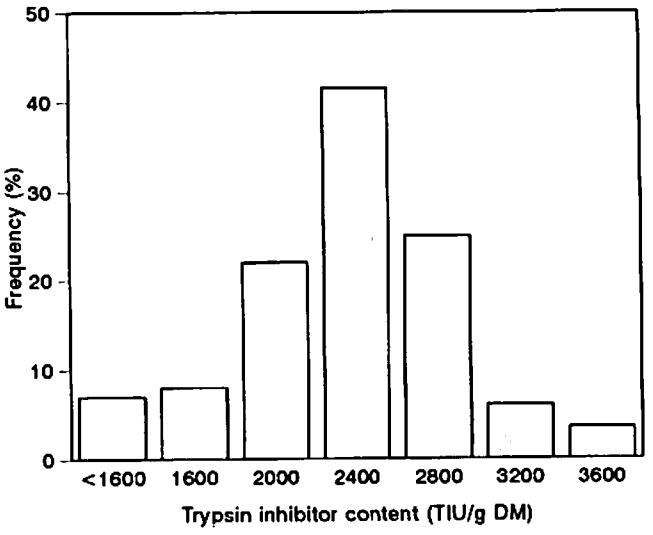

Figure 2. frequency distribution for trypsin inhibitor content in 113 lines/varieties of faba bean.

Protein content

The variability observed for protein content included types with 22–26% (20% of the lines/varieties analyzed), as well as lines/varieties with 32–37% (8% of the total lines). The modal class for this distribution was 26–28%. About 65% of the lines had 26–30% protein content. The mean was 28.27 ± 0.24% with 8.95% coefficient of variation (CV).

Trypsin inhibitor content

A considerable amount of variability was observed among the lines/varieties, with values ranging between 804 and 3613 TIU/g DM. The highest frequency was in the class 2400–2800 TIU/g DM (40% of the lines analyzed). The mean was 2549 ± 53% (CV = 22.07%).

Significant differences (P≤0.01, F test) were observed among lines/varieties for both the characters analyzed. In Table 2, the best lines for protein content (≥30%) and trypsin inhibitor content (<2000 TIU/g DM) are reported; their progeny will constitute the material for subsequent selection work. Considerable differences have been reported within and between faba bean cultivars for protein content and level of trypsin inhibitor in seeds (Frolich et al. 1974; Picard 1977; Baudet and Mosse 1980; Griffiths and Lawes 1977; Hanelt et al. 1978; Filippetti 1979; Filippetti et al. 1985; Bhatty 1974; Bond 1977; Lafiandra et al. 1981).

Table 2. Mean values for protein content (≥30%) and trypsin inhibitor content (<2000 TIU/g DM) for the better lines/varieties of faba bean analyzed.

Line/variety	Protein (%)	Trypsin inhibitor (TIU/g DM)
Sann. 5	30.00	
Torre lama chiaro	30.18	
Sann. 2	30.20	
Felicia	30.24	915
Vesuvio	30.39	1985
Sann. 8	30.40	
Conamore	30.43	
34-Marokko	30.55	
Minica	30.61	1266
Dwarf minor	30.63	
Dwarf 2	30.86	
91/42	31.14	
Sann. 11	31.20	
Rowena	31.32	804
Sann. 12	31.70	
Giza 4	31.75	1427
Sann. 9	31.80	
135-Ethiopien	32.12	
91/36	32.41	
Dwarf 1	32.48	
Rebaya	32.48	
Hedosa	32.96	
H2OS	33.59	
Bond	34.33	868
14-Cagnote	34.41	1749
91/25	36.93	
112925		1979
Aguadulce		1917
109265		1886
L8-23		1833
L8-12		1777
AD-E-4		1606
LA-44/3		1528
LA-27 major		1503
Overall mean	28.27	2549
Range	22.92 – 36.93	804 – 4140

The range of variation for trypsin inhibitor in pea and faba bean seeds is now well established, by many authors, between 700 and 12,000 TIU/g DM. The range 3300–6200 TIU/g DM is given by Valdebouze et al. (1980) for 26 cultivars of faba bean, along with 2700–5400 TIU/g DM and 5700–11700 TIU/g DM for spring and winter pea varieties, respectively. Intra-varietal variability is much less than inter-varietal variability: about 1:2 for the first as compared with 1:6 for the second (Leterme et al. 1993). There is no correlation between trypsin inhibitor content and protein content ($r=-0.19$, $P>0.05$). Faba bean seeds are widely used in animal nutrition because of their high protein content; they and pea have low trypsin inhibitor activity compared with other legumes (e.g. soybean has 40,000–70,000 TIU/g DM). However, a few varieties of faba bean have even lower trypsin inhibitor content, e.g. Minica, Felicia, Bond and Rowena. The results of this study reveal the existence of high variability in faba bean seeds, mainly for trypsin; significant differences were observed among the lines/varieties analyzed. Based on the available data for *Vicia faba*, it seems possible to select and maintain a higher protein level and a low trypsin inhibitor content, after some generations of selection.

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Variety Release Notice

Shambat 616, A Faba Bean Cultivar for Khartoum State and the New Areas of Faba Bean Production South of Khartoum

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Abstract

With the proposal to extend the faba bean (*Vicia faba* L.) growing area in Sudan south of Khartoum, there was a need to develop suitable cultivars. Lines were derived from bulked F_1 crosses made at Hudeiba Research Station, single-plant selections from agricultural schemes in Northern Province, farmers' fields and certain breeding nurseries. Evaluation was through the standard procedure of preliminary, advanced, national and verification yield trials (in comparison with local varieties Hudeiba 72 and BF 2/2). Shambat 616 was approved for release in December 1993. This new cultivar flowers in 41 days and matures in 114 days; it is classified as early/medium maturing. The 100-seed weight is 41–49 g. Shambat 616 has fairly good tolerance of the root rot/wilt complex and to the viral diseases leaf mottle and leaf roll.

Key words: *Vicia faba*; faba beans; varieties; adaptation; disease resistance; root rots; wilts; viroses; maturation; Sudan.

شمباط 616، صنف من الفول للزراعة في محافظة الخرطوم والمناطق الجديدة لإنتاج الفول في جنوبي الخرطوم

الملخص

تنفيذاً للاقتراح بالتوسع في زراعة الفول في جنوبي الخرطوم بالسودان، كان لابد من استنباط أصناف ملائمة. اشتُقت سلالات من مجموعة تهجينات الجيل الأول F1 المنفذة في محطة بحوث الهضيبة، ومن انتخاب النباتات الفردية التي تمت في المشاريع الزراعية في المحافظة الشمالية، ومن حقول المزارعين وبعض مشاتل التربية. وقد تمت عمليات التقييم عبر الإجراءات القياسية لتجارب الغلة الاختبارية الأولية المتقدمة الوطنية (مقارنة بالصنفين المحليين هضيبة 72 و BF 2/2). تمت الموافقة على اعتماد شمباط 616 في شهر كانون الأول/ديسمبر 1993. ويزهر هذا الصنف الجديد في 41 يوماً وينضج في 114 يوماً، وقد صُنف على أنه صنف باكوري/متوسط النضج. يبلغ وزن المئة بذرة 41-49 غ. كما أنه يتمتع باحتمال جيد تماماً للمرض المركب من تعفن الجذور/الذبول وللمرضين الفيروسيين، تبرقش الأوراق والتفاف الأوراق.

Introduction

The faba bean region of cultivation in Sudan extends from the north of the Dongola area (19°10' N, 30°28' E) to Khartoum State (15°36' N, 32°31' E) and south to Gezira, El Rahad and New Halfa. This area covers agro-ecological zones with comparatively cooler and longer winters in the north, and shorter ones in the south. It is anticipated, therefore, that faba bean genotypes would differ in their adaptation in these zones. With the proposed expansion of the faba bean production area in Sudan south of Khartoum, there is also a need to breed new varieties suitable for growing in these areas. The characteristics needed include early flowering and maturity, tolerance to heat, salinity and high exchangeable sodium in the soil, resistance to root rot/wilt complex and a high yielding ability.

According to these criteria, two cultivars – Shambat 75 and Shambat 104 – were released for the new areas in 1991 (Salih and Mohamed 1992; Salih 1992).

Materials and Methods

The genotypes included in these trials were a combination of the bulk of F_1 crosses made at Hudeiba Research Station and progenies of single-*plant selections* collected from the agricultural schemes in Northern Province,

farmers' fields and certain breeding nurseries. The lines were evaluated for seed yield according to the conventional procedure of testing in preliminary, advanced, national and verification trials. Most of the preliminary trials were conducted at Shambat, but the other trials were carried out in a number of locations. The design usually used was randomized complete blocks with various numbers of replicates. The tested inbred lines were compared with either or both of the standard varieties, Hudeiba 72 and BF 2/2. Inter- and intra-ridge spacings were 0.6 and 0.2 m, respectively. Sowing was on both sides of the ridge. The trials were planted in the second half of October or the first half of November and irrigated every 7–10 days. Weeding and other cultural operations were done as necessary.

The attributes measured included seed yield, 100-seed weight, pods/plant and some quality characters.

A statistical stability test was computed for each genotype included in the yield tests of 1990/91, 1991/92 and 1992/93 seasons. The method used was the linear regression coefficient (b) obtained by regressing genotypes' mean yields on the seasonal (environmental) index obtained as the difference between the marginal means and the overall means (Eberhart and Russell 1966).

Results and Discussions

Seed yield

In the 14 yield experiments, line 00616 was best in seven, second in three and third in three experiments; it was thirteenth in one experiment. It out-yielded BF 2/2 or Hudeiba 72, the traditionally grown varieties, by an average of 17.7% (Table 1).

The average grain yield of the genotypes along with their regression coefficient and ranking position are presented in Table 2. On the basis of these two parameters of stability, genotype 00616 was the most stable genotype. It is early flowering and early maturing, which fits well with the short growing-period of Khartoum and the new areas south of Khartoum; it has fairly good tolerance of the root rot/wilt disease complex and viral diseases (leaf mottle virus and leaf roll).

Quality assessment

Quality assessment was done mainly for the eight genotypes included in the national verification trial of 1992/93. Data in Table 3 show that line 00616 had the

Table 1. Line 00616 and mean of Hudeiba 72 and BF 2/2 (checks) yields in different experiments and seasons (kg/ha).

Season	Genotypes		Difference (%)
	00616	Checks	
1982/83	2966	2358	+25.8
1983/84	1750	1302	+34.4
1984/85	1380	1588	-15.0
1985/86	3679	3026	+21.6
1986/87	2078	1725	+20.5
1987/88	3084	2256	+36.7
1988/89	2734	2391	+14.5
1989/90	2785	2868	-2.9
1989/90	2461	1925	+27.8
1989/90	2250	1795	+25.3
1990/91	1033	658	+57.0
1990/91	1436	1252	+14.7
1991/92	3593	3212	+11.9
1992/93	4020	3600	+11.7
Mean	2518	2140	+17.7

Table 2. Mean seed yield, regression coefficient and ranking position average.

Genotype	Mean yield	Regression coefficient (b)	Ranking (average)
00616	3138	0.98	2.58
BB 7	3742	0.82	2.87
00104	1765	1.31	3.00
00634	2933	0.99	3.83
557/80	3831	0.92	4.25
00594	3539	1.21	4.37
00633/II	1498	0.50	4.75
Bulk 1/3	3920	0.95	4.75
00648	2320	0.97	5.12
Hudeiba 72	2796	0.94	5.42
BF 2/2/8/1	2732	1.00	5.50
SM-L	1514	1.05	7.25
H. 72/7	1318	1.00	8.25

Table 3. Yield component and yield quality attributes of faba beans included in the national verification trial of 1992/93.

Genotype	No. of pods/ plant	100-seed weight (g)	Non-soakers (%)	Total defects (%)	Testa (%)	Hydration coefficient (%)	Cookability	Tannic acid (%)	Protein
00616	17.8	44.2	8.9	11.4	13.0	207.3	28.7	0.775	29.18
BB7	20.1	48.4	6.9	9.7	13.0	204.3	24.2	0.830	34.68
Bulk 1/3	21.1	48.4	7.6	9.5	13.6	210.1	27.7	0.870	35.45
00594	20.1	42.4	9.4	13.4	13.2	205.1	25.1	0.860	34.98
00634	20.8	42.3	7.9	11.5	13.5	207.3	23.5	0.520	33.93
557/80	18.7	43.0	8.9	11.8	13.0	206.1	26.1	0.745	35.06
BF 2/2/8/	22.8	40.4	5.2	7.8	12.6	216.9	28.9	0.580	34.94
Hudeiba 72	18.7	40.9	8.2	14.7	13.3	210.0	27.4	0.770	34.55
Mean	20.0	43.7	7.9	11.2	0.20	208.4	26.4	0.743	34.10
S.E.±	0.77	0.30	0.35	0.63	13.1	0.75	1.04	0.02	—

All values included in this table are averages of four locations.

third largest seed making it preferable to consumers compared to small-seeded genotypes. Although line 00616 had the lowest crude protein content in 1992/93, it had the second highest (37.59%) in 1991/92. Line 00616 had the second lowest testa fraction, and the fourth lowest percentage of defective seeds. It had the sixth highest percentage of hard seed (non-soaker), and the fifth highest tannic acid content. It also had the highest percentage of cookability and the fourth highest percentage of hydration coefficient.

Conclusion

On 12 December 1993, the Variety Release Committee of the Agricultural Research Corporation approved the release of line 00616 as a commercial cultivar for Khartoum State and the new faba bean areas south of Khartoum under the name Shambat 616.

Pedigree of cultivar Shambat 616

Shambat 616 was originally a bulk of seed sample collected from a farmer at El Saadanya village in Nile State in 1978. This seed material with unknown origin was registered in the record of the plant breeding section of Shambat Research Station under the designation 00616.

Description of variety Shambat 616

The variety has a vigorous growth habit with a height of 90–110 cm, and an indeterminate stem. Stem thickness ranges between 10 and 15 mm. The plants are resistant to lodging and have basal branching of nearly 3–4 branches per plant. Its leaf is formed from 4–6 leaflets. The leaflet shape is intermediate (sub-elliptic) and of medium size. Inflorescences are multi-flowered and the majority have three flowers formed in the axils of the leaves. The flowers have white petals with wings spotted dark purple.

The genotype takes an average of 41 days to flower and 114 days to mature, hence it can be classified as having early/medium maturity.

The mature pods are erect, flattened, constricted, yellowish-brown and with matted surface. The pod length is about 6 cm and each pod has 3–4 seeds. The majority of the pod-bearing nodes have 2–3 pods. The 100-seed weight ranges from 41 to 49 g. The seeds are angular, mostly with light brown testa and black hilum.

Acknowledgement

I thank ICARDA/Netherlands NVRP for the generous financial support that enabled the execution of this work.

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Salih, F.A. and M.B. Mohamed. 1992. Shambat 75, a faba bean cultivar for El Rahad area of Sudan. *FABIS Newsletter* 30: 17–19.

News

Editors' notes

Publication delays

This double-issue of *FABIS Newsletter* (cover date 1994) is going to press in mid-1995. We apologize for the delays in production. Please note that it will also be necessary for us to produce a double-issue for 1995 in order to bring the sequence back on schedule in 1996.

Reporting of mutants

Papers which report new mutants will not be accepted for publication, unless (1) the mode of inheritance has been determined, (2) seed of the homozygous mutant is provided to the ICARDA Gene Bank (heterozygous for lethal and semi-lethal mutants), and (3) a gene symbol is proposed in line with the system outlined for *Pisum* L. in *Pisum Newsletter* 9: 67–70 (1977).

Faba bean in AGRIS

Recipients are reminded that this title ceased publication after 1990 (vol. 6). As most of you are aware, faba bean references are now published in an annual supplement to *FABIS Newsletter*.

Variety yield trial data

It is the editors' policy not to publish papers which use data from variety trials grown at one location in one year. We may, however, be prepared to consider such papers when exceptional circumstances are involved, such as when a large number of entries are used, when the genetic diversity is particularly high, or when an unusual trait is discussed.

Conferences

1995

***American Phytopathological Society Annual Meeting*, Pittsburgh, PA, USA.** Contact: APS Headquarters, 3340 Pilot Knob Road, St Paul, MN 55121, USA.

1996

***6th International Parasitic Weed Symposium*, Cordoba, Spain, tentative.** Contact: Dr Maria Teresa Moreno, Centro de Investigacion y Desarrollo Agrario, Apartado 4240, 14080 Cordoba, Spain [Fax +34-57-202721; Telex 76686].

1997

***International Food Legume Research Conference III*, Adelaide, Australia, 22–26 September 1997.** Contact: Dr F.J. Muehlbauer, Chair: IFLRC-III, 303W Johnson Hall, Washington State University, Pulman, WA 99164-6434, USA [Tel. +1-509-335-9521; Fax +1-509-335-8674]; or, Prof. R.J. Summerfield, Program Chairman IFLRC-III, Department of Agriculture, University of Reading, Early Gate, Reading, Berkshire RG6 2AT, UK [Tel. +44-734-318482; Fax +44-734-352421; Telex 847813].

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Kenya
Korea (Republic)
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Here is your opportunity to make your set of *FABIS Newsletters* and *Faba bean in AGRIS* complete! ICARDA Distribution Office needs to make space, so we are going to dispose of all pre-1990 stocks of our three crop-oriented newsletters and bibliographies. We are therefore offering to dispatch near-complete sets and odd back issues to faba bean researchers and libraries who have missing copies, or who only recently began to subscribe to the newsletter. Hurry! Do not delay! Stocks will not last and some issues are very rare. Write *today* to: FABIS Newsletter, ICARDA, P.O. Box 5466, Aleppo, Syria.

STOP PRESS: Copies of some issues rediscovered! However, the following are **definitely** out of print.

FABIS Newsletter Nos. 12, 26.
Faba bean in AGRIS Vols. 2-5.

ICARDA Publications and Services

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

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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.

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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.

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The ICARDA library maintains bibliographic databases for the use of researchers at the center and elsewhere. FABIS and LENS databases contain 5000 and 1500 references, respectively, extracted from AGRIS since 1975. Literature searches can be conducted by the library staff and results downloaded to diskette or hard copy. Photocopies of articles identified in a literature search can be provided to users, if available. Researchers can request a literature search by letter or telex to: Library.

Visuals
ICARDA has produced three slide/tape modules dealing with legume hybridization techniques. The three programs, *Hybridization Techniques in Chickpea*, *Hybridization Techniques in Lentil*, and *Hybridization Techniques in Faba Bean*, discuss the morphology of the flowers, crossing block layout, and emasculation and pollination techniques.

A further module, *Introduction to Biological Nitrogen Fixation*, explains the role of nitrogen in agriculture, what biological nitrogen fixation is, how it benefits farmers, how it can be practised and how other crops benefit from it.

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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.

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Manuscript

Contributions should be sent to FABIS/CODIS, ICARDA, P.O. Box 5466, Aleppo, Syria. The name, address, 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. Figures should be original drawings, good-quality computer 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, Materials and Methods, Results, Discussion, Conclusions and References. Articles will be edited to maintain uniform style, but substantial editing will be referred to 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. 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, eds). Martinus Nijhoff Publ., 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, eds). 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-032/1500/ August 1995

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