



# FABIS

## Faba Bean Information Service

NEWSLETTER

No. 7

DECEMBER 1983

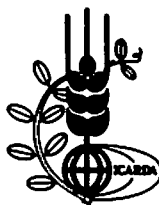


THE INTERNATIONAL CENTER FOR AGRICULTURAL RESEARCH IN THE DRY AREAS  
(ICARDA)

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## GENERAL ARTICLES

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### ANTIMETABOLITES IN FABA BEANS: THEIR METABOLIC SIGNIFICANCE

Ronald R. Marquardt

*Department of Animal Science, University of Manitoba  
Winnipeg, Manitoba R3T 2N2 CANADA*

#### Introduction

Faba beans are an excellent food for humans, being high in protein and easily digestible. They contain compounds, however, that may be deleterious. This review attempts to identify the possible antimetabolites and to assess their overall importance with regard to the nutritive value of faba beans.

#### Phytates

The phytate contents of several cultivars of whole and dehulled faba beans (cotyledons) were reported to be 1.50 and 1.82% (Griffiths, 1982). The difference among cultivars is generally small but considerable interplant variation has been observed (Griffiths, 1982; Griffiths and Thomas, 1981). In general this variation has been associated with a variation in phosphorus content of the seed. In another study the phytic acid content of dry beans (*Phaseolus vulgaris*), soya beans, barley, wheat and rapeseed were found to be 1, 1.0-1.5, 1.0, 0.6-1.4 and 3.5%, respectively (Maga, 1982). These results would suggest that the phytate content of faba beans is not excessively high compared to other common foodstuffs and therefore may not seriously affect its nutritional value.

#### Unavailable carbohydrates

Pritchard *et al.* (1973) using chemical analysis estimated the percentage of carbohydrates in 8 cultivars of faba beans that were unavailable for utilisation by monogastric animals at 19-20% in winter-sown beans and 22-37% in spring-sown beans. The unavailable carbohydrates were composed of approximately 3-6% water-soluble polysaccharides with the predominant carbohydrate being uronic acid, 3-13% hemicelluloses, 4-6% cellulose and 5-7% lignin. These carbohydrates are unavailable for utilisation by monogastric animals, which include humans, and may also inhibit the utilisation of other nutrients in a manner similar to that described for other carbohydrates (Leeds, 1982). The actual digestibility of these carbohydrates, however, and the degree to which they interfere with nutrient utilisation, have not been established.

#### Hypocholesterolemic factor

Studies by Jaya *et al.* (1981) demonstrated that faba bean protein concentrate was able to reduce serum cholesterol levels in rats fed a high-fat, high-cholesterol diet; there was a marked increase in the excretions of faecal bile acids, particularly cholic acid, in animals fed faba bean as compared to the control. They hypothesised that saponins, which have a cholesterol-lowering effect, were the causative agent as they are present in faba beans at rather high levels (3.7%). They also suggested that the fibre component was not the responsible agent, but this may not be completely correct in view of the rather high levels of fibre-like material in faba beans and its effect on fat digestibility (Leeds, 1982). Additional research will be required to establish the relative importance of different compounds. Overall it may be concluded that faba beans contain a lipid lowering factor that selectively reduces their nutritional value. This may be advantageous in humans since excessive caloric intake, particularly in the form of fat, is generally considered to be undesirable.

#### Protease inhibitor

Substances that inhibit proteolytic activity of certain enzymes are found throughout the plant kingdom, particularly among the legumes. Much of the research on the mode of action and effects of protease inhibitors has been carried out on soya beans (see Liener and Kakade, 1980, for a review).

Faba beans have been reported to contain a heat labile trypsin inhibitor at approximately 10-20% of the concentration found in raw soya beans (Wilson *et al.*, 1972; Marquardt *et al.*, 1975 and Ward *et al.*, 1977) which would suggest that its effect would be minimal compared with soya beans. The low trypsin inhibitor activity of faba beans also does not appear to affect animal performance. Wilson *et al.* (1972 and 1972a) isolated an extract with a high trypsin-inhibiting activity from faba beans and observed that it had no effect on chick growth when incorporated into the diet. Results reported by Marquardt *et al.* (1976) support these conclusions, as there was not a corresponding improvement in the nutritional value of faba beans with heat treatment and the destruction of the trypsin inhibitor. Abbey *et al.* (1979) using the rat as the experimental animal also concluded that proteinase inhibitors were not the main factors responsible for the growth depression observed when raw faba bean meal is fed.

It may be concluded that the trypsin inhibitor in faba beans is of minor concern to humans as it would have only a slight effect on nutrient utilisation if consumed in its raw form and would be readily inactivated when subjected to cooking temperatures.

#### Lectin (haemagglutinins)

Lectins are proteins that possess the remarkable ability to agglutinate erythrocytes and other cell types. They occur widely in nature, but are mainly found in the seeds of plants, particularly those of the legumes. The toxicity of the thermolabile lectins depends upon the source of the lectin and the experimental animal. In order to exhibit a toxic action when ingested orally, a lectin must resist digestion (Jaffe, 1980).

Faba bean lectins, which are located in the cotyledon but not testa portion of the bean (Ward *et al.*, 1977), have been isolated in pure form and some of the physical and chemical properties have been established (Wang *et al.*, 1974). Very little research has been carried out on their toxic effects. Raw faba beans, however, when fed to chicks (Marquardt *et al.*, 1976) or rats (Abbey *et al.*, 1979) at levels of greater than 85% and 50% respectively at worst depressed the rate of growth but did not increase mortalities in any experiment. These results would suggest that faba bean lectins are either nontoxic or are inactivated in the stomach. Studies by Marquardt *et al.* (1976) also demonstrated that there was not a direct inverse relationship between loss of lectin activity and corresponding improvement in chick performance when faba beans were autoclaved for different time periods.

It may be concluded that oral ingestion of faba bean lectins does not adversely affect chick or rat performance and probably does not affect humans. Even if native lectins do affect humans, they would not be a problem as they would be denatured by cooking.

#### Condensed tannins (proanthocyanidins)

Tannins are characterised by their ability to interact with and precipitate proteins. They appear to be responsible for the astringency of many plant materials and decrease the nutritive value of certain foodstuffs. The presence of tannins in grain crops is relatively rare, occurring only in selected strains of sorghum and barley, and in faba beans. Dickinson *et al.* (1957) originally suggested that faba beans contained condensed tannins and that the suitability for canning of white-flowered varieties was due to the absence of condensed tannins in the seed coat.

Studies on the nutritional significance of condensed tannins have been carried out by different research groups. Bond (1976) demonstrated that *in vitro* dry matter digestibility of whole grain from tannin-free white-flowered cultivars of faba beans was about 5% higher than the tannin-containing colored-flowered varieties and that this difference was due largely to differences in digestibilities of the testa. Marquardt *et al.* (1978) demonstrated that the testa from tannin-free cultivars of faba beans had higher concentrations of acid-detergent-fibre and cellulose, a lower concentration of lignin and a lower percentage of dry matter than the testa from tannin-containing cultivars.

Feeding trial studies by Marquardt *et al.* (1977) and Martin-Tanguy *et al.* (1977) demonstrated that purified condensed tannin from faba beans, when incorporated into a chick diet, markedly reduced feed intake, weight gains, efficiency of feed utilisation and dry matter and amino acid retentions. In another study Marquardt and Ward (1979) demonstrated that similar responses were obtained for birds fed tannin-free as compared to tannin-containing cultivars of faba beans. Heat treatment of both tannin-containing and tannin-free cultivars resulted in improved animal performance, though to a lesser extent in the latter case. This suggests that part of the response to heat treatment was due to the destruction of condensed tannins and part to an effect of heat treatment on some other factor. Heat treatment would have denatured nearly all of the protein, which would presumably have increased its digestibility and therefore its nutritive value.

Solvolysis studies by Cansfield *et al.* (1980) of condensed tannins, purified by using Sephadex LH-20 chromatography, demonstrated that they contained different proanthocyanidin molecules. They differ from one another in the length of the polymer chain (degree of polymerisation), composition of the polymer chain (relative proportions of cyanidin and delphinidin generating species), sequencing of monomers in the polymer and oxidation effects. Flavan 3-ols (catechins) and flavan 3,4-diols (leucoanthocyanidins) were not detected, in contrast to results presented by Martin-Tanguy *et al.* (1977) who used a different isolation procedure.

It may be concluded that condensed tannins are present in the testa of faba beans in moderate amounts and that they reduce its nutritive value by decreasing the availability of certain nutrients, particularly amino acids. Condensed tannins in faba beans, however, can be eliminated by the use of appropriate processing methods such as dehulling (Ward *et al.*, 1977), imbibition of basic, neutral or acidic aqueous solutions (Reichert *et al.*, 1980) or heat treatment, or by the use of tannin-free cultivars. With

regard to human foods, condensed tannins presumably do not greatly affect the nutritional value as most of their effects would be eliminated when prepared for consumption.

### Vicine and Convicine

A number of papers and reviews on the mode by which vicine and convicine induce favism in humans and metabolic disturbances in animals have been published (Mager *et al.*, 1980; Marquardt, 1982; Muduuli *et al.*, 1981 and 1982; Chevion *et al.*, 1982; Hegazy and Marquardt, 1983a; Frohlich and Marquardt, 1983). The results of these studies would suggest that vicine and convicine, which are present in faba beans, are not only a potential hazard when consumed by favism-susceptible individuals but possibly may also have some long-term effects on human health. Studies with animal models, particularly the laying hen, should assist in clarifying some of the potential chronic effects of faba bean consumption.

One of the reasons why vicine and convicine are a potential problem in faba beans is that they are not readily destroyed by heat treatment (Olaboro *et al.*, 1981) or when prepared for human consumption (Hegazy and Marquardt, 1983). Vicine and convicine can be removed from foodstuffs by extraction in an aqueous media (Hegazy and Marquardt, 1983) or by hydrolysis of the glycosides with the enzyme,  $\beta$ -glucosidase, (Kim and Hoehn, 1981). These procedures are time consuming and will need considerable refinement before they are ready for wide use. A more satisfactory solution would be the development of cultivars of faba beans that are free of vicine and convicine but, to date, no such lines have been identified.

### Conclusions

It would seem that there are several potential anti-metabolites in faba beans, including phytates, certain unavailable carbohydrates, a hypocholesterolemic factor, a protease inhibitor, lectins, condensed tannins, vicine, convicine and possibly other unidentified factors. Certain factors such as the protease (trypsin) inhibitor, lectins and condensed tannins are readily destroyed by cooking, and therefore should not be considered to be a serious problem when faba beans are used as a human food. The hypocholesterolemic factor and possibly certain unavailable carbohydrates may be beneficial rather than deleterious in that they would tend to reduce cholesterol absorption and thereby depress plasma cholesterol levels, a factor associated with heart disease in humans. A specific carbohydrate component, however, may also cause flatulence. Phytates which complex certain cations are present in faba beans but

their concentrations are not excessively high compared with other legumes and grain seeds, and may therefore have only a minor effect on the nutritional value. Vicine and convicine, however, are potentially harmful; they may cause favism and induce other metabolic effects in humans. Additional research should be carried out to clarify the effects of vicine and convicine in favism-susceptible and favism-nonsusceptible individuals and to improve the means of reducing or eliminating them.

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# SHORT COMMUNICATIONS

## General

### THE FABA BEAN CROP IN MEXICO

Luis Osoria,  
*Agricultural Research Station, Chapingo, MEXICO*

#### Introduction

According to Box (1961) the faba bean (*Vicia faba*) was first cultivated by Neolithic Man in the Mediterranean basin, spreading later to more northern countries by the Bronze and Iron Ages. The nutritional value of the faba bean was particularly appreciated by the Egyptians, Greeks and Romans. The faba bean was introduced to the New World after the discovery of America, the first recorded crop being in 1602 on the eastern seaboard of North America.

#### Production areas in Mexico

At present the faba bean crop occupies some 48,000 hectares of land in the central regions of the country at altitudes of between 1800 and 2600 meters above sea level.

Average yield from the crop is 725 kg/ha, with the greater proportion of the production coming from Mexico, Puebla and Tlaxcala states. Of these, Mexico state is the largest single producer, in terms of both area planted and total yield (Table 1), providing 38% of the national output. The state has a number of advantages for production of the crop; favourable climate and soil conditions, and its proximity to Mexico City, which is the single most important outlet for the produce and the centre for distribution to the rest of the country.

In Mexico State the climate is humid, warm temperate in nature, with annual average temperatures of 16°C and dry winters. The predominant soils on which the crop is grown are of volcanic origin. Texture varies from clay crumb to sandy crumb to sand.

#### Uses of the crop

Faba bean is only used for human consumption in Mexico. Average annual per capita consumption is approximately 800 g, national production currently meeting demand. Although detailed statistics are not available, it has been observed that the majority of the consumption is in the form of dry seeds rather than fresh green seeds.

Table 1. Dry faba bean production in Mexico.

States	Area (ha)	Average yield (kg/ha)	Percentage of total production
Mexico	18,212	1,088	38
Puebla	8,662	651	18
Tlaxcala	5,318	1,645	11
Hidalgo	5,477	500	11
Veracruz	5,027	802	10
Michoacan	2,145	731	4
Subtotal	44,841		93
Other states	3,181	600	7
Total	48,022		100

\*Anuario Estadístico 1978, SARH, Mexico.

#### Cultivation

Most of the crop is grown on a subsistence basis, for consumption by the farmer and his family. Generally, no fertiliser is used, and availability of water for irrigation is restricted.

Planting is usually along rows 80 cm apart, with 30 to 40 cm between hills and two to three seeds per hill. However, in the western part of the country (Jalisco and Guanajuato States) inter- and intra-row spacings of 42 and 40-60 cm, respectively, are the norms.

Planting date depends upon the type of product required. For fresh consumption the crop is planted in October to November, while planting for dry seed production takes place in February through to the end of May. In the latter case planting is not mechanised, since the seeds must be placed very deep in order to utilise residual moisture from the previous season's rains.

Faba beans are also grown as an inter-crop with corn (*Zea mays*). In this case, the rows are 80 to 100 cm apart, with inter-plant spacing of 20 to 30 cm, alternating between bean and corn plants. As for monoculture, two to three seeds are planted per hill. Planting takes place in February and April, and irrigation may be used to establish the crop.

With spring planting the incidence of disease is low due to the low relative humidity during the plant development phase.

## Diseases

The most prevalent diseases of the faba bean crop in Mexico are the foliar diseases caused by *Alternaria* spp., *Botrytis* spp., *Uromyces fabae* and *Erysiphe* spp., with virus diseases and *Fusarium* root rots being of lesser importance (Campos, 1976).

## Pests

There are two pests of economic importance; *Aphis fabae*, which damages the growing tips of the plant, and *Macrodactylus* sp., which attacks the leaves of the plant.

## Breeding

Research work on the faba bean started at the National Institute of Agricultural Research (INIA) some six years ago. Germplasm from local ecotypes and introduced material has been undergoing evaluation for physical and agronomic characteristics.

The farmers of the region largely plant a local cultivar, "Criolla", of the *major* variety, from which many ecotypes have been selected empirically.

Amongst this material considerable variability has been found for plant height (1.5 to 2.5 m), number of seeds per pod (one to four), seed colour (yellow, cream, purple and variegated) and days to maturity (120 to 150). However, all the material tested was susceptible to the diseases mentioned above.

Selections have been made from the local material for use in the breeding programs. However, the lack of resistance to the diseases noted above, and the low yield potentials of the cultivars currently in use highlights the need to expand the germplasm available to the breeding programs by introduction of cultivars from other sources.

Yield trials on the local cultivars have identified a number of lines which yield well either in monoculture or intercropped with corn. Foremost among these are Puebla-15, Tlaxcala-13 and Mexico-2 for monoculture, and Pueblo-11 and Mexico-2 for intercropping (Crispin, Sanchez, and Perez, 1978).

## Conclusion

While there are a number of problems associated with the cultivation of faba beans in Mexico at present, there is considerable scope for overcoming them through the introduction of improved cultivars and through breeding for improved yield and disease resistance among the local cultivars.

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## FABA BEANS IN GREECE: PAST AND FUTURE

D.G. Roupakias

*Department of Genetics and Plant Breeding, School of Agriculture, Aristotelian University of Thessaloniki, GREECE*

Faba bean (*Vicia faba*) has been cultivated in Greece since ancient times. During the era of Homer (8th century BC) faba bean seeds were known by the name of *Kyamos* (Iliad N, 589), and *Kyamos Hellenicos* (broad beans from Greece) were known as a national hellenic crop since the Bronze Age (Panos, 1960). Homer refers to faba beans being black seeded (Iliad N, 589). From what is reported by Theocritus (Idyll VII, 66, cited by Kavadas, 1956) it is concluded that faba beans were used for human consumption either as green pods or dry seeds, both raw and cooked. Backer-Dillinger (1929, cited by Panos, 1960) reported that the crop was dedicated by the Athenians to a God whose name day was celebrated in June. In ancient Greece faba beans were also used for voting purposes, putting the seeds as white or black "ballots" (Thucydides 8, 66, cited by Kavados, 1956).

Although faba beans have been cultivated in Greece for centuries and have been included in experimental trials since 1933, the area covered is still very limited. Faba beans have never been widely cultivated in modern Greece (Fig. 1), and the area has been further reduced during the last 30 years. Varieties grown were the broad bean (*Vicia faba* var. *major*), and were mainly used for human consumption (Panos, 1960). Field beans (*V. faba* var. *minor*) were introduced to the country in 1958 (Stylopoulos, personal communication), and were studied intensively at the Forage Crops Research Institute of Larissa from 1962 to 1969. At that time, a severe infection of sclerotinia stem blight (*Sclerotinia sclerotiorum*) made the researchers change their goal from breeding for high yield to breeding for resistance to sclerotinia stem blight.

In the trials from 1962 to 1969, two selections were found which were well adapted to the conditions in Greece; "Polycarpe", selected from the small-seeded Italian variety "Vesuvio"; and "Tanagra", selected from the Moroccan variety "10-868". However, field beans were not grown commercially in Greece until recently.

The main reasons for the decline in the area under faba beans in modern Greece are as follows:

1. During the 1940s and 1950s faba beans were used as a protein source for human consumption. However, with the subsequent rapid increase in the standard of living during the 1950s and 1960s, people began to turn to other protein sources such as meat, eggs, and dairy products. In addition, faba beans lost their importance as a horticultural crop since people preferred green beans (*Phaseolus vulgaris*), of which new varieties had become available suitable for canning and freezing.
2. With the mechanisation of Greek agriculture after the Second World War, farmers switched to mechanically harvestable crops, mainly cereals. In addition, the country was striving to achieve self-sufficiency in cereal production at that time, and the Government actively supported cereal production, resulting in neglect in pulse production.
3. The relatively low yields of faba beans compared to other crops (Fig. 1), and a lack of new varieties coming on the market to off-set this, due to the lack of research on the crop throughout the world.

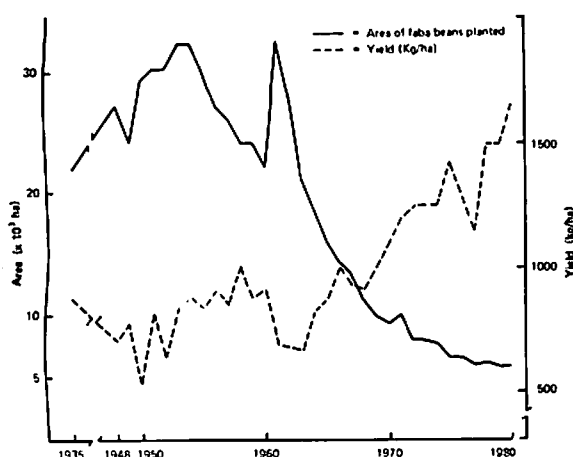


Fig 1. Changes in area planted and yield (kg/ha) for faba beans in Greece 1935-1980.

4. Susceptibility to a number of diseases. The first drastic decline in the acreage of faba beans (1956-60, Fig. 1) coincided with an increase in ascochyta blight infection (Stylopoulos, personal communication).

5. Field beans were never grown in Greece as a protein source for animals, this being provided largely by imported soya bean.

The last three factors are related to the limited extent of research on the crop.

The abrupt increases in the price of soya bean in the early 1970s led to an increase in faba bean research in many countries which are not traditionally soya bean producers, e.g., Canada and the European countries. These studies have shown faba beans to have a yield potential equal, or even superior, to that of wheat (Evans *et al.*, 1972), and to possess satisfactory nutritional value for animal feed (Marquardt *et al.*, 1974; Stothers, 1974; Ingalls *et al.*, 1974; Petersen and Schultz, 1980).

There is a need for a leguminous crop in the rotation system of the rainfed area of Greece, which amounts to 56% of the agricultural land and which is mostly under continuous cereals at present. There is also a need for a high protein animal feed to replace the importation of expensive soya bean.

Soya bean has little potential in Greek agriculture, being a spring crop, and thus requiring irrigation, coming into direct competition with other crops which give a higher return, e.g., cotton, maize, sugar beet, tomatoes. It is also of no applicability to the rain-fed areas.

However, faba beans as a winter-sown crop is suitable for rain-fed areas, and may be able to fulfill the needs for both high protein animal feed, and the rotation.

Recently there has been an increase in the interest in the crop from the farmers. However, the area is likely to remain limited until the following requirements are met:

1. Development of cultivars suitable for mechanical harvesting.
2. Development of cultivars with improved disease resistance.
3. Availability of suitable herbicides for weed control.
4. Use of faba beans in industrial formulation of animal feeds.

The last point is important to ensure an outlet for the produce.

If the above requirements are met, faba beans could be grown on more than 300,000 hectares, assuming that the area presently under cereals (1,354,400 ha) will include faba beans in the rotation one year in four.

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## EFFECT OF SOIL SALINITY AND ALKALINITY ON FAB BEAN GROWTH IN THE SUDAN – A REVIEW

Farouk A. Salih

Shambat Research Station, P.O.Box 30, Khartoum North, SUDAN

Faba beans are traditionally grown in Sudan on fertile "Gureir" soils, a silt loam deposited in a narrow strip along the banks of the Nile. Due to the scarcity of fertile soils, and the high demand for faba beans, coupled with its ever increasing price, new lands of lower fertility, like "Kuru and Terrace" which are affected by various degrees of salinity and alkalinity are coming into production in the Nile, Northern and Khartoum Provinces. El Karouri (1966) showed that salinity and alkalinity in these soils were of common occurrence, and that the ionic composition of salts and their distribution varied with depth and site. Sodium and calcium were the two main cations found in the soils of the Hudeiba region. Magnesium was present in

all profiles in varying concentrations. Potassium was also always present, but in low concentrations. Sulphates and chlorides were the dominant anions. The bicarbonate content was relatively low, and carbonate generally absent except in a very few restricted areas.

Heipko and Kaufman (1964) reported that exchangeable sodium percentage (ESP) for Hudeiba soils varied between 4.5 and 17.5%. Any increase in ESP significantly reduced yield, but did not have any effect on the 1000-grain weight or drymatter. Shedding of flowers was more frequent in alkaline soils, in which more severe water stress occurred.

El Karouri (1979) carried out some investigations on salt tolerance of faba beans on Aridisols at the Soba Research Farm in Sudan. The soils have a high salt content with a dominance of  $\text{Na}_2\text{SO}_4$ . The pH of the soil was 8.2. His results showed that a 50% reduction in drymatter yield corresponded with a soil salinity level of 10.5 mmho/cm. The negative correlation between yield and  $\text{EC}_e$  ( $r = -0.883$ ), together with the field observations indicated that soil salinity may be responsible for the variation in growth and yield of faba beans in the salt-affected areas of the Northern Province. Also, there was a negative correlation between soil salinity and shoot drymatter yield. Extrapolating from these data, the author concluded that faba beans could not survive when the  $\text{EC}_e$  of the saturation extract approached 22 mmho/cm.

Varieties of faba bean differed in their ability to tolerate salinity (Ayoub, 1970), the line BF2/2 tolerating salinity better than Baladi and Selaim. The adverse effects of salinity were manifested mainly in delayed emergence of seedlings in the field. However, Babiker (1975, 1976, 1977) using "Gureir", "Karu", and "High Terrace" soils in pots, found no effect of salinity on the growth and yield of faba beans.

Environmental conditions were found to modify salt tolerance of faba beans (El Karouri, 1977). Faba bean sown in October in saline soils at Soba had a lower rate of plant survival, drymatter production, and yield than a crop sown in November. November sowing had the advantage of relatively lower temperatures coupled with high humidity. Evapotranspiration is reduced under these conditions, as is soil moisture depletion rate, thus reducing the salt concentration in the root zone.

Heipko and Kaufman (1964) attributed the adverse effects of alkalinity in Hudeiba soils (where Na level was low) entirely to its effect upon soil water availability; where the ESP of the soil is very high it will adversely affect the soil structure.

The production of faba beans on these vast areas of "Kuru" and "Terrace" soils may be limited if no soil improvement measures are taken. El Karouri (1976) reported that application of farm yard manure (FYM) at 25.6 t/ha gave increased yield at Soba Research Farm. Yousif (1982) found that application of chicken manure (12.8 t/ha) plus nitrogen (46.5 kg N/ha) gave the largest increase in grain yield, of 48.7% over the control. FYM alone or nitrogen alone both decreased grain yield. Yield increases from sewage application were intermediate. The additive effect of N when incorporated with chicken manure or sewage was not significant. In another experiment, 25.6 t FYM/ha significantly increased yields by 47% over the control.

*It is clear that organic additives can improve the yield of faba bean. Chicken manure has the most conspicuous effect since it decomposes easily, and contains some organic acids, more nitrogen and more available phosphorous, as measured by tissue analysis (Yousif, personal communication). This effect may be due to the reduction in exchangeable sodium and improvement of the soil's physical and chemical properties. Ayoub (1975) reported that nitrogenous material such as  $\text{NH}_4$  and nitrates were found to increase Na translocation to the stems of bush beans, either because of their nitrogen supply to the plants or their acidifying effect on the soil.*

Few studies have been conducted on the effects of potassium levels on salt tolerance of faba beans, but K levels do not seem to have a significant effect (Ayoub, 1971; Babiker, 1975, 1976, 1977).

The situation for phosphorus is complex. El Karouri (1969) at Hudeiba found that soil acidification by sulphuric acid, urea and potassium dihydrogen orthophosphate resulted in a progressive decline in soil pH, and an increase in phosphate solubility.

Ayoub (1971) found that gypsum significantly improved the permeability of the deep clay soils (50 to 80% clay), but that this did not increase the yields of faba beans.

Straw mulch application decreased Na accumulation in dry beans (Ayoub, 1975); the effect was similar to that of gypsum, but less. Mulching is known to keep the soil both cooler and more moist. Ayoub (1975) found that the average daily temperature at 1400 hrs during October and

November in the top 5 cm of the soil was reduced by 4.1°C by mulching. The mulch also reduced the rate of soil moisture depletion to a depth of 30 cm by about 10%. The reduction in soil temperature and conservation of moisture seem to be crucial factors in enhancing the survival of the crop on sodic soils under arid conditions. The increased soil moisture under the mulch maintains the dilution of sodium ions, and may reduce sodium uptake by the plant.

Salih and Ageeb (1982) reported that mulching the faba bean crop with grass straw or groundnut shells led to increases in grain yield of 21 and 15% over the non-mulched crop at Wad Medani and Shambat, respectively.

In addition to the studies reviewed, work needs to continue on monitoring the soil salinity and alkalinity at different sites in the northern region, and relating this to the growth and development of the faba bean crop. The level of ESP at which yields are reduced needs further study. Screening of varieties and breeding lines for salt tolerance needs to be expanded.

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# Breeding and Genetics

## AN UNUSUAL PROPORTION AMONG MORPHOLOGICAL CHARACTERS IN A *VICIA FABA MINOR* X *VICIA FABA MAJOR* PROGENY

C. De Pace<sup>1</sup>, and A. Filippetti<sup>2</sup>

<sup>1</sup> Agricultural Biology Institute, Univ. of Tuscia, Viterbo, ITALY

<sup>2</sup> Plant Breeding Institute, Univ. of Bari, Bari, ITALY

There are many reports that seed yield of *Vicia faba* L. can be increased by selecting plant types structured for a more efficient exploitation of photosynthate (Nagl, 1981). In many cases this means plants that show an increase in the partitioning for seed yield.

Unfortunately, available genotypes do not possess all the desired combinations of morphological traits (no. of seeds per pod, no. of pods per node, leaf area index, harvest index) which are required in a breeding program aimed at improving yield through a change in plant type.

Thus, a prebreeding of the biological material through mutagenesis or interspecific hybridization is suggested.

Mutagenesis has been proved to be a source of useful morphological mutants (Sjodin, 1971; Nagl, 1981; Filippetti *et al.*, 1982) while wide hybridization of *Vicia faba* with other *Vicia* species has not been successful.

However, an intraspecific cross, although problematic (Abdalla, 1977), is possible, and a *Vicia faba minor* x *Vicia faba major* cross has been attempted in order to combine the desirable genetic characteristics present separately in the two botanical types (Poulsen and Knudsen, 1980).

While breeding through mutagenesis relies on the induction of a simple useful mutation, *major* x *minor* crosses could allow selection for new character combinations involving several plant features.

We have attempted at the Plant Breeding Institute of Bari to breed new lines of *Vicia faba* with unusual character combinations by crossing two morphologically extreme *Vicia faba minor* and *Vicia faba major* lines. We report here on some morphological features observed in the F<sub>1</sub> plants.

A single plant progeny from *Vicia faba minor* cv. "Herra" was used as the female parent in a cross with the *V. faba major* line "MGBF 250". The cross was made in March 1979, and only 10% of the pollinated flowers gave pods with mature seeds.

Parental values, F<sub>1</sub> means, and deviation of the F<sub>1</sub> means from the mid-parent values and from the *major* parent for 11 characters are given in Table 1. The F<sub>1</sub> shows intermediate pod and seed size, and pod bearing stem portion compared to the parents with a tendency towards the *minor* parent. In addition, the F<sub>1</sub> shows a positive increase for all the yield components with respect to the

Table 1. Parental means and heterotic values estimated as the difference of the F<sub>1</sub> mean values from the average values of the parental lines and from the mean value of the *major* parental line, of *Vicia faba minor* x *Vicia faba major* cross.

Character	Parent mean		F <sub>1</sub>	Difference from parental mean %	Diff. from <i>major</i> parent %
	<i>minor</i>	<i>major</i>			
Pods per plant	12.0	10.4	15.6	+39	+ 50
Seeds per plant	34.7	31.9	52.5	+ 60	+ 65
Seeds per pod	2.9	3.1	3.4	+ 13	+ 10
Podding nodes	6.6	9.7	9.0	+ 10	- 7
Pods/podding node	1.8	1.1	1.7	+ 31	+ 55
Seed yield per plant (g)	11.8	58.4	38.2	+ 9	- 35
100 seed weight (g)	34.4	185.0	73.6	- 33	- 60
Pod length (cm)	7.0	17.5	10.3	- 16	- 41
Seed length (cm)	1.2	2.8	1.5	- 25	- 46
Seed width (cm)	0.9	1.9	1.0	- 29	- 47
Secondary stems per plant	0.6	1.6	0.3	- 73	- 81

mid-parental value, and an increase for number of pods and seeds per plant, seeds per pod, and number of pods per podding node with respect to the *major* parental line. In spite of these increases, the overall seed yield of the F<sub>1</sub> was 35% less than that of the *major* parent. However, with such radically altered proportions between seed yield components, and changes in plant morphology in the *minor* x *major* F<sub>1</sub> compared to that of the parents, it is expected that segregation in F<sub>2</sub> will allow identification of a new plant type with an overall increase in seed yield as compared to the *major* parent.

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## PRELIMINARY STUDIES ON GENETIC DETERMINATION OF ELECTROPHORESIS ALLOENZYME BANDS IN *VICIA FABA* L.

T. De Mora<sup>1</sup>, J.A. Gonzalez<sup>2</sup>, and J.M. Serradilla<sup>2</sup>

<sup>1</sup>Dpto. de Leguminosas, CRIDA 10, INIA, Cordoba, SPAIN

<sup>2</sup>Dpto. de Genetica, Escuela Tecnica Superior de Ingenieros Agronomos, Cordoba, SPAIN

Polyacrylamide electrophoresis has been used to ascertain enzyme variation in the *Vicia* genus (Ladizinsky, 1975; Abdalla and Gunzel, 1979; Yamamoto and Plitmann, 1980), in studying homogeneity of interspecific hybrids (Yamamoto, 1975, 1979 and 1980), in differentiating between cultivars of *Vicia faba* L. (Stegeman *et al.* 1980), and in identifying inbred lines (Gates and Boulter, 1980). Starch gel electrophoresis, in spite of being a convenient technique for this type of study, has only been used for the purpose of identifying the forms of glutamine synthetase in field beans (Barrat, 1981).

We are applying these starch gel electrophoresis techniques to the study of *Vicia faba* L. populations with the following objectives;

- to ascertain genetic variability within populations;
- to compute genetic distances between populations of different geographic origins and between botanical varieties;
- to estimate the allogamy coefficient using material from a large collection of entries.

The methods used are those of Shaw and Koen (1968), Scandalios (1969), Shaw and Prasad (1970), and Brewer and Sing (1970), suitably modified for our material. Enzymograms have been obtained for alcohol dehydrogenase (ADH), esterases (EST), leucine aminopeptidase (LAP), malate dehydrogenase (MDH), phosphoglucomutase (PMG), 6-phosphogluconate dehydrogenase (6-PGDM), superoxidase dimutase (SOD), glutamate oxaloacetate transaminase (GOT), and xanthine dehydrogenase (XDH).

Preliminary studies on seeds from F<sub>1</sub> plants from crosses between widely differing inbred lines have thrown some light on the genetic determination of the electrophoresis patterns of these enzymes.

Figure 1 shows the patterns obtained. ADH shows two banding areas, probably controlled by two different loci; segregation is clear in locus one, and not consistently

shown in locus two. GOT bands are also in two groups; most often segregation is clear in locus two, and occasionally it appears in locus one. MDH shows a complex group of numerous bands whose segregation is shown in the figure. SOD apparently has two loci; only number two segregates.

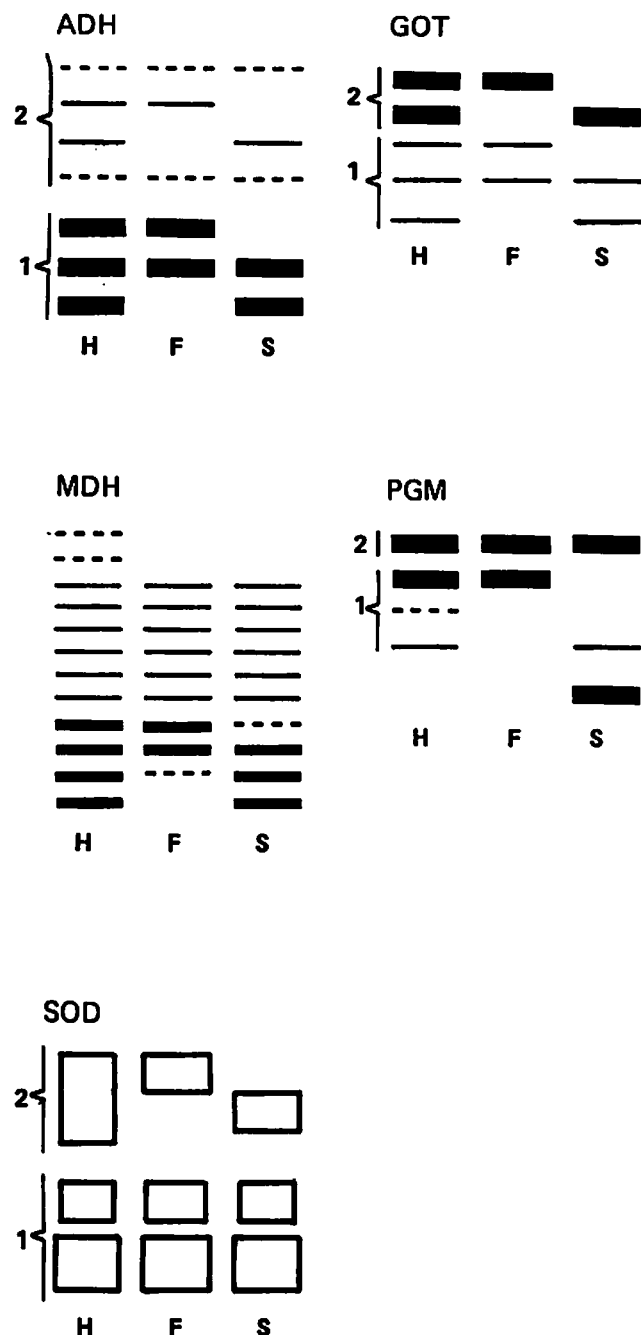


Fig 1. Electrophoresis patterns for alcohol dehydrogenase (ADH), glutamate oxalacetate transaminase (GOT), malate dehydrogenase (MDH), phosphoglucumutase (PGM), and superoxidase dimutase (SOD).

Finally, PGM shows a clear segregation in locus one, and very fuzzy, but probably segregating, bands in locus two.

These parental and F<sub>1</sub> patterns demonstrate the genetic determination of the enzyme systems analysed. F<sub>2</sub> studies will test if these results are maintained, and the genetic relationships (independence or linkage) among the loci involved.

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## A METHOD FOR DIRECTED PRODUCTION OF DEFINITE ANEUPLOIDS OF *VICIA FABA* L.

I. Schubert, R. Rieger and A. Michaelis

*Zentralinstitut für Genetik und Kulturpflanzenforschung  
der Akademie der Wissenschaften, DDR-4325 Gatersleben,  
G.D.R.*

A large variety of primary and secondary karyotype reconstructions have been described in *V. faba minor* on the diploid level. Primary reconstructions (reciprocal translocations and inversions) arose spontaneously or were induced by mutagen-treatment (Michaelis and Rieger, 1959, 1971; Sjödin, 1971a, b; Dobel *et al.*, 1973; Schubert *et al.*, 1981). So-called secondary karyotype reconstructions were obtained from plants heterozygous for two primary karyotype reconstructions, with one chromosome involved in both of them, by meiotic crossing over between partially homologous regions of the primarily translocated or inverted chromosomes (Schubert *et al.*, 1982).

Contrary to the situation with primary and secondary karyotype reconstructions, it is rather difficult to obtain ploidy variants in *V. faba*. Only two tetraploid lines (Poulsen and Martin, 1977; Schubert *et al.*, 1982), and some trisomics descended from one of them (Martin, 1978; Gonzales-Garcia *et al.*, 1981) which could be propagated via seeds have been reported so far.

In free pollination experiments (see Schubert *et al.*, 1982), we found plants with  $2n = 14$ , instead of  $2n = 12$ , chromosomes among the progeny of differently reconstructed karyotypes. This change in chromosome number was stated to be a case of pseudoaneuploidy due to aberrant meiotic chromosome segregation (4:2) from a hexavalent formed in plants heterozygous for two translocations, both of which involved the short (satellite) arm of the former wildtype chromosome I.

This hypothetical explanation was confirmed by crossing two other translocation lines, each involving the satellite arm of standard chromosome I, and selfing the heterozygous  $F_1$  plants (Fig. 1). The homozygous parental karyotypes used for crossing were symbolized A (with a reciprocal translocation between the satellite arm of chromosome I and the long arm of chromosome III), and J (with a reciprocal translocation between the satellite arm of chromosome I and the short arm of chromosome V), respectively. The plants obtained by selfing of the heterozygous AJ plants had karyotypes with 12 (J hom, A hom, AJ het), 13 or 14 chromosomes (Table 1).

Most of the karyotypes with 13 chromosomes arose from fusion of a gamete with 6 chromosomes (either from karyotype A or I) and a gamete possessing 7 chromosomes but no metacentric chromosome (both arms of the wildtype metacentric chromosome are represented by the two reconstructed acrocentric chromosomes IJ and IIIA, Fig. 2a, and 2b).

Most karyotypes with 14 chromosomes were structurally homozygous, having no metacentric chromosome, and are thus clearly the result of combination of 2 gametes with 7 acrocentric chromosomes. They are tetrasomic for the short arm and the centromere of chromosome III, and for the telomere of the short arm of chromosome V (Fig. 2c). In several other species (e.g., *Drosophila*, maize, barley, see Hagberg, 1962), duplications of certain chromosome segments were found in some  $F_2$  individuals after crossing of two translocation lines both involving the same two pairs of chromosomes.

Karyotypes with 11 or 10 chromosomes containing 3 or 4 metacentrics were not found. Probably the plants do not tolerate mono- or nullisomy for those regions which are tri- or tetrasomic in karyotypes with 13 or 14 chromosomes.

The observed numbers of individuals with the different karyotypes are shown in Table 1.

In addition to the pseudoaneuploids described above, 11 plants with 13, and 3 plants with 14 chromosomes (together, 1.6% of the progeny), which represented true aneuploids, were found. The chromosomal composition of these karyotypes is shown in Table 1 (lower part) and Fig. 2 d-g.

These aneuploids are either primary trisomics for standard chromosomes III or V (Fig. 2e, f), or tertiary trisomics for the long arm (IJ) or the satellite arm (IIIA) of the metacentric standard chromosome (Fig. 2d, g) in a homozygous (A or J) or heterozygous (AJ) karyotype. All chromosomes obtained as trisomics from this source contributed to the hexavalent at meiosis of the heterozygous parental AJ karyotype. The tertiary trisomics seem to be especially valuable, since until now all efforts to obtain trisomics for the complete metacentric chromosome were unsuccessful.

The aneuploid karyotypes with 14 chromosomes represent trisomics for chromosome III, V or IIIA in karyotypes composed of a chromosome set from one of the original translocation lines, and a set of 7 acrocentric chromosomes (see Table 1).

**Table 1.** Observed frequency of different karyotypes among the progeny of heterozygous AJ plants after selfing.

	Karyotype formula						Number of chromosomes	Number of metacentrics	Observed frequency	
	I	II	III	IV	V	VI			%	absolute
<b>Diploids</b>										
A hom	IA <sup>m</sup>	+	IIIA	+	+	+	12	2	13.6	119
	IA <sup>m</sup>	+	IIIA	+	+	+				
J hom	IJ	+	+	+	VJ <sup>m</sup>	+	12	2	16.0	137
	IJ	+	+	+	VJ <sup>m</sup>	+				
AJ het	IA <sup>m</sup>	+	IIIA	+	+	+	12	2	30.4	261
	IJ	+	+	+	VJ <sup>m</sup>	+			60.2	517
<b>Pseudoaneuploids</b>										
A + 7	IA <sup>m</sup>	+	IIIA	+	+	+	13	1	16.7	143
	IJ	+	+	+	+	+				
J + 7	IJ	+	+	+	VJ <sup>m</sup>	+	13	1	17.6	151
	IJ	+	+	+	+	+				
AJ 14	IJ	+	+	+	+	+	14	0	3.9	33
	IJ	+	+	+	+	+				
A + 5	IA <sup>m</sup>	+	IIIA	+	+	+	11	3	—	—
	IA <sup>m</sup>	+	—	+	VJ <sup>m</sup>	+				
J + 5	IJ	+	+	+	VJ <sup>m</sup>	+	11	3	—	—
	IA <sup>m</sup>	+	—	+	VJ <sup>m</sup>	+				
AJ 10	IA <sup>m</sup>	+	—	+	VJ <sup>m</sup>	+	10	4	—	—
	IA <sup>m</sup>	+	—	+	VJ <sup>m</sup>	+			38.2	327
<b>Primary trisomics</b>										
A hom + III	IA <sup>m</sup>	+	IIIA	+	+	+	13	2		2
	IA <sup>m</sup>	+	IIIA	+	+	+				
J hom + V	IJ	+	+	+	VJ <sup>m</sup>	+	13	2		1
	IJ	+	+	+	VJ <sup>m</sup>	+				
AJ het + V	IA <sup>m</sup>	+	IIIA	+	+	+	13	2		2
	IJ	+	+	+	VJ <sup>m</sup>	+				

(Continued)

**Table 1.** Observed frequency of different karyotypes among the progeny of heterozygous AJ plants after selfing.

	Karyotype formula						Number of chromosomes	Number of metacentrics	Observed frequency	
	I	II	III	IV	V	VI			%	absolute
AJ het + III	IA <sup>m</sup> + IJ	+	IIIA + +	+	+	VJ <sup>m</sup> +	13	2		2
A + 7 + III	IA <sup>m</sup> + IJ	+	IIIA + IIIA	+	+	+	14	1		2
<b>Tertiary trisomics</b>										
A hom + IJ	IA <sup>m</sup> + IA <sup>m</sup> + IJ		IIIA IIIA	+	+	+	13	2		1
J hom + IIIA	IJ	+	+	+	VJ <sup>m</sup>	+	13	2		1
	IJ	+	IIIA	+	VJ <sup>m</sup>	+				
AJ het + IIIA	IA <sup>m</sup> + IJ	+	IIIA + IIIA	+	+	VJ <sup>m</sup> +	13	2		2
J + 7 + IIIA	IJ	+	IIIA +	+	VJ <sup>m</sup>	+	14	1		1
	IJ	+	IIIA	+	+	+			1.6 total	14 858

+ = wildtype chromosome

m = metacentric chromosome

Up to now no seeds could be obtained from these aneuploids since some died as seedlings from a fungal infection, while the remainder proved to be infertile when grown in a phytotron during the winter. It should be possible, however, to propagate at least some of the aneuploids if the sample for selection becomes large enough.

The advantage of the method described here is that from these crosses, within two generations definite trisomics are obtainable in predictable frequency. Via the

same experimental route, primary and tertiary trisomics were produced in various plant species (for review see Khush, 1973). Since suitable translocation lines are now available, it should be easy to obtain, by means of appropriate crosses, trisomics for the other chromosomes (II, IV and VI) of *Vicia faba*. Preliminary results support this inference. It should therefore be no problem in future to produce any desired type of trisomy in *Vicia faba*. In the past this attempt was time consuming and successful by chance only.

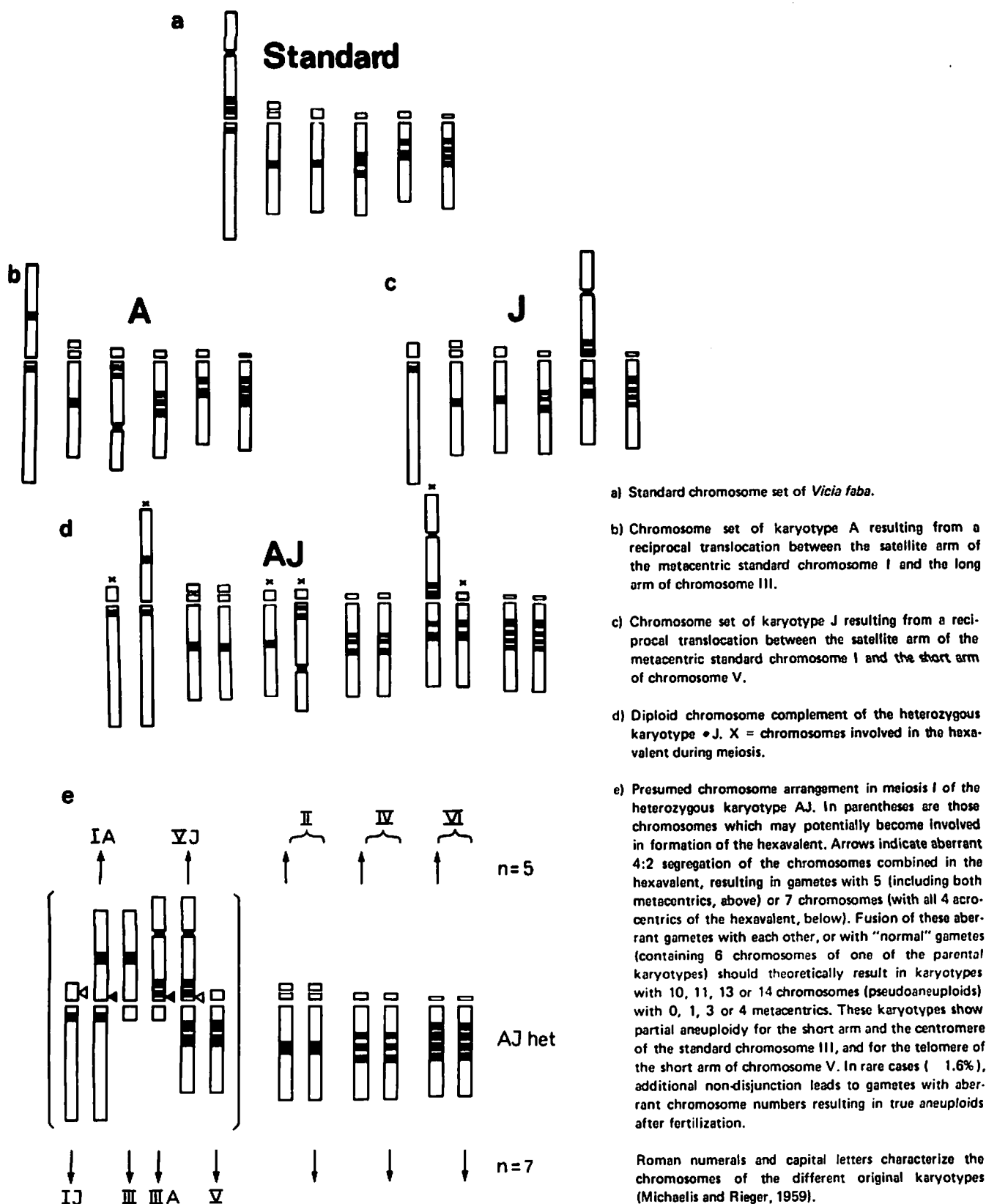
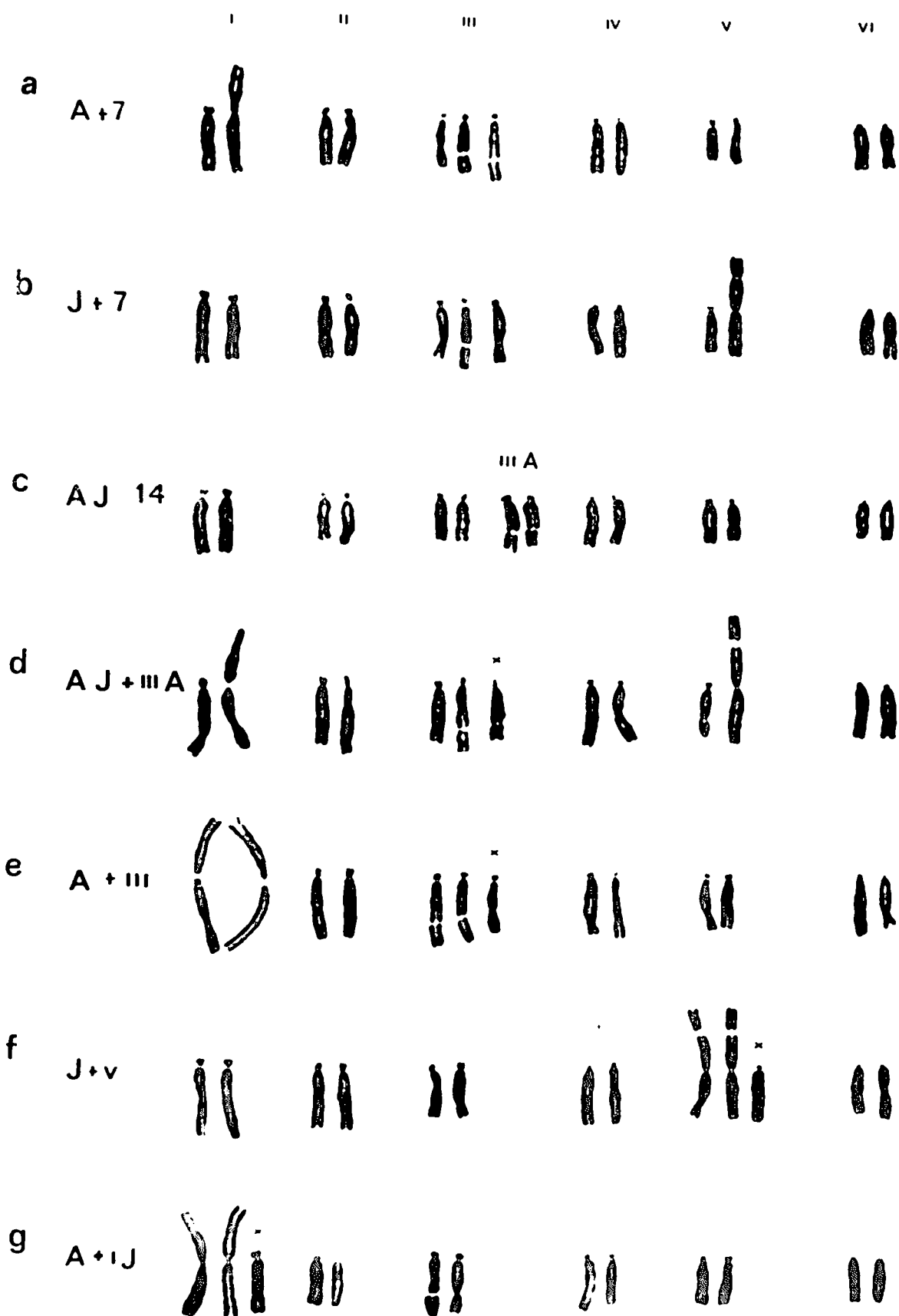


Fig. 1. The origin of pseudoaneuploids and true aneuploids from translocation heterozygotes in *Vicia faba*.



**Fig 2.** A sample of karyotypes with 13 and 14 chromosomes (Feulgen staining).

- a) A + 7 = one chromosome set of karyotype A plus 7 acrocentric chromosomes.
- b) J + 7 = one chromosome set of karyotype J plus 7 acrocentric chromosomes.
- c) AJ14 = homozygous karyotype with 14 acrocentric chromosomes.
- d) AJ + IIIA = heterozygous karyotype AJ plus additional chromosome IIIA (asterisk) representing the satellite arm of the standard metacentric chromosome translocated to the long arm of chromosome III as a tertiary trisomic.
- e) A + III = homozygous karyotype A plus an additional standard chromosome III (asterisk).
- f) J + V = homozygous karyotype J plus an additional standard chromosome V (asterisk).
- g) A + IJ = homozygous karyotype A plus additional chromosome IJ (asterisk) representing the long arm plus centromere of the standard metacentric chromosome and the telomere of the short arm of chromosome V as a tertiary trisomic.

## Summary

A method for the production, in predictable frequency, of definite trisomics by crossing of two appropriate translocation lines and selection of progeny after selfing of the karyotypes heterozygous for both translocations is described. Within two generations all acrocentric chromosomes may be obtained as primary trisomics, and both arms of the metacentric satellite chromosomes as tertiary trisomics.

## Acknowledgement

We are grateful to Dr. G. Kunzel for critical reading of the manuscript.

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# SUBSPECIFIC CLASSIFICATION OF *VICIA FABA* L. BY PROTEIN AND ISOZYME PATTERNS.

H.R. Kaser and A.M. Steiner

*Institut für Pflanzenzüchtung, Saatgutforschung und  
Populationsgenetik (350), Universität Hohenheim, D-7000  
Stuttgart 70, GERMAN FED. REPUBLIC*

The subspecific classification of *Vicia faba* into *minor equina*, and *major* is still controversial (cf. Hanelt, 1972). In this study, 7 *minor* and 10 *major* cultivars of the 1977 recommended varieties list of the German Federal Republic, together with 5 cultivars and 49 land races from all over the world were studied for physical seed characters and disc electrophoretic protein-, and isoelectric focusing isozyme patterns (Fig. 1) (Kaser, 1982).

No discontinuities were observed in seed weight, size and shape, with the variability being greater within and between the race populations, as compared to the cultivars.

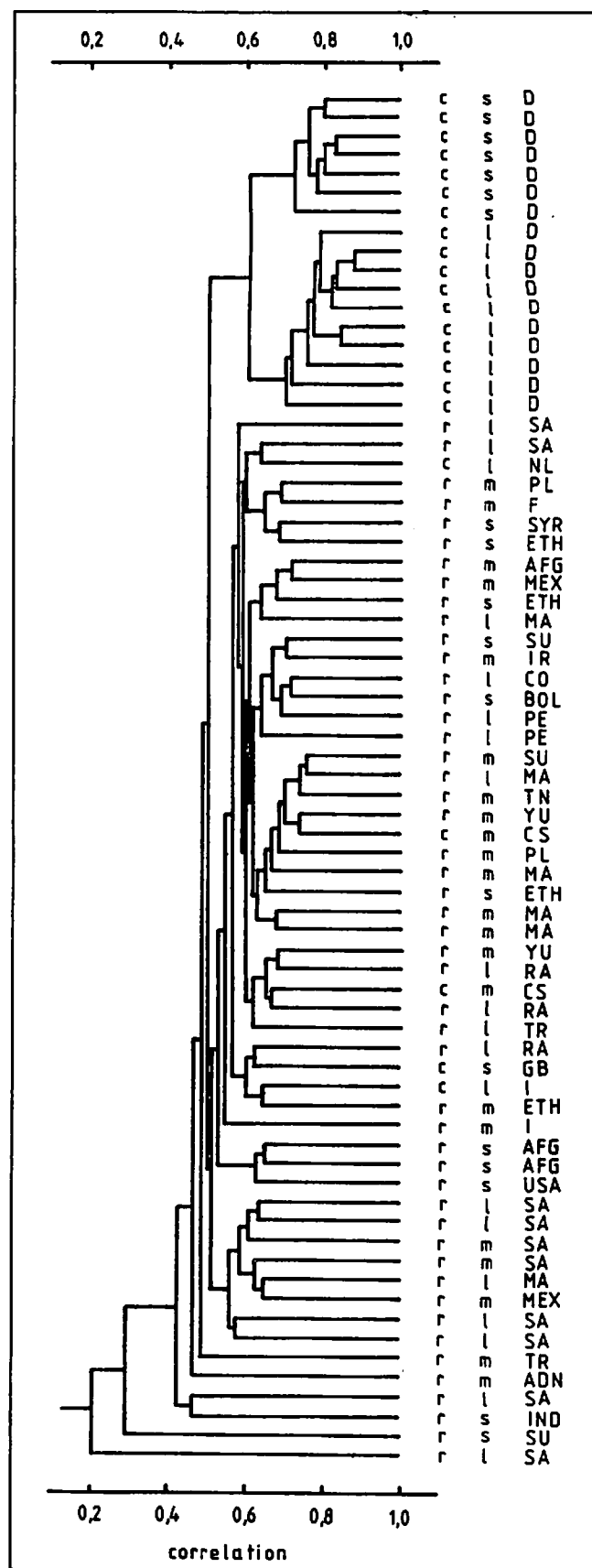
The populations could be best characterized and discriminated by the patterns of the seed proteins and esterases, root esterases and peroxidases, seedling cotyledon esterases and phosphatases, epicotyl esterases, peroxidases and phenoloxidasen. Seed patterns were stable irrespective of year and site, but seedling patterns differed with the developmental stage. The 9 patterns showed 233 different bands in the cultivars and 430 in the races. There was a total of 466 different bands suitable for taxonomic characterization.

The polymorphism indices indicated that the variability within the populations of cultivars and races estimated by 20 separate single seed analyses was of the same order of magnitude as the variability between the populations estimated by a combined random 20 seed sample analysis. In cultivars, variability decreased with generation from pre-basic to certified seed. The 17 German cultivars could be most easily discriminated using the seed protein and esterase patterns.

Fig 1. Phenogram of 71 populations of *Vicia faba* L. based on WPGMA cluster analysis of correlation coefficients on 466 protein and isozyme characters. c = cultivar, r = land race; s = small -, m = medium -, and l = large-seeded.

Countries are designed by traffic codes.

SA = first generation South American races grown at Hohenheim.



The variability is remarkable: e.g. in cv. Ackerperle 257, single seeds drawn from 12 lots of certified seed revealed 165 different esterase patterns demonstrating failure in single seed identification. Of the isozyme bands, X 30% in the cultivars, while only X 5% in the races, were monomorphic. 85-100% of the isozyme bands of the cultivars were found in the races, but only 31-75% of those of the races in the cultivars. The number of bands per isozyme was more variable in the races than in the cultivars. The band numbers of 10 assorted geographic groups were between 161-249 and only 49% of all 466 bands occurred in more than 3 groups. Infrequent bands indicated diversity in the Near East-Iran-Afghanistan area and in South America. X-ray mutants showed additional or missing bands.

A numerical-taxonomic analysis revealed that the classification of the 71 populations as shown by phenograms largely depends on type, number and combination of the 9 different patterns taken for calculation. A phenogram based on all 466 characters, however, shows the German *minor* and *major* cultivars as two separate groups, which are closer related within and between each other than in the case of any race population or within race groups, indicating a local gene pool (Fig. 1).

Seed weight, size and shape show no discontinuities; populations are discernible by protein and isozyme patterns. Except for the German cultivars, there is no correlation between seed physical, protein, and isozyme characters. Hence, it is possible to define operational systems for classification, but it was not possible to reveal a natural system of subspecific taxonomy.

#### Acknowledgement

The provision of samples by public institutions and breeders, especially Pflanzenzucht Oberlimpurg Dr. P. Franck, and the support of the Deutsche Forschungsgemeinschaft (DFG) is gratefully acknowledged.

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## A DIALLEL CROSS ANALYSIS OF SOME CHEMICAL CONSTITUENTS OF FABA BEAN.

R.A. Mitkees and H.F. Hassan

Field Crop Research Institute, Agricultural Research Center, Orman, Giza, EGYPT.

### Introduction

Legumes are nutritionally important because of their relatively high protein contents. Seidl *et al.* (1969) found a range in protein content from 18 to 32% in the seeds of legumes, with the exception of soybean and groundnut. In comparison to beef, legume protein is high in lysine, while low in total sulphur amino acids and tryptophan (Tandon *et al.*, 1957; Ali *et al.*, 1982). Dickson and Hackler (1972) found that inheritance of total protein and individual sulphur amino acids in *Phaseolus vulgaris* is polygenic, and generally recessive.

The aim of this study was to ascertain detailed information on the genetics of inheritance of protein, amino acids, and ash contents in faba bean. Differences between genotypes were analysed for genetic attributes according to the method described by Hayman (1954).

### Materials and Methods

The study utilised a half diallel cross among six faba bean lines: Giza 1, Giza 2, Rebaya 34, F 292, F 338, and F 383. The experiment was laid out in a randomized complete block design, with five replicates, each consisting of 21 plots of one row 3.5 m long. Each plot represented one of the possible 15  $F_1$  genotypes, and their six parents. A bulk sample was taken from each to determine the following:

- protein percentage, by Kjeldahl method (A.O.A.C., 1970)
- amino acids, as percentages of total protein (Chung and Lavy, 1954)
- ash percentage (A.O.A.C., 1970)

Significant differences among genotypes were analyzed for type of gene action and genetic ratios were determined, by the methods of Jones (1965) and Mather and Jinks (1971).

Field and laboratory work were performed at Giza Agricultural Research Station during the 1979/80 and 1981/82 seasons.

### Results and Discussion

The relative contents of 16 amino acids, crude protein and ash percentage were determined. Only methionine, arginine, lysine, protein and ash contents showed significant differences among genotypes, and the genetic analysis was restricted to these characters.

Both additive and dominance gene effects were significant for all characters except lysine, where most variation was attributable to dominant gene action (Table 1a). Investigation of parental and progeny variances, covariances, and F estimates suggests epistatic effects acting upon ash content (Table 1b). However, estimation of variance components revealed dominant gene action to be prevalent for all characters. Environmental and additive variances shared equally the remaining variance for all characters except lysine and ash contents. The amount of additive variance for lysine (20.6% ) compared to that for environment (2.5% ) suggests the possibility for successful selection for this factor. Protein and arginine contents were similar in this respect, in that they possessed additive variance components of 24% and 36.7% , respectively. Over 98% of the total variation in ash content was related to the dominance type of gene action. Leleji *et al.* (1972) found only a small effect of additive genetic action in the determination of protein content in dry beans, while Kelly and Bliss (1975) found the opposite.

The  $b_1$  items indicate directional dominance for low amounts of methionine, arginine, and ash percentage (Table 1a). Protein and lysine contents show ambidirectional dominance. Variance ratios indicate over-dominance for all characters (Table 1c). Partial dominance gene action in the inheritance of methionine in dry beans has been observed (Leleji, 1971).

An unequal distribution for plus and minus genes was observed for all factors except lysine, as indicated by  $b_2$  values. Ratios of UV reveal equal gene distributions for methionine and lysine (Table 1c).

The  $K_D/K_R$  ratios indicate a greater proportion of dominant genes for protein and arginine, but a pre-dominance of recessives for methionine, lysine, and ash contents. Thus, selection for higher methionine, lysine and ash contents should be possible, by selection for recessive genes.

A number of groupings of 4 to 5 genes, or gene blocks, of dominant type, K, were observed in the inheritance of protein content, indicating its quantitative inheritance. The other characters may be more simply inherited, with no more than a single dominant gene. Protein and arginine may possess additional modifiers.

**Table 1. Results of Genetic Analysis.**

Source of Variation	Degrees of Freedom	Protein a - Mean Squares	Methionine	Arginine	Lysine	Ash
additive, a	5	4.3278**	0.0780**	0.4777**	0.0052	0.2829**
dominance, b	15	8.9137**	0.3482**	0.2634**	0.0266**	0.2791**
b <sub>1</sub>	1	1.0861	0.0242**	2.0604**	0.0021	0.6693**
b <sub>2</sub>	5	8.9530**	0.0521**	0.2075**	0.0040	0.4890**
b <sub>3</sub>	9	9.7615**	0.0264**	0.0948	0.0418**	0.1142**
error, e	80	0.5011	0.0045	0.0555	0.0063	0.0350
<b>b - Variance Components</b>						
additive, D		1.3494**	0.0542**	0.0134	0.0014	0.1216
dominance, H <sub>1</sub>		7.4794**	0.2021**	0.4525**	0.1485**	1.4789**
H <sub>2</sub>		5.3836**	0.2053**	0.2965**	0.1646**	2.0199**
D, H covariance, F		2.2865**	0.0446**	-0.0830*	-0.0190	-0.4147**
average dominance, h <sup>2</sup>		25.8180**	0.0095	0.0256	-0.0003	0.0809
environment, E		0.5011**	0.0009	0.0555**	0.0013	0.0070
<b>c - Variance percentages and genetic ratios</b>						
additive, D <sub>R</sub> %		24.0	7.8	36.7	20.6	0.0
dominance, H <sub>R</sub> %		55.0	84.8	36.2	76.9	98.7
environment, E <sub>R</sub> %		21.0	7.4	27.1	2.5	1.3
UV ratio		0.18	0.25	0.16	0.28	0.34
dominance degree		2.35	1.88	5.81	10.30	3.49
K <sub>D</sub> /K <sub>R</sub> ratio		2.12	0.11	1.28	0.21	0.27
number of genes, K		4.38	0.19	0.35	0.01	0.16

\*, and \*\* indicate significance at 0.05 and 0.01 levels, respectively.

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# **POLLEN TUBE GROWTH AND TIME OF DAY IN INTERSPECIFIC *VICIA* CROSSES.**

G. Ramsay, J.K. Jones & B. Pickersgill  
*Department of Agricultural Botany, University of Reading,  
 Whiteknights, Reading RG6 2AS, UK*

During attempts to cross *Vicia faba* with wild *Vicia* species, data on pollen tube growth in interspecific crosses were also obtained to determine whether or not the pollen tube growth varied with time of pollination in the UK.

Throughout the summer season of 1982 plants of various accessions of *Vicia faba* grown in pots in a greenhouse at Reading were emasculated shortly before anthesis, pollinated immediately with other species of section *Faba* and covered with a gelatin capsule. Only one flower was pollinated at each node and all unused flowers were removed. Flowers were harvested after 24 hours and prepared for fluorescence microscopy. The presence or absence of pollen tubes in the ovary was scored.

Pollen tubes were frequently seen in the ovary but only rarely observed entering micropyles. The results for pollinations during each of three 2-hour periods are displayed in Table 1. There was little variation in the percentage of ovaries with pollen tubes during the three different time periods and a  $\chi^2$  goodness-of-fit test, with correction for continuity, confirmed that no differences were detectable. This indicates that germination of pollen grains and growth of pollen tubes of these species in the stigma and style of *V. faba* is very similar during the three time periods used.

they found an improvement in pod set following pollination near midday. Experimental circumstances and environmental factors differed considerably and could have influenced the results, but if the studies are comparable then it seems that pod set and pollen tube growth are not closely linked. Chapman *et al.* (1979) found that many fertilised flowers were abscised and in certain conditions, i.e., removal of photosynthate sinks, unpollinated flowers will persist on the plant.

Other aspects of this work will be published shortly. Financial support from the Overseas Development Administration for this cooperative research project with ICARDA and assistance from colleagues Linda Hammond and Heather Stewart are gratefully acknowledged.

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Table 1. Effect of time of day on numbers of ovaries with pollen tubes (PTs) following pollination of *V. faba* stigmas with pollen of species of section *Faba*.

Time	No. of Pistils					
	With PTs	Total	%			
9.00 - 11.00	17	30	57	$\chi^2$	=	0.23
11.00 - 13.00	20	43	47	d.f.	=	1
14.00 - 16.00	34	71	48	p	>	0.5
Total	71	144	49			

Omar and Hawtin (1980) and Omar *et al.* (1981) compared pod set in crosses between breeding lines of *V. faba* through similar time periods. It is interesting that

# Physiology and Microbiology

## THE EFFECT OF SOIL SALINITY ON DINITROGEN FIXATION AND YIELD OF FABA BEAN (*VICIA FABA* L.)

R.H. Lockerman, T.J. Kisha, J.R. Sims<sup>1</sup>, and A.S. Abdel-Ghaffar<sup>2</sup>

<sup>1</sup> Associate Professor, Research Assistant and Professor, Plant and Soil Science Department, Montana State University, Bozeman, Montana, U.S.A.

<sup>2</sup> Professor, Soil & Water Science Department, Alexandria University, EGYPT.

Soil salinity affects crop production in many areas of the world, including the Northern Great Plains and the Nile River Flood Plain (Chapman, 1971). Saline soils not previously utilized in agriculture are becoming more important as new lands are brought into production to meet the demands of an increasing population, or to replace land lost to urban development. Crops tolerant to saline conditions need to be evaluated for production on marginal lands. Additionally, the rising costs of nitrogen fertilizer increases the importance of plant species capable of biological dinitrogen fixation. The response of leguminous crops, such as faba bean (*Vicia faba* L.), to soil salinity and the effects of soil salinity on symbiotic dinitrogen fixation have become increasingly important in land resource management.

### Materials and Methods

The effects of soil salinity and applied fertilizer nitrogen on growth and dinitrogen fixation in faba bean are being evaluated through a cooperative project between the Montana Agricultural Experiment Station, USA, and Alexandria University, Egypt. Equal equivalents of NaCl

and CaCl<sub>2</sub> were incorporated in 6 m<sup>2</sup> plots to create four levels of electrical conductivity (2, 5, 11, and 14 mmhos/cm EC<sub>g</sub>) prior to planting. Half of each plot received NH<sub>4</sub>NO<sub>3</sub> at a rate of 200 kg N/ha. Indigenous nitrate nitrogen in plots not receiving fertilizer application was 10 kg/ha. Faba bean, cv. Ackerperle, was planted at a rate of 12 seeds/m with 30 cm row spacing. Plants were evaluated at 40, 60, 80 and 100 days after planting for nodulation, shoot and root dry weights and acetylene reduction. Ninety plants from each plot were harvested at the conclusion of the experiment for determination of total seed dry weight.

### Results and Discussion

The effects of four salinity levels on shoot, root, and seed dry weights at four harvest dates are given in Table 1. There was no significant difference between treatments with and without fertilizer nitrogen. Preliminary greenhouse experiments showed that plants utilizing soil nitrate nitrogen had an advantage over those completely dependent upon symbiotic dinitrogen fixation. The small amount of nitrogen indigenous to the field experimental plots was apparently sufficient to supplement biological fixation and provided for healthy vegetative growth. Shoot and root dry weights at the highest soil salinity level were reduced by 65 and 70%, respectively. There was no significant difference between 2 and 5 mmhos on shoot and root dry weight until after 60 days after planting. Total seed dry weight decreased as soil salinity increased, resulting in a 50% reduction in maximum yield at a conductivity level of approximately 11 mmhos/cm. These data agree with earlier results involving the effect of soil salinity on faba bean growth in the Sudan (El Karouri, 1979). Nitrogen had no effect on seed dry weight. However, the percentage seed nitrogen was higher in low-nitrogen plots where there was greater dependence on symbiotic fixation.

Table 1. Effect of four salinity levels on growth and yield of *Vicia faba* L.

Conductivity (mmhos/cm)	Days after planting									
	40	60	80	100	40	60	80	100	100	
	Shoot dry wt. (g)				Root dry wt. (g)				Seed dry wt./m <sup>2</sup> (g)	
2	2.2	12.9	20.5	26.9	0.5	2.2	3.9	4.3	200.1	
5	2.2	12.8	13.6	13.3	0.5	2.0	3.0	2.0	146.0	
11	1.7	7.8	11.0	14.3	0.4	1.3	2.1	2.1	111.5	
14	1.2	5.8	10.0	9.3	0.3	1.0	1.9	1.3	30.4	

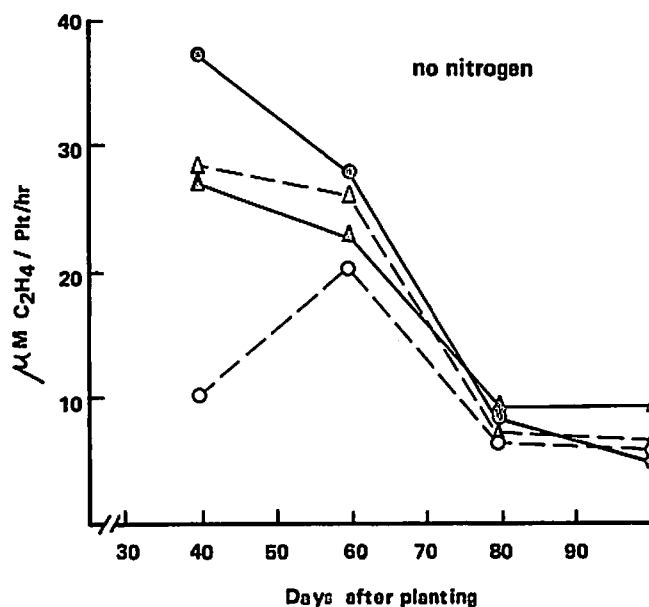
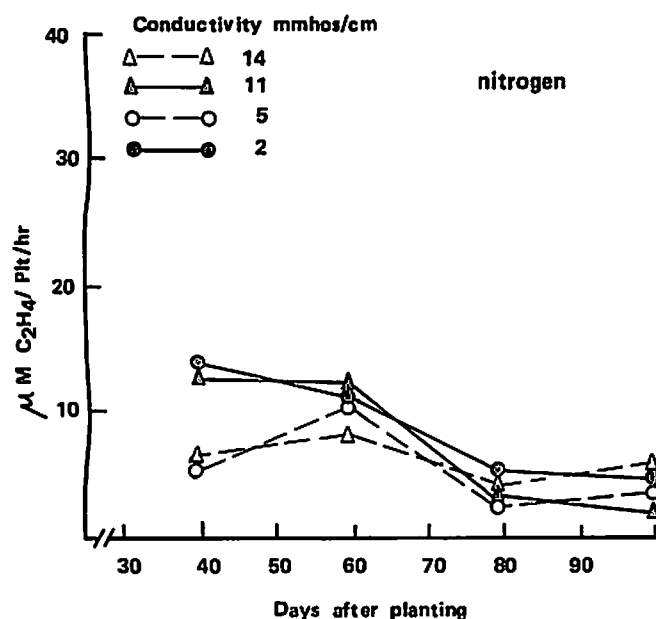
Each shoot and root observation is the mean of 16 plants.

Acetylene reduction throughout the growing season was qualitatively similar in both low and high nitrogen treatments (Fig. 1). Quantitatively, there was approximately a 50% reduction in nitrogen fixation in treatments that received fertilizer nitrogen. Dry weights and total nitrogen content decreased proportionately with increased soil salinity, and acetylene reduction activity reached a maximum at approximately 5 mmhos. This indicated that the shoot and root had a greater dependence upon symbiotically-fixed nitrogen in moderately saline conditions. Acetylene reduction activity was proportionate to nodulation. Increased nodulation and the subsequent increase in acetylene reduction activity at moderate salt concentrations may have been the result of soil aggregation and improved gaseous exchange in the root zone.

## Conclusion

These data indicate that the faba bean plants provided all the required plant nitrogen when properly inoculated in low nitrate soils. Dean and Clark (1977) reported that a small amount of soil nitrogen was beneficial to faba bean growth. Faba bean is moderately salt tolerant, decreasing to 50% of maximum yield at approximately 11 mmhos. Soybean (*Glycine max* L.), lentil (*Lens culinaris* L.), green pea (*Pisum sativum* L.), and dry bean (*Phaseolus vulgaris* L.) yields decreased to 50% at soil salinity levels of EC 9, 4, 4, and 4 mmhos/cm, respectively (Abel and McKenzie, 1963; Ayoub, 1977; Das and Mehrotra, 1971; Richards, 1969). Our data suggest that faba bean has a greater production potential on moderately saline soils than many other large seeded grain legumes.

Fig 1. The effect of salinity on acetylene reduction in faba beans. Each point is the mean of 16 plants.



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## FLOWER REMOVAL AND NODULE ACTIVITY IN FABA BEAN (*VICIA FABA* L.)

R.M. Brook and J.I. Sprent

Scottish Crop Research Institute and Dept. of Biological Sciences, University of Dundee, U.K.

### Introduction

It is well established that legume root nodules fixing atmospheric nitrogen rely upon an adequate supply of carbohydrate to function, and that both root nodules and developing fruits are major sinks for assimilates (Lawrie and Wheeler, 1975; Pate and Herridge, 1978). The aim of the present work was to determine if prevention of flowering could prolong nodule activity beyond the time of pod setting, when nodule activity normally declines, as more assimilate would be available for nodules.

### Materials and Methods

The trial was carried out at the Scottish Crop Research Institute, Dundee, in 1977, using spring bean cv. 'Minor', grown in the field at a population density of 44 plants/m<sup>2</sup>.

Flowers were removed from treated plants as soon as was possible without damaging the plants. Controls were not deblossomed. There was a tendency for treated plants to form tillers and more flowers, and these were removed as they appeared. At each of five harvest dates, five treated and five control plants were dug up, except on June 22, when only control plants were taken, as treatments had not begun then. The root systems were detached, shaken free of loosely adhering soil, placed in 900 ml 'Kilner' jars, and sealed. Ethylene production by the nodules was then measured using a Varian series gas chromatograph (Sprent, 1972). Nodules were counted and weighed.

### Results and Discussion

Deblossoming affected nodule numbers, and nitrogenase activity (Table 1). In the treated plants, nodule mass was greater than in controls from July 5, and there were more nodules from July 19, but neither of these variates were significantly different from controls until September 14.

The differences in absolute and specific rates of nodule activity between the treated and control plants were not clear cut until the final harvest. Whereas nodules on control plants had all but ceased activity by the end of the season, nodules on treated plants were most active. The low

rate of activity on July 5 was probably because of drought at the time of harvest. Water stress is known to depress nodule acetylene reduction activity (Sprent, 1972). Senescence was also markedly delayed in the treated plants.

Table 1. Values of unit leaf rate (ULR) and plant growth rate (PGR) for control and deblossomed plants.

		June 22- July 5	July 5- July 19	July 19- Aug 9	Aug 9- Sept 14
ULR (mg/g/d)	Contr.	128	112	108	15
	Treat.	139	95	42	-20
PGR (mg/d)	Contr.	426	535	499	30
	Treat.	480	521	371	-180

In faba beans, root growth ceases during pod filling (Day *et al.*, 1979). Sprent and Bradford (1977) found that in a normally growing faba bean crop, nodule activity declined from July onwards, a pattern found in the controls in the present work. As both root growth and nodule activity are dependent on an adequate supply of carbohydrate from the shoot, then a diversion of such supplies to the pods during the reproductive phase will result in senescence of roots and nodules. Brook *et al.* (in prep.) have shown that the developing pods are sufficiently strong sinks to warrant the remobilisation of previously stored reserves from the stem and tap roots.

In the present work, when flowers were removed, nodule numbers, mass, and activity continued to rise to a maximum by September 14, when the experiment was terminated. The most probable explanation of this behaviour is that in the absence of developing pods, and delayed leaf senescence, assimilates were available for the roots, permitting nodule growth and activity to continue.

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## EFFECTS OF FLOWER REMOVAL ON GROWTH IN FABA BEANS (*VICIA FABA* L.)

R.M. Brook and J.I. Sprent  
*Scottish Crop Research Institute and Dept. of Biological Sciences, University of Dundee, U.K.*

### Introduction

In a series of experiments on field grown faba beans (cv. 'Minor'), source-sink relationships were examined. This article describes an experiment in 1978 which looked at the effects of complete removal of flowers on the carbon economy.

### Materials and Methods

The layout and treatments have already been described (Brook and Sprent, 1983). Stem, leaf and fruit dry weights were recorded from the second harvest onwards. The experiment was terminated on September 14.

### Results and Discussion

From the second harvest onwards stem and leaf dry weights in the treated plants were greater than in the controls, although these differences were not significant until the final harvest for the stems, and the penultimate harvest for leaves (Fig. 1). Senescence was delayed in the treated plants and on August 9 they had nearly three times the dry weight of leaves of the controls. At the final harvest, although leaf weight had decreased, it was still more than the maximum recorded for control plants (July 19).

In the deblossomed plants, although leaf weight was greater, unit leaf rate (ULR) on a leaf weight basis was lower than in controls from mid-July onwards, reducing the rate of dry matter accumulation (Table 1). The greater leaf mass of treated plants was evidently insufficient to compensate for the lower ULR.

In August and September, plant growth rate (PGR) of the treated plants was actually negative, largely due to abscission of lower leaves. Despite the complete loss of leaves in control plants by the final harvest, PGR never became negative, due to the increase in fruit dry matter. Igwilu (1982) found similar results in depodded faba beans.

As flowers are not photosynthetic structures and fruits are a net loss to the C-economy (Brook and Sprent, 1983), the prevention of formation of reproductive sinks must have affected photosynthesis indirectly. Hill-Cottingham (1976) found that in depodded spring beans, RUDP carboxylase activity was lower than in normally fruiting plants, suggesting that the rate of photosynthesis was diminished.

Wareing *et al.* (1968) attributed a greater rate of photosynthesis in various partially defoliated species to an increase in the supply of cytokinin per leaf. Mondal *et al.*, (1978) found that a decrease in photosynthesis in response to depodding in soya beans started within 8 h, and Koller and Thorns (1978) observed a quadrupling of leaf stomatal resistance in 48 h in response to depodding in the same crop.

It appears that the faba bean, in common with a number of species, is able to control the rate of photosynthesis so that it is commensurate with the 'load' placed upon the leaves.

Table 1. Values of unit leaf rate (ULR) and plant growth rate (PGR) calculated for control and deblossomed plants.

		June 22- July 5	July 5- July 19	July 19- Aug 9	Aug 9- Sept 14
ULR (mg/g/d)	Cont.	128	112	108	15
	Treat.	139	95	42	-20
PGR (mg/d)	Cont.	426	535	499	30
	Treat.	480	521	371	-180

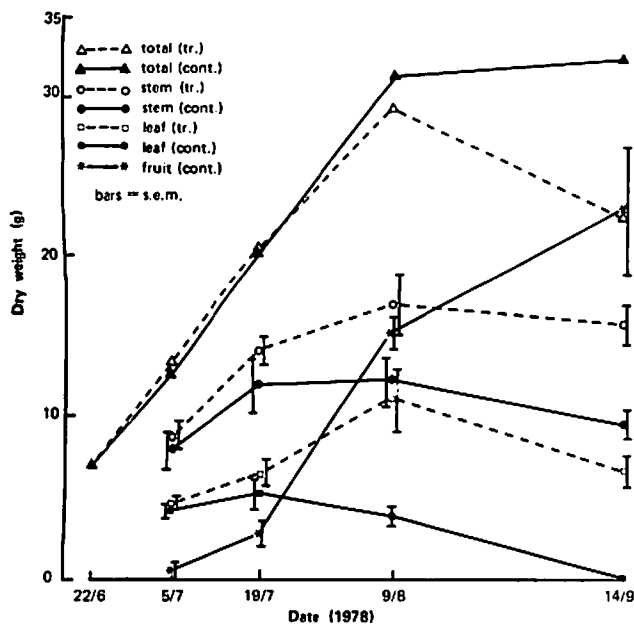


Fig 1. Changes in dry weight of whole plant, stem, and leaves for deblossomed (tr.) and control (cont.) plants, and in fruit of control plants.

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## INFLUENCE OF DROUGHT STRESS ON NITROGEN CONTENT OF FABA BEAN SEED

Peter Schroeder

Institute of Crop Science, Technical University, Berlin West, 1000 Berlin 33(Dahlem),  
GERMAN FEDERAL REPUBLIC

The nitrogen content of faba bean seed varies considerably within varieties (El Sayed, Nakkoul & Williams 1982). Little is known of how agricultural practices influence the nitrogen content. Stock, Kaufhold & Klein (1980) report that there is no change in the protein content of faba bean seed with additional irrigation, despite a yield increase of more than 250%. Different levels of N supplied by mineral fertilizer did not affect the nitrogen content of seed, compared to seed from plants which received their N supply exclusively from rhizobium bacteria (Hofer & Jaggi, 1978). The experiments reported here study the impact of limited drought stress (a period of about a fortnight) on growth and yield of faba beans.

## Materials and Methods

Two pot experiments were performed outdoors under a plastic film roof in 1979 and 1980.

Experiment I: Drought stress was imposed by controlling the weight - i.e. the available water - of the pot. During the stress period the available moisture was kept at 20-30%. Otherwise the pots were regularly irrigated, keeping the available moisture near 100%. The soil was composed of mixture of 5.5 kg compost, 300 g peat, and 3 kg quartz sand.

Experiment II: Water availability was controlled via the soil-water potential. This mechanism operates automatically via a porous ceramic candle which is introduced into a loamy silt soil. The candle is connected with a pending water column to a storage bottle, and with an automatic subpressure system, by which the soil water potential can be kept constant at any level (Fig. 1) (Sommer and Tanasescu, 1981; Schroeder, 1982). During the unstressed period the water availability was equivalent to field capacity conditions ( $-0.08$  bar). During the stress period, the soil water potential was kept at  $-0.55$  bar.

Three varieties were tested:

Kleine Skladia, *Vicia faba minor*, from the Federal Republic Germany (FRG).

Herz Freya, *Vicia faba minor*, FRG

No 213, *Vicia faba major*, Morocco.

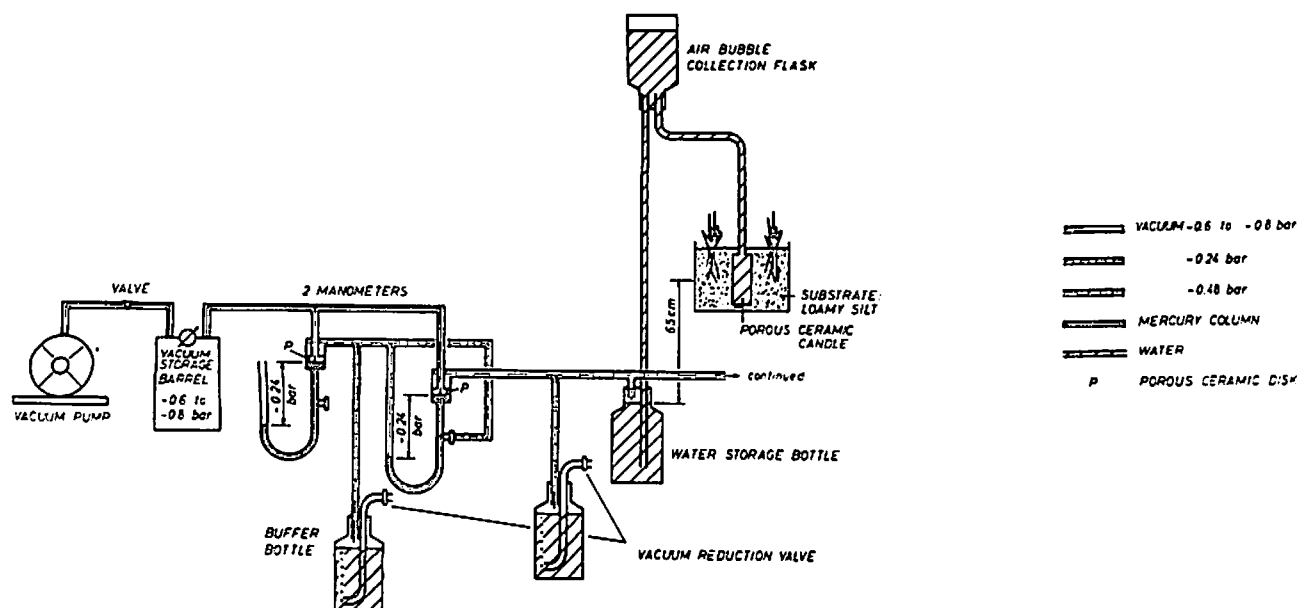


Fig. 1. Schematic diagram of apparatus used in Experiment II.

The limited drought stress treatments were applied for equal periods from the beginning of flowering to the end of the growth period (Table 1). In experiment II, a permanent drought stress treatment, from the beginning of flowering to the end of the growth period, was also imposed (treatment 6, Table 2).

## Results and discussion

From the mean of the three varieties (Experiment I) the data indicate that limited drought stress tends to reduce N content (Table 1). However, this trend is not confirmed by the data of experiment II; stress applied at the beginning of flowering resulted in an N content (4.32%) significantly higher than that of the control (4.08%). This may have been due to random error. The permanent drought treatment led to a significantly lower N percentage (3.67%). The variation in N content between years (0.37%) was similar for both experiments.

Table 1. N content (%) in seed dry matter in Experiment I, means of 1979 and 1980 data.

Period of drought	Kl. Skladia	Herz Freya	No 213	Mean of varieties
1 Beginning of flowering	4.08	4.00	4.45	4.18
2 Mid-flowering half-way	4.01	4.08	4.47	4.18
3 End of flowering	4.17	4.11	4.51	4.26
4 Beginning of pod setting	4.18	4.33	4.30	4.27
5 Beginning of pod filling	3.89	4.07	4.47	4.14
6 Mid-pod filling	3.91	3.97	4.42	4.10
7 End of growth	4.08	4.02	4.54	4.21
8 Control	4.23	4.18	4.66	4.35
LSD 5%		0.24		0.14+

+ corresponding F - values differ significantly with P = 5%

**Table 2.** N content (%) in seed dry matter in Experiment II, 1980.

Period of drought	Kl. Skladia	Herz Freya	Mean of varieties
1 Control	3.96	4.19	4.08
2 Beginning of flowering	4.26	4.38	4.32
3 End of flowering	3.98	4.08	4.04
4 Pod filling	3.96	4.08	4.03
5 End of growth	4.02	4.13	4.08
6 Permanent drought	3.52	3.80	3.67
LSD 1%	0.18		0.12++

++ corresponding F-values differ significantly with  $P=1\%$

According to Rawsthorne *et al.* (1980), competition for photosynthates occurs between bacteroids, nodule tissue, and various vegetative and reproductive sinks in the host plant. The productivity of rhizobium bacteria depends both on their genetic capacity (van den Berg, 1977; Amarger, 1974 a,b) and on conditions in the soil. Water deficits have a negative effect (Sprent, 1972). Apart from reducing the multiplication of, and causing direct damage to, the rhizobium bacteria during drying of the soil (Sprent, 1979), there is a decrease in the availability of nutrients (cf Patrick & Fontenot, 1976) and the plants have a lower uptake of mineral nutrients (Maertens, 1981).

Pod drop is increased and yield depressed in faba beans subjected to limited drought stress (Schroeder, 1983). These, and the reduced nitrogen content of the seeds, are due to the fact that, in a stress situation, the plant is unable to supply enough photosynthates to all the competing sinks i.e., flowers, leaves, pods, roots, and nodules. The report by Stutte & Weiland (1978) of a considerable gaseous nitrogen loss associated with transpiration in soybeans, and other crop and weed species, suggests that N loss is more sensitive than transpiration to temperature variations, and that these processes probably operate independently. Thus, while the gaseous N loss in these experiments might have been reduced due to reduced transpiration during the drought, the limiting factor for N conversion into dry matter remains the ability of the plant to supply the necessary photosynthates. Consequently, the N content in faba bean seed is not a sensitive indicator of limited stress situations, and only long-term droughts result in lower N percentage of dry seed weight in faba beans.

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# Agronomy and Mechanisation

## EFFECT OF SOWING DATE AND PLANT POPULATION ON SEED QUALITY OF FABA BEAN.

Abdalla Elmubarak Ali and Asma Mohamed Ali  
Food Research Centre, Shambat, Khartoum North, SUDAN

### Introduction

The faba bean growing season is short in Sudan, being limited by high temperatures and disease stress at both the beginning and the end. Also relatively high temperatures during the growing season tend to limit yields. Under such conditions, timely sowing of the crop is of paramount importance in achieving good yields of high quality seeds.

No work had been done previously directly relating seed quality to plant population and sowing date. Thus seed samples from a trial concerning seed rate and plant population at Selaim, Hudeiba and Zeidab (Taha *et al.*, 1982) were evaluated for quality characteristics. The results from the three sites were very similar, and thus only the results from the Selaim site are presented here.

### Materials and Methods

#### a. Assessment of the physical quality of the seeds.

One thousand seeds from each treatment were selected at random, weighed and the volume measured before and after soaking. The hydration coefficient (H) was calculated from the equation.

$$H = \frac{\text{weight of soaked beans} \times 100}{\text{initial weight}}$$

The percentage hull and the percentage total defects, including unimbibed seeds, undersized seeds and insect damage, were also determined.

#### b. Cooking of faba bean seeds.

Faba bean seeds were soaked, drained, blanched in boiling water for six minutes, placed into cans with brine and heated in a retort for 60 minutes at 230° F (110° C).

The cookability and increase of weight after processing (I.W.P.) were determined using the following equations:-

$$\text{Cookability} = \frac{\text{drain weight} \times 100}{(\text{drain weight} - \text{initial weight})}$$

$$\text{I.W.P.} = \frac{(\text{drain weight} - \text{filling weight}) \times 100}{\text{filling weight}}$$

### Results and Discussion

Little effect of plant population was observed for any of the parameters measured, hence results presented are for the medium (33.3 plants/m<sup>2</sup>) seed rate.

The highest seed weight (Fig. 1) was obtained from the earliest planting date and seed weight progressively decreased as planting was delayed.

The lowest defect rate (Fig. 2) recorded was from the earliest planting date and defects increased as planting was delayed. These results are in general agreement with those reported by Pandey (1981) and Taha (1982); the latter reported maximum 1000 seed weight from October 10 and 20 planting dates.

The planting date had no significant effect on the hull percentage, hydration coefficient, I.W.P., or cookability of the beans (Table 1). Cookability and I.W.P. were found to be highly correlated ( $r = 0.85$ ).

Yousef (1978) showed that cooking quality of faba beans, as measured by a tenderometer, is highly correlated with certain physical properties of the seeds, such as 100-seed weight, hull percentage, viscosity, hydration coefficient, specific gravity, total solids, soluble solids and insoluble solids in stewing liquor, and colour.

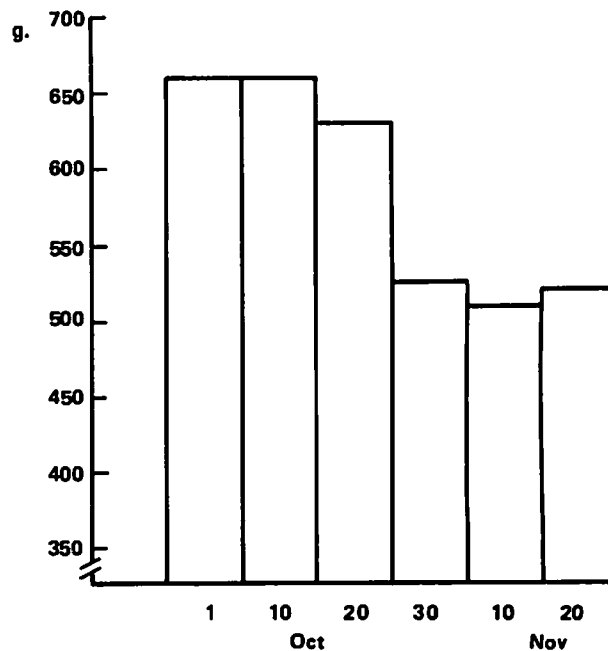


Fig 1. Effect of sowing date on 1000 seed weight.

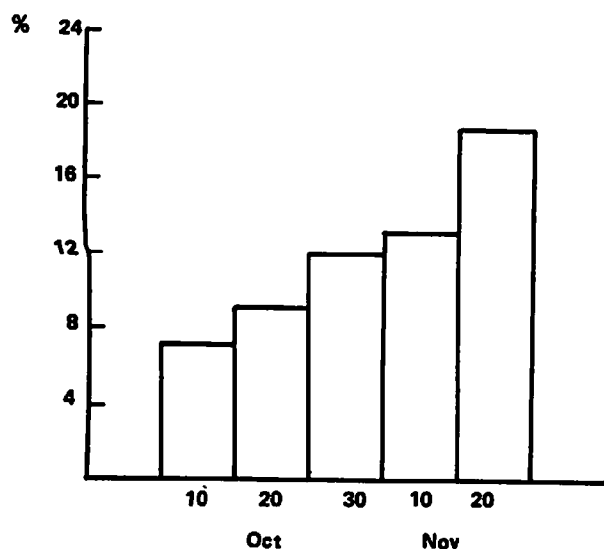


Fig 2. Effect of sowing date on percentage total defects.

Table 1. Effect of sowing date on seed quality in Selaim area. (33.3 plants/m<sup>2</sup>).

Quality factor	Sowing date				
	10 Oct	20 Oct	30 Oct	10 Nov	20 Nov
Hull %	12.4	12.4	13.2	14.8	13.2
Hydration Coefficient	166	162	171	167	200
Cookability	59.40	57.68	59.57	54.76	55.40
I.W.P.	46	36	47	21	38

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## INFLUENCE OF SOWING DATE, SEED RATE, AND SEED SIZE ON YIELD AND YIELD COMPONENTS OF FABA BEANS

Farouk Ahmed Salih

Shanbat Research Station, P.O.Box 30, Khartoum North, SUDAN

An experiment was performed during the 1979/80 season at the Hudeiba Research Station in Sudan to assess the effects of sowing date (October 25 and November 24), seed rate (59.5, 119.0, and 178.5 kg/ha), and seed size (small, medium, large, and ungraded), and their interactions, on the grain yield and yield components of faba bean cultivar Hudeiba 72.

#### Materials and Methods

Seeds of the cultivar were sorted by hand into small, medium and large categories, with 1000-seed weights of 291 g, 368 g, and 437 g, respectively. The ungraded seed had a 1000-seed weight of 338 g. The treatments were combined factorially and laid out in a randomised block design with four replicates. Planting was on ridges 60 cm apart, with seeds evenly spaced along the rows. Irrigation was applied 10 times during the season, at 10 to 12 day intervals.

#### Results and Discussion

No significant interactions between sowing date, seed size, and seed rate were observed.

The main effects of the treatments on seed yield and yield components are given in Table 1. The earlier planted crop significantly outyielded the later planted crop by 37%, although it gave a 20% reduction in the plant population at harvest. Increased 1000-seed weight, number of pods per plant, and number of seeds per pod resulted in increased seed yield in the earlier planted crop. This is largely as would be expected, since late planting subjects the crop to unfavourable environmental conditions, (particularly high temperatures toward the end of the growing season) which hasten maturity. This usually results in fewer pods per plant and smaller seeds. Seed yield was significantly increased by increasing the seed rate, with the highest seed rate (178.5 kg/ha) outyielding the 119.0 kg/ha and 59.5 kg/ha seed rates by 17 and 26%, respectively. The number of pods per plant was reduced with increasing seed rates, but this effect was more than off-set by the increase in the plant population. The number of seeds per pod, and the 1000-seed weight were not significantly affected by seed rate.

Seed size had no significant effect upon seed yield, although the trend was for the highest yields to be obtained from planting medium-sized seed. Seed size showed no significant effect on any of the other parameters, except the number of pods per plant; plants grown from small seed giving fewer than those of the other three seed batches.

**Table 1.** Effects of sowing date, seed rate, and seed size on the seed yield and yield components of the faba bean cultivar Hudeiba 72.

	Grain yield (kg/ha)	Plant pop. ( $\times 10^3$ /ha)	1000-seed weight (g)	No. of pods/plant	No. of seeds/pod
<b>Sowing date</b>					
October 25	1019	78.1	346	19.5	2.53
November 24	743	93.5	334	13.2	2.41
SE	$\pm 47.6$	$\pm 3.81$	$\pm 4.81$	$\pm 0.72$	$\pm 0.05$
<b>Seed size</b>					
Small	873	89.5	339	14.9	2.38
Medium	969	89.5	336	16.8	2.44
Large	864	79.2	344	17.2	2.49
Ungraded	819	85.0	343	16.4	2.58
SE	$\pm 67.8$	$\pm 5.38$	$\pm 6.80$	$\pm 1.02$	$\pm 0.07$
<b>Seed rate (kg/ha)</b>					
59.5	764	76.6	334	19.0	2.50
119.0	850	82.3	350	16.4	2.38
178.5	1028	98.5	337	13.6	2.54
SE	$\pm 58.5$	$\pm 4.66$	$\pm 5.89$	$\pm 0.88$	$\pm 0.06$

## LEAF TOUCHING AND SOME GROWTH CHARACTERISTICS OF FABA BEAN CROPS SUBJECTED TO DIFFERENT DEGREES OF WATER STRESS

A.J. Karamanos

Laboratory of Crop Production, The Agricultural College  
75 Iera Odos, Athens 301, GREECE

### Introduction

The non-destructive measurement of leaf area on marked plants is a useful method when small changes in leaf expansion rates are to be detected. This method reduces considerably the variation between successive samplings and thus smoother and more accurate curves can be fitted to the time courses of both total leaf area and the area of individual leaves. Furthermore, the method is essential whenever field or laboratory space restricts the possibilities for destructive sampling. Nevertheless, there is evidence that routine handling has consequences for plant growth. Mechanical perturbation has induced a number of physiological changes (Audus, 1935, 1939; Godwin, 1935) which eventually have led to an alteration of plant morphology, usually expressed as a decrease in size of the manipulated plant or organ. Thus, shaking reduced height in tomato, coleus and marigold (Mitchell *et al.*, 1975) and leaf area in marrow (Turgeon & Webb, 1971). Furthermore,

rubbing of the internodes induced a reduction of the stimulated internode in *Phaseolus vulgaris* (Jaffe, 1976). Jaffe & Biro (1979) found that mechanical handling was associated with a hardening of *Phaseolus vulgaris*, *P. aureus*, *Glycine max* and *Zea mays* to drought and freezing stress.

In this paper, possible effects of regular leaf touching for non-destructive leaf area measurements on a number of important plant characteristics are examined for a faba bean crop (*Vicia faba* L., cv. Maris Bead) subjected to three different irrigation treatments. The characteristics examined were leaf area, growth of secondary stems, and final yields, on which the effects of touching were assessed in association with the different degrees of water stress experienced by the plants.

### Materials and Methods

The crop was sown on May 6, 1975 on a sandy loam soil at the Reading University Farm (Sonning, Berks.) under polythene rainshelters. Soil water was kept at three different levels by irrigating at different frequencies. Irrigation was timed by the plant water potential just before sunrise ( $\Psi_d$ ).  $\Psi_d$  was allowed to fall to  $-0.3$  MPa before water was applied in the wet (W) treatment, and to  $-0.5$  MPa in the medium (M) treatment. No irrigation was applied to the dry

(D) treatment. The three irrigation treatments were randomized in four replicates. In each treatment, four plants were randomly chosen for non-destructive leaf area measurements when plants had three unfolded leaves (day 29 after sowing). The measurements were taken every second day until 77 days after sowing when plants had the pods well formed. Leaf area of individual leaflets was estimated by measuring their linear dimensions (length and maximum breadth) with a ruler with 0.5 mm divisions. The product of the two dimensions was then multiplied by a proportionality constant (K) which did not vary with leaf age. Separate constants were used for different leaf positions and irrigation treatments (Karamanos, 1976). The values of K were in the range of 0.65 to 0.73. The total leaf area of a plant was estimated by summing the area of individual leaflets of all leaves.

At five day intervals from day 37 to day 72, three plants per treatment were sampled for destructive determination of leaf area. In this case, leaf area was measured in the laboratory with a leaf area photometer (EEL Unigalvo, Type 20). The total number of secondary stems, and biological and economic yields were evaluated for the regularly touched plants, and for the rest of the crop at the beginning of September when all pods became dark.

## Results and Discussion

The relation between the total leaf areas taken on the same days from both touched and untouched plants is shown for all irrigation treatments in Figure 1. From regressions fitted separately for each treatment (Fig. 1) it was found that the slopes did not differ significantly among treatments. When the slopes of the regressions were compared to that of the 1:1 line, only the regression coefficient of the W treatment was found to be significantly lower than unity. Judging from the regression line of the W treatment, it appeared that the touched plants exhibited larger leaf areas during the earlier growth stages, with a tendency to approach those of the untouched plants in the later stages.

The same conclusion can be reached when examining the common regression line from all three treatments (Fig. 1). The slope of the straight line (0.82) was significantly lower than unity ( $t = 3.72$ ,  $p < 0.001$ ). These results contrast with those reported by Bull (1968), who did not detect any obvious differences in leaf area between touched and untouched plants in faba beans and also with the findings of Dennett (1975), who found that regular handling was associated with greater leaf areas at the later stages of plant growth.

Handling increased the number of plants with secondary stems compared to the untouched plants (Table 1). This agrees with the results of Dennett (1975).

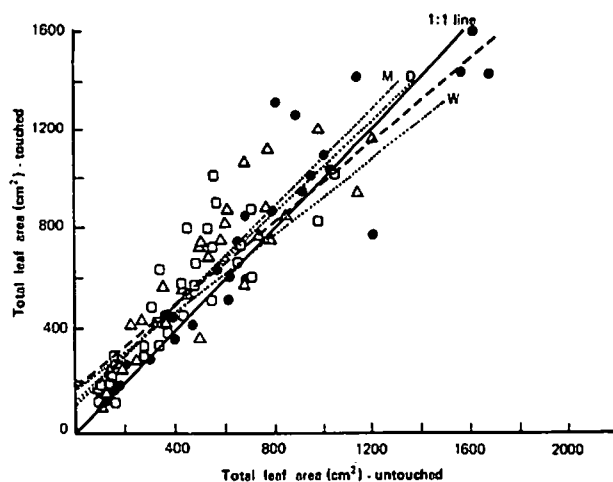


Fig. 1. The relation of the total leaf areas per plant measured on the same days in touched and untouched faba bean plants. Results from three irrigation treatments (○ : wet (W), △ : medium (M), □ : dry (D) in four replicates on eight sampling occasions. The dashed line shows the fitted linear regression for all three treatments ( $Y = 166.3 + 0.82X$ ,  $r = 0.88$ ), while the dotted lines show the fitted linear regressions for each irrigation treatment (W:  $Y = 174.7 + 0.76X$ ,  $r = 0.88$ ; M:  $Y = 109.9 + 0.98X$ ,  $r = 0.91$ ; D:  $Y = 122.0 + 0.94X$ ,  $r = 0.86$ ). The continuous line indicates the 1:1 line.

Table 1. The number of plants with secondary stems for touched and untouched plants for three irrigation treatments.

Treatment		With stems	Without stems	$\chi^2$
W	Touched plants	11	5	10.75 (**)
	Untouched	252	564	
M	Touched plants	10	6	9.64 (**)
	Untouched	204	531	
D	Touched plants	9	7	8.33 (**)
	Untouched	210	631	

\*\* = significant at the  $p < 0.01$  level.

The relations of the biological and economic yields between touched and untouched plants for all treatments are shown in Figures 2 a and b. In both cases, the points from the M and D treatments were more or less uniformly distributed along the 1:1 line, whereas in the W treatment the yields of the untouched plants were consistently higher than those of the touched ones. As a result of the different relationships among treatments, the slopes of the linear regressions for all three treatments were significantly lower than unity in both cases ( $t = 3.64$ ,  $p < 0.01$  for the biological, and  $t = 4.06$ ,  $p < 0.01$  for the economic yield).

The routine handling of maize plants considerably reduced stem dry weight, and to a lesser extent the total leaf area, in comparison with unhandled plants (Beardsell, 1977). In our case, a reduction in the total leaf area of the touched plants was observed only in the well-hydrated plants of the W treatment during the later growth stages. Such a reduction can be partly ascribed to a significant acceleration of leaf senescence and death observed in the touched plants (Table 2).

Although such responses are already known to occur in other plants, probably as a result of enhanced ethylene production (Salisbury, 1963; Jaffe & Biro, 1979), it is worth noting that the stressed plants of the M and D treatments *did not* reduce their leaf area to the same extent as the plants in the W treatment. Since touching is known to make the plants harder to several kinds of stress (Jaffe & Biro, 1979), the reduction in leaf area by means of leaf

senescence observed in the W treatment might eventually be regarded as an adaptive mechanism for reducing the transpiring area of the touched plants. The effect was less pronounced in the harder plants of the M and D treatments. In the W treatment the dry weights of the stems of touched plants were also lower, though not significantly, while the differences were trivial in the M and D treatments.

The leaf area reduction of the touched plants in the W treatment during pod filling may also be responsible for the lower seed yields of the touched plants. The lack of consistent differences in seed yields between touched and untouched plants in the M and D treatments may be due to the virtually similar leaf areas in those treatments.

Table 2. The mean of dead leaves for both touched and untouched plants in the three irrigation treatments on day 72. The standard errors of the means ( $S_x$ ) and the levels of significance are also shown (ns: not-significant).

Treatments	Touched		Untouched		Level of significance
	$\bar{x}$	$S_x$	$\bar{x}$	$S_x$	
W	1.56	0.24	0.17	0.11	$p < 0.001$
M	2.19	0.23	1.58	0.29	ns
D	2.06	0.19	1.50	0.42	ns

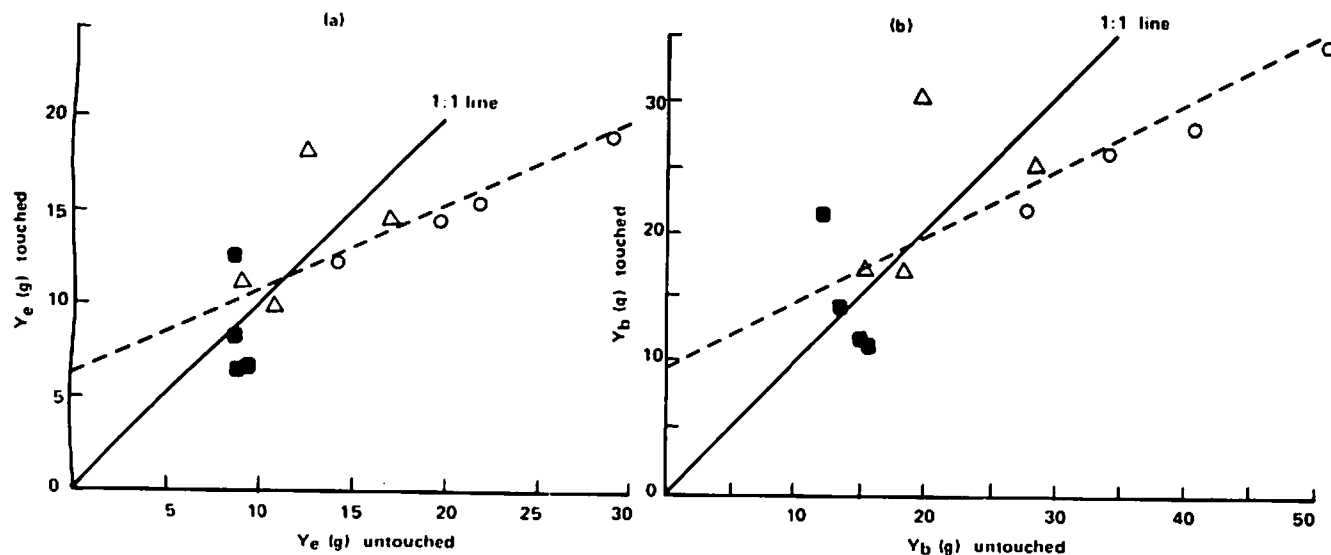


Fig. 2. The relations of the economic ( $Y_e$ ) and biological yields ( $Y_b$ ) between touched and untouched faba bean plants at harvest. The dashed lines show the fitted linear regressions while the continuous ones the 1:1 lines in each case. (a) Economic yield:  $Y = 6.2 + 0.44X$ ,  $r = 0.73$ . (b) Biological yield:  $Y = 9.4 + 0.50X$ ,  $r = 0.80$ .

The considerable increase in the production of secondary stems observed in the touched plants may be related to an alteration in the balance of the hormones associated with apical dominance (Tucker and Mansfield, 1973) leading eventually to cancelling of the inhibition of the basal buds. Judging from the values of  $\times 2$  for each treatment, it appears that increasing water stress slightly reduced the number of secondary stems induced by touching (Table 1). The fact that water stress alters the hormonal balance of plants (Vaadia, 1976) may complicate the possible effect of leaf touching on the inhibition of the basal buds and, thus, makes an interpretation extremely difficult.

### Conclusions

The regular touching of leaves for non-destructive leaf area measurements of faba bean crops subjected to three different degrees of water stress induced alterations in some growth characteristics in comparison to untouched plants. Leaf touching considerably increased the production of secondary stems. Total leaf area per plant was lower in the touched plants only in the well-hydrated treatment after the beginning of pod-filling. This was partly ascribed to a significant increase in the number of dead leaves of the touched plants. Biological and economic yields per plant were lower from the touched than the untouched plants only in the well-hydrated treatment, but did not differ in the drier treatments. It appears that lower degrees of water stress are associated with an increase in the effects of leaf touching on the examined characteristics.

### Acknowledgement

I wish to thank Mr. R. Silver for technical assistance.

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# INFLUENCE OF PLANT DENSITY, GIBBERELIC ACID AND SITE ON THE YIELD AND YIELD COMPONENTS OF DIFFERENT FABA BEAN GROWING TYPES

J. Burkhard and E. R. Keller,  
Swiss Federal Institute of Technology, Institute of Crop  
Science, Universitatstrasse 2, CH-8092 Zurich,  
SWITZERLAND.

In *Vicia faba* it is noticeable that a significant proportion of the flowers and young pods do not develop into mature pods (Keller, 1974). By means of  $^{14}\text{C}$  labelling, Jaquierey and Keller (1978, 1980) showed strong competition for assimilates between leaves, stem apices, and young pods. By removing the stem apex, it was possible to restrict vegetative growth in favour of reproductive growth. However, with this method the photosynthetically active leaves of the stem apex were absent during the pod filling phase (Gehriger and Keller, 1979).

Bellucci *et al.* (1982) using growth regulators, tried to modify the partitioning between vegetative tissues and pod formation so as to lead to a better development of yield components. It was shown that the application of gibberellic acid ( $\text{GA}_3$ ) led to greater pod growth and to higher seed yields. It remains to be seen whether this can be confirmed at other locations and with different varieties.

A stable and relatively high harvest index (ratio between the dry matter of seeds and the total dry mass of the plants - in the present study the root dry matter was excluded) was found in all experiments with faba beans at the Swiss Federal Institute of Technology. From this it would appear that the plant ideotype one should seek would combine early maturity with sturdy growth. With suitable agronomic practices (i.e., stand), such a plant would produce more total dry matter, and thus greater seed yield.

Our work aims to answer the following questions:

- \* What demands must the faba bean ideotype meet ?
- \* Does the harvest index remain stable under changing environments ?
- \* Can a constant harvest index combined with a high planting density improve the production of total dry matter per unit area and thus the seed yield ?
- \* Is the yield fluctuation of faba bean related to the site ?
- \* Can bloom and pod drop, and with it the yield of faba bean, be influenced by the use of gibberellic acid ( $\text{GA}_3$ ) ?

The experiment consisted of field trials in the years 1979 to 1981, and a green house experiment in 1982. In 1979 we examined the influence of plant density (10-80 plants/ $\text{m}^2$ ) on yield and yield components of four different growing types (Herz Freya, Maris Bead, Minica, Svalof 0621), according to the methods of Nelder (1982). Concurrently, the influence of site upon yield was investigated at 11 sites, with crops of uniform plant density. In 1980 and 1981 we tested selected varieties (Herz Freya, Maris Bead, and Minica) and plant densities (30-70 plants/ $\text{m}^2$ ) at two sites, along with the effects of  $\text{GA}_3$  on the yield components. In 1982 the reaction of different varieties to  $\text{GA}_3$  treatment under standardised greenhouse conditions was investigated.

## Results

The results of the experiments indicate that the faba bean ideotype should have the following characteristics:

- high seed yield per plant.
- high total dry matter yield per unit area, combined with a high harvest index.
- a large number of fertile internodes and a long fertile stalk. Preferably the flowering nodes should be carried high on the stalk of the plant.
- resistance to, or tolerance of, pests and diseases.
- early maturity, and lodging resistance.

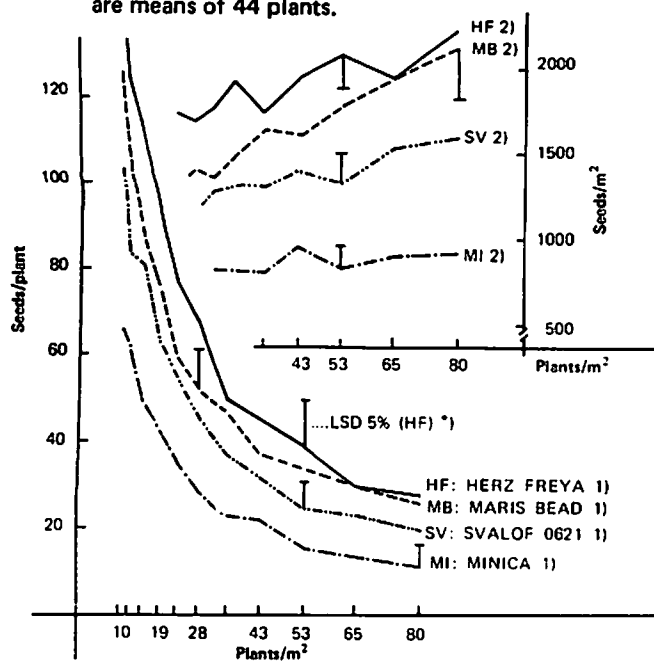
Only weak correlations were found between seed yield and plant height, seed number per pod, and protein content of the seed (Table 1). The tillering capacity of the plants was suppressed at high planting densities.

The crops having the highest yields all showed high seed and pod numbers per plant (Fig. 1), and a high single pod weight.

Crude protein contents of the seed ranged from 25 to 31%. Differences were observed between sites and varieties. Minica was notable in that at high plant populations it gave high yields allied to high protein contents of the seed.

The harvest index varied widely with variety, planting density and site, and between years. With increasing planting density, stalk and seed weight per plant, and harvest index decreased. However, given sufficient lodging resistance, the total seed yield was maintained or increased. Thus the harvest index is a measure of the individual plant performance, but not necessarily of whole crop performance.

**Fig 1.** Influence of plant density on the number of seeds per plant and number of seeds/m<sup>2</sup> (inset). Values are means of 44 plants.



\*) The LSD-values of each variety are valid for all plant densities.

**Table 1.** Selected correlations (*r*) from the Langenthal site 1981.

	Herz Freya	Seed yield Maris Bead	Minica
Plant height	.22**	.19*	-.01 ns
Height of the topmost pod	.57***	.66***	.28***
Height of the lowest pod	.08 ns	.13 ns	.00 ns
Length of the pod-bearing stem	.43***	.60***	.27***
No. of i. (i = internodes)	.44***	.50***	.09 ns
No. of pod-bearing i.	.51***	.72***	.48***
No. of i. to the topmost pod	.46***	.66***	.36***
No. of i. to the lowest pod	-.00 ns	.06 ns	-.07 ns
No. of pods/ plant	.55***	.76***	.51***
No. of seeds/ pod	.35***	-.00 ns	-.05 ns
No. of seeds/ plant	.63***	.77***	.50***
No. of seeds/ m <sup>2</sup>	.90***	.96***	.74***
Thousand seed weight	.49***	.42***	.51***
Seed weight/ plant	.77***	.84***	.67***
Haulm weight/plant	.54***	.69***	.56***
Haulm weight/m <sup>2</sup>	.80***	.75***	.69***
Harvest-Index	.74***	.70***	.53***

**Table 2.** Summary of experimental effects, 1981.

The underlined symbols (\*) in the column headed "variety" are valid for the comparison between Herz Freya and Maris Bead, and not for the comparison between all varieties (including Minica).

	Variety	Plant density	GA3
Plant height	***	ns	ns
Height of the topmost pod	***	ns	ns
Height of the lowest pod	***	**	ns
Length of the pod-bearing part	***	**	ns
No. of i. (i = internodes)	***	**	ns
No. of pod-bearing i.	***	**	ns
No. of i. to the topmost pod	***	**	ns
No. of i. to the lowest pod	***	***	ns
No. of side shoots	ns	ns	ns
No. of pods/ plant	***	***	ns
No. of pods on side shoots	ns	ns	ns
No. of seeds/ pod	***	*	*
No. of seeds/plant	***	***	ns
No. of seeds/ m <sup>2</sup>	***	***	ns
Thousand seed wt.	***	ns	ns
Seed weight/plant	**	***	ns
Seed weight/ m <sup>2</sup>	**	*	ns
Haulm weight/plant	***	***	ns
Haulm weight/ m <sup>2</sup>	***	***	ns
Harvest-Index	***	*	ns
Protein content	**	*	ns

ns = not significant

\*, \*\*, and \*\*\* = significant at the 5%, 1% and 0.1% P level, respectively.

Increasing the planting density significantly reduced many parameters in the individual plants (Table 2). However, this effect may be offset by the increased number of plants per unit area, in the range of planting densities studied. Given the cost of seed of lodging resistant varieties, the economic optimum yield is achieved at approximately 50 plants/m<sup>2</sup>.

The fluctuation in yields observed was largely independent of site, given that no disease, pest or storage problems occurred.

The varieties tested all reacted in the same manner to GA<sub>3</sub> application; internodes extended a few days after application, with short internodes, followed by further extended internodes developing some three weeks after the application. The only plant parameter which was significantly affected was the number of flowers per plant, and the seed yield was not affected.

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#### CRACKING FREE THRESHING PRINCIPLE FOR DRY FABA BEANS.

J. Diekmann, J. Papazian, and H. Mutran,  
ICARDA, P.O.Box 5466, Aleppo, Syria.

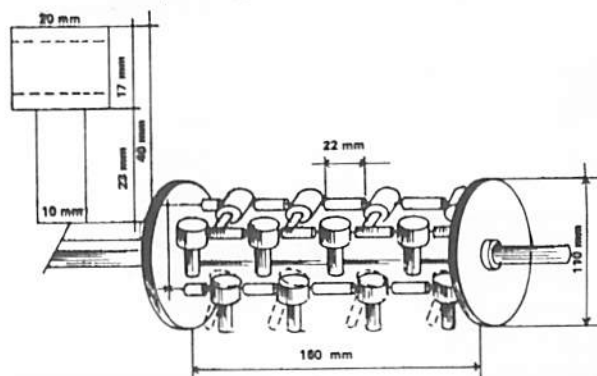
A method which allows threshing of faba beans without breakage of the seeds has been developed. The concept is based upon ideas of Mr. H. Mutran, ICARDA's Agricultural Workshop Supervisor. The method uses a flail-finger threshing drum, instead of the standard rasp-bar drum and concave, or rubber roller principles previously used.

The new drum (Fig. 1) has been installed in stationary single-plant threshers and plot threshers (both Vogel-type) at ICARDA's experimental farm. The main use is the threshing of hand harvested plot material, but the principle could be adapted for commercial threshers. The main advantage over the rasp-bar drums or rubber rollers is to give breakage-free threshing of faba beans. The system is effective for chickpeas and lentils as well. The drum will be tested for use with other legumes, i.e., ground nuts.

In the single plant thresher with a 3 hp (2.25 kW) engine, a drum 160 mm long and 110 mm in diameter, with four bars and four fingers per bar, is used. The fingers are 10 mm diameter and 40 mm long. A drum speed of 1200 to 1500 rpm is used.

For the plot size thresher with an 8 hp (6 kW) engine the drum is 600 mm long by 350 mm in diameter, with six bars and 12 fingers per bar. The fingers are 12 mm diameter and 95 mm long. The drum speed used is 700 to 1000 rpm.

The fingers are round and made of aluminium. The system has been installed in 10 machines so far, and was used successfully during the 1982 harvest season.



The flail-finger threshing drum installed in a single plant thresher, and a sample of threshed beans.

# Pests and Diseases

## THE DISTRIBUTION OF *DITYLENCHUS DIPSACI* (KUHN) FILIPJEV ON FABA BEAN IN SYRIA

Bernd Augustin

Institut für Pflanzenkrankheiten, Nussallee 9, 5300 Bonn 1  
GERMAN FEDERAL REPUBLIC

### Introduction

The stem nematode, *Ditylenchus dipsaci* (Kuhn) Filipjev is a polyphagous nematode with a host range exceeding 450 different plant species. Nineteen biological races of *D. dipsaci* have been described according to their host preference (Decker, 1961), and two additional races have been described using body length and chromosome number (Grimaldi de Zio, 1975). *Vicia faba* L. is the most common host of the faba bean race, although several other races are able to multiply on this host (Whitehead *et al.*, 1979; Sturhan, 1964, 1965, 1966). In Syria the giant race seems to be dominant on faba beans.

The amount of damage caused by this nematode varies considerably according to host, nematode population, and environment, and can range from displaying no symptoms to plant death (Decker, 1961).

The stem nematode has been reported on *V. faba* from many mediterranean countries: Morocco (Schreiber, 1977), Tunisia (Schreiber, 1978), Jordan (Hashim, 1979), Cyprus (Phillis and Siddiqi, 1976), Syria (Hanounik and Sikora, 1980), and from Algeria, Italy, Malta, France and Spain (Lamberti, 1981).

The first observation of *D. dipsaci* on faba bean in Syria was the stimulus for investigating the problem by evaluating the distribution of this parasite throughout the country and the degree of infestation of local seed exchanged on markets and between growers.

### Materials and Methods

Some 108 faba bean fields were checked for *D. dipsaci* attack during the 1981/82 season; 100 plants/ha were examined for symptoms of nematode damage, and the presence of *D. dipsaci* was verified microscopically. The disease intensity was assessed using the following key:

0 = no symptoms

1 = discolouration with moderate damage

2 = malformation with severe damage

The disease intensity index is given by the equation:

$$(n_1 \times 0) + (n_2 \times 1) + (n_3 \times 2)$$

$$n_1 + n_2 + n_3$$

where  $n_1$  = number of plants showing no symptoms;  $n_2$  = number showing discolouration; and  $n_3$  = number malformed.

Twenty-two samples from farmers' seed lots, and 19 samples from different local markets were tested. The seeds were soaked individually in water at 5°C, and the nematodes released into the water after 24 hours were counted. Except for six samples, each sample consisted of 300 seeds. In addition, the presence of nematode larvae in plant debris and soil particles of each sample was investigated.

### Results

Table 1 shows the occurrence, distribution and intensity of *D. dipsaci* infestation in faba bean fields in Syria. The nematode was detected in 48 out of 108 fields. The incidence and intensity of attack in each field varied greatly. The nematode occurred mainly in the western part of Syria, along the coast (Lattakia and Tartous) and around the larger cities (Damascus, Hama, Idlib), traditional faba bean growing areas. Infested fields were not found in the drier and warmer areas of eastern Syria (Raqqah, Deir ez Zor, Hassake).

The levels of *D. dipsaci* infestation of seed from farmers' seed lots and from local markets are presented in table 2; 53.7% of the seed samples examined were infested with nematodes. The nematode was found more often, and in larger numbers, in farmers' samples than in those taken from the market. Whereas 1.3 to 2.8% of the farmers' seeds were infested, only 0.3 to 0.6% of the seeds from the market samples proved to be infested. The only exception was a sample from the local market of Chisr al Choughour with 16.3% infested seeds. Besides the seed, plant debris and soil particles in seed lots proved to be an important source of the nematode.

### Discussion

The results of the survey of *V. faba* fields and seed samples in Syria 1981/82 demonstrate that *D. dipsaci* is well established within the country. This is especially true in the western part where faba beans are grown under rain-fed or partially irrigated conditions. The distribution of the nematode seems to be greatly influenced by environmental conditions. The frequency of *D. dipsaci* decreased with increasing temperature and decreasing moisture levels. In eastern Syria around Al Hassake and Deir ez Zor no infesta-

tions were found. The occurrence of the nematode around Tartous and Dar'a was relatively low. In the area near Homs, cultural practices, i.e., crop rotation, may have reduced nematode distribution.

Fifty four percent of all *V. faba* seed samples were infested with *D. dipsaci*. There were large differences in frequency of occurrence and nematode density in the seed, depending on the origin of the seed. On average, 0.6 - 2.2% of the seeds were infested. The results are similar to those obtained by Schreiber (1977; 1978) who found 1.25-3.54% infestation in Morocco and Tunisia.

Most of the faba bean seed production in Syria is located around Homs and Aleppo, where only moderate *D. dipsaci* infestation was found. On that basis, it would be advisable to concentrate the seed production in these areas, which would help in reducing the spread of the seedborne nematode. Together with appropriate crop rotation and weed control, it should be possible to minimise the *ditylenchus* problem on *V. faba* in Syria.

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Table 1. Occurrence, distribution and intensity of *Ditylenchus dipsaci* on faba bean in Syria.

Region/ Village	field No.	approx. size (ha)	incidence (%)	intensity index (0-2)
Lattakia	1	0.3	17	0.17
Billo	2	0.2	72.5	1.40
Joplo	3	0.5	91.6	1.07
	4	0.005	50.0	
	5	0.03	22.5	0.23
	6	0.025	42.0	0.42
Kabir	7	0.05	100.0	1.0
	8	0.01	48.0	0.52
Astal	9	0.75	64.0	0.82
	10	0.05	90.0	1.04
Lattakia	11	2.0	47.5	0.51
Haffe	12	0.15	44.0	0.44
	13	0.8	47.0	0.49
	14-17		0	
			x 43.3	
Tartous, Banyas	1	0.5	58.0	0.63
Banyas	2	0.5	5.0	0.05
	3	0.2	60.0	0.60
	4	0.4	8.8	0.10
	5	0.1	30.0	0.30
	6-10		0	
			x 16.2	
Hama, Soran	1	0.025	0	
Hama	2	0.08	62.0	0.70
	3	0.015	70.0	0.70
			x 44.0	

(Continued)

Table 1. Occurrence, distribution and intensity of *Ditylenchus dipsaci* on faba bean in Syria.

Region/ Village	field No.	approx. size (ha)	incidence (%)	intensity index (0-2)
Homs	1	0.075	0	
Al Quseir	2	0.15	0	
	3	0.1	12.0	0.12
	4	0.075	2.0	0.02
	5-11		0	
			$\bar{x}$ 1.3	
Damascus, Aka	1	0.2	2.0	0.02
Aka	2	0.1	22.0	0.34
	3	0.05	6.0	0.06
Akaria	4	0.6	15.0	0.26
Al Abun	5	0.05	84.0	1.60
Arbin	6	1.0	69.0	0.69
Harasta	7	1.0	90.0	0.90
Douma, Rihan	8	0.09	34.0	0.34
Zabadani	9	0.05	100.0	1.00
	10	0.025	40.0	0.40
	11	0.4	38.0	0.44
	12	0.6	11.0	0.11
	13	0.08	98.0	1.00
	14	0.4	30.0	0.30
Randenun	15	0.6	12.0	0.13
Rhabareb	16	0.01	76.0	0.76
	17-25		0	
			$\bar{x}$ 29.1	
Dar'a, Ataman	1	0.015	24.0	0.26
	2-6		0	
			$\bar{x}$ 4.0	
Idleb	1	0.075	38.0	0.40
Maara	2	0.1	18.0	0.18
			$\bar{x}$ 28.0	
Aleppo, Afrin	1	0.6	16.0	0.16
El Bab	2	0.75	6.0	0.07
	3	0.6	26.0	0.26
	4	0.7	2.0	0.03
	5	0.3	58.0	
Om Assaf	6	0.3	24.0	0.28
Ain el Arab	7	0.025	4.0	0.04
	8-20		0	
			$\bar{x}$ 6.8	
Raqqa, Deir Ezzor, Hassaka	1-14		0	
			$\bar{x}$ 0	

Table 2. Frequency and intensity of *Ditylenchus dipsaci* infestation of faba bean seed samples collected in Syria, 1982

Region/ village	sample No.	source	infested seeds (%)	max. No. larvae/seed	No. larvae in plant debris and soil part
Idleb, Al Jar	1	farmer	0.33	2	3
Harem	2	"	0.33	49	
Istrabrag	3	"	5.66	438	4
Harem	4	"	2.99	147	98
	5	"	1.99	39	
			$\bar{x}$ 2.3		
Hama, Ayo	1	"	8.99	4875	108
	2-4	"	0		
			$\bar{x}$ 2.3		
Homs, Zor el Pasha	1	"	4.99	2175	13
Tel Biseh	2+	"	11.99	875	9
Tel Kalakh	3	"	5.33	76	
Ismailieh	4	"	1.33	4150	81
	5-9	"	0		
			$\bar{x}$ 2.8		
Damascus + Dael Dar'a	1	"	1.33	5	41
Dar'a, Aftares	2	"	3.33	128	67
	3-4	"	0		
			$\bar{x}$ 1.3		
Aleppo	1	market	1.99	300	47
	2	"	0.66	7	5
	3	"	0.66	69	214
	4	"	1.33	100	44
	5-9	"	0		
			$\bar{x}$ 0.52		
Idleb	1	"	1.66	335	700
	2-3	"	0		
			$\bar{x}$ 0.56		
Lattakia	1	market	0.33	10	
	2	"	0.66	58	9
	3	"	0		
			$\bar{x}$ 0.33		
Tartous	1	"	0.33	1	
			$\bar{x}$ 0.33		
Chisr al	1	"	16.33	8600	2670
Choughour	2	"	0		
			$\bar{x}$ 8.2		

+ less than 300 seeds tested

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## EFFECTS OF ALDICARB ON *DITYLENCHUS DIPSACI* IN *VICIA FABA*.

S.B. Hanounik  
ICARDA, P.O. Box 5466, Aleppo, SYRIA.

### Background and objectives

The stem nematode, *Ditylenchus dipsaci* (Kuhn) Filipjev, is a destructive seed and soil borne pathogen of faba beans, *Vicia faba* L., in many parts of the temperate region (Goodey, 1941; Hooper, 1971). Infested seeds play an important role in its survival and dissemination. Although methyl bromide is highly effective against free larvae of *D. dipsaci*, dosages needed for complete eradication of eelworms from infested seeds cause a substantial decrease in germination (Powell, 1974). Therefore, this test was conducted, at Lattakia, Syria, to study the effect of aldicarb (Temik-10G) on yield and seed infestation of faba beans grown in soils with different population densities of *D. dipsaci*.

Table 1. Effect of aldicarb soil treatment on yield and seed infestation of faba beans grown in soils with different *Ditylenchus dipsaci* population densities.

Nematode density (Larvae/1000 cm <sup>3</sup> soil)		Dry seed Yield* (g/m <sup>2</sup> )		% plants attacked		% infested seeds	
Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated
0	0	528 a	560 f	0.0	0.0	0.0	0.0
40	16	506 a	554 f	2.8	1.4	5.0	0.0
100	28	448 b	500 g	7.0	1.4	6.6	0.0
300	80	364 c	490 g	17.0	2.8	10.3	0.0
720	240	312 d	484 g	37.0	5.7	13.3	0.0
6500	2400	170 e	416 h	65.7	14.0	20.0	1.3

\* Within each column, means followed by different letters are statistically different ( $P < 0.05$ ) according to Duncan's Multiple Range Test.

### Materials and Methods

*D. dipsaci*-infested stems of the susceptible faba bean cultivar ILB 1815 were cut into 2 cm segments, mixed thoroughly with soil at a ratio of 1:1, then watered daily in the field. After 15 days, infested soil was diluted with nematode-free soil until population densities of 0, 40, 100, 300, 720 and 6500 larvae of *D. dipsaci* per 1000 cm<sup>3</sup> soil were obtained. Faba bean seeds were sown in rows 1m long and 50 cm apart, then covered to a depth of 15 cm with this series of infested soils. Aldicarb, a systemic nematicide, was then added in bands 10 cm wide to the surface of the infested soil, using 10kg a.i./ha and mixed thoroughly to a depth of 5 cm. The plots were then irrigated with water. A split plot design was used with nematode densities in the main plot and chemical treatment in the split, with 3 replications.

### Results and conclusions

As the population density of *D. dipsaci* was increased from 0 to 6500 larvae per 1000 cm<sup>3</sup> of soil, there was a significant increase ( $P < 0.05$ ) in the number of both infested plants and seeds, and also a significant decrease ( $P < 0.05$ ) in faba bean yield. Decreases in the population density of *D. dipsaci* in aldicarb-treated soils were associated with a significant decrease ( $P < 0.05$ ) in disease incidence and a significant increase in faba bean yield at all nematode densities. Yield increases in aldicarb-treated over untreated plots ranged from 90% with the lowest, to 144% with the highest nematode density. Seeds obtained from treated plots were nematode-free at all population densities except from plots with 6500 larvae/1000 cm<sup>3</sup> soil. Plants grown in untreated plots yielded infested seeds even at the lowest nematode population levels.

Thus, aldicarb can be used to reduce losses due to *D. dipsaci*, and also to prevent nematode survival and dissemination in seeds from plants grown in soils with 720 larvae/1000 cm<sup>3</sup> soil or lower.

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## A SCANNING ELECTRON MICROSCOPE STUDY OF GERMINATION AND EARLY STAGES OF DEVELOPMENT OF *OROBANCHE CRENATA* FORSK.

M. Aber and G. Salle

Universite Pierre et Marie Curie, Laboratoire de Cytologie et Morphogenese Vegetales, Batiment L, 4 Place Jussieu, 75230 PARIS Cedex 05, FRANCE

In many regions faba bean is severely attacked by the flowering parasitic weed, *Orobancha crenata* Forsk., causing severe losses. Since *O. crenata* was first identified in Tunisia (Boeuf, 1905), Algeria (Ducellier, 1923) and Morocco (Bleton, 1943), it has been spreading throughout the mediterranean countries.

A study of the early stages of development of *O. crenata* is vital for the understanding of the biology of this obligate parasite. By planting the seeds of *O. crenata* and faba bean in close proximity (Aber and Salle, 1983), it has been possible to observe the earliest stages of germination of the parasite, and its further growth.

The brownish seeds of *O. crenata* are minute (Fig. 1), 350 to 450  $\mu$ m long, and are therefore easily dispersed by field machines, wind or water. They are oval (Figs. 1 and 2), narrow towards the micropyle, and broad at the funicle end. Observed with the scanning electron microscope (SEM), the seed coat shows alveolae which are large dead cells in which the collapsed outer wall has often disappeared (Fig. 2). Inner and radial walls are rich in true pits which are more or less round, with an average diameter of 1.5  $\mu$ m.

When *O. crenata* seeds are in the rhizosphere of *Vicia faba*, they begin to germinate 40 days after planting. Two weeks before the host flowers, many seeds are germinated (Fig. 3).

Common abbreviations: H, host root; Pr, procaulome; S, seed.

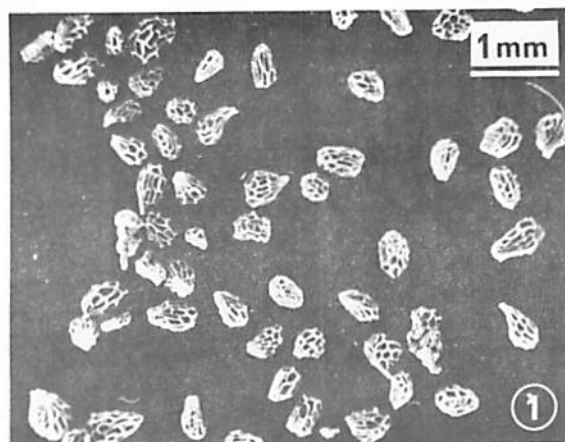


Fig. 1. Seeds of *O. crenata* at low magnification.

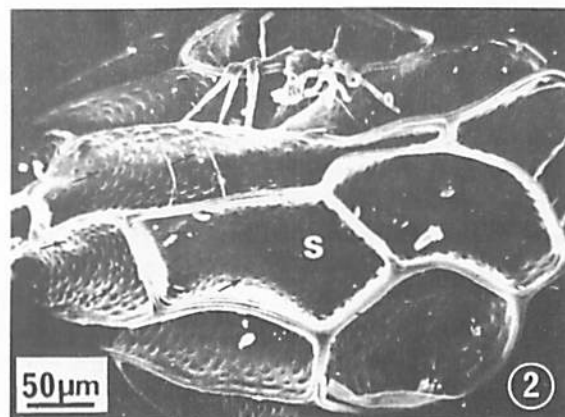


Fig. 2. Detail of a seed of *O. crenata* at higher magnification. Numerous pits are present in the alveolae of the testa (arrows).

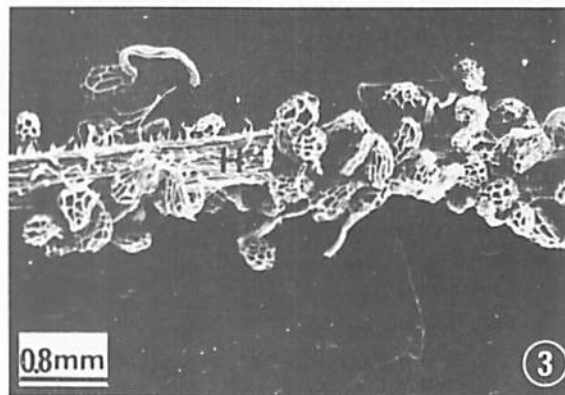


Fig. 3. Seeds of *O. crenata* germinating in the vicinity of a host root.

Upon germination a delicate parenchymatous filament, the procaulome,  $800\text{ }\mu\text{m}$  long and  $80\text{ }\mu\text{m}$  wide, arises from the region of the radicle of the relatively undifferentiated embryo (Fig. 4). The procaulome has a terminal group of rapidly dividing cells; occasionally fungi are observed in this region.

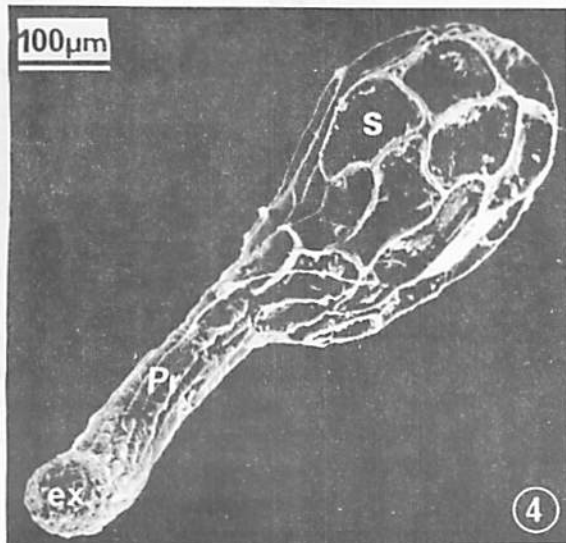


Fig. 4. Detail of early stage of germination with the procaulome and its extremity, ex.

The tip of the procaulome swells and differentiates into many papillae (Fig. 5) as it grows towards the host root which come into contact with the outer root cells. The attachment takes place in two to three days, the rapidity suggesting that enzymatic processes are involved.

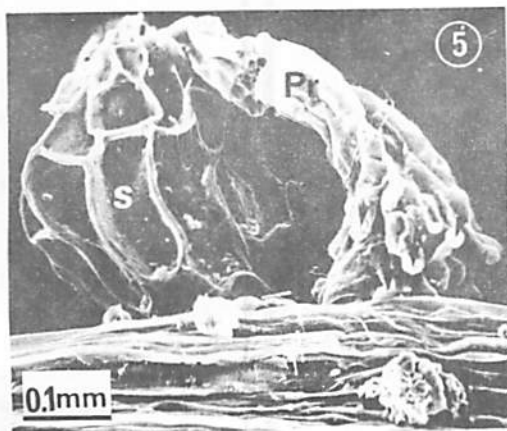


Fig. 5. Very beginning of attachment to the host. The extremity of the procaulome differentiates into many papillae (arrows).

Some of the contact cells penetrate through the host cortex in the direction of the central stele, where the connections to the vascular system of the host are established. Meanwhile, the external part of the seedling grows to form a small tubercle ( $\approx 600\text{ }\mu\text{m}$  in diameter) which can be easily distinguished from the host nodules by its yellowish colour and the presence of the testa (Fig. 6). At this time, the host root cortex has been more or less torn off by the growing endophyte. The distal part of the host root becomes thinner due to the sink effect of the parasite. The rate of development of the young tubercle is closely related to the climate and to the vigour of the host; the more vigorous the host, the larger the tubercle. Its diameter can exceed 5 mm. This correlation emphasises the importance of the availability of nutritive substances from the host root.

The number of tubercles on the root varies according to the number of *Orobanche* seeds that have germinated in the vicinity, and with the number of procaulomes attached to the root. Two to three weeks after the parasite becomes attached to the host, the surface of the tubercle develops small domes, the future short roots (Fig. 7). Simultaneously, the procaulome degenerates. Later, several crowns of roots develop around the leaf primordia of the *Orobanche* shoot apex (Fig. 8).

This SEM study, together with a cytological and cytochemical one (Aber and Salle, 1983), provides new information on the developmental stages of *Orobanche crenata*, and, hopefully, fresh insights into means of controlling this parasite.

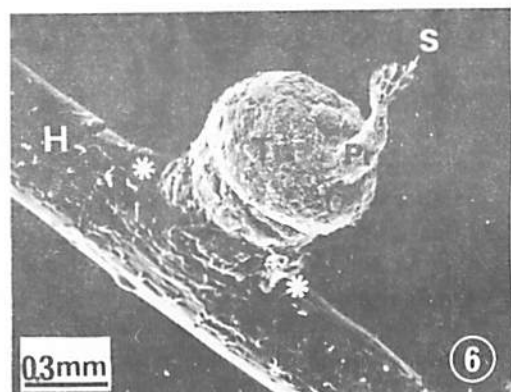


Fig. 6. Young tubercle, Tu, with procaulome and seed still present. \*, the host root cortex has been torn off by the development of the endophyte.

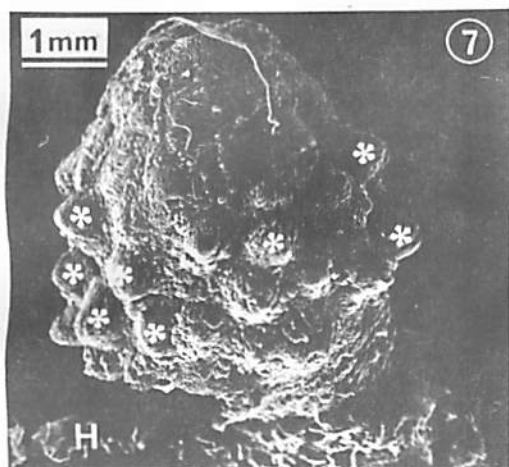


Fig. 7. Tubercule with many domes (\*) corresponding to the future roots.

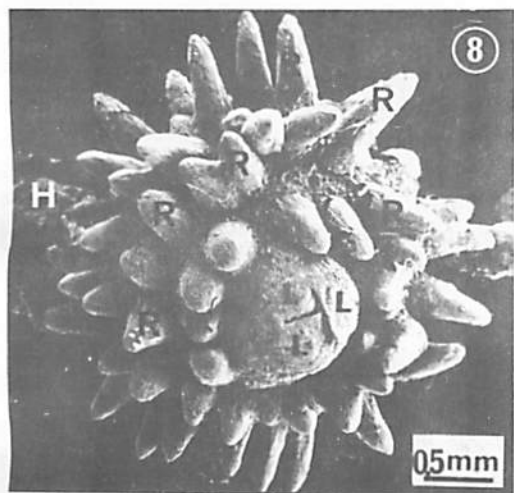


Fig. 8. Bud of *O. crenata* with many crowns of roots, R, and leaves, L, protecting the shoot apex.

From the present study, and the ontogenetic and structural study of Aber and Salle (1983), it appears that the procaulome cannot be likened to a root (Koch, 1878), a germ (Blanchard, 1952), or a "germ-tube like organ" (Kadry and Tewfic, 1956). Lacking in conducting elements, it is only a vector which brings the active terminal cells into contact with the host root. It is particularly fragile, and is thus likely to be sensitive to certain chemical substances. Moreover, the observation of the presence of fungi growing in association with the procaulome tip opens the possibility of biological control measures which should be investigated.

The attachment of the parasite to the host occurs so rapidly that we have not yet been able to propose any appropriate methods of control during this phase.

On the other hand, the tubercule phase is very interesting for two reasons. First, the meristematic cells of the young tubercule show important histogenetic and organogenetic properties, in producing the internal organ (the endophyte), and the external organ with a unique shoot apex and crowns of roots (Aber and Salle, 1982). Second, parenchymatous cells of the tubercule contain considerable quantities of starch used during the growth of the spike.

Tubercules are sinks for nutrients from the host. The work of Kassasian (1973), Schmitt *et al.* (1978), and Schluter and Aber (1980) indicates that the tubercule stage is the most sensitive to herbicide action, particularly glyphosate. It has been shown by Whitney (1973), working with 2, 4-D, that two doses of low concentration applied to the foliage of the host results in the chemical accumulating to toxic levels in the tubercules and buds of the parasite. It is believed that this mechanism may also apply with glyphosate. In our program we plan to use cold and labelled glyphosate in order to investigate this hypothesis. Moreover, it is hoped to demonstrate the effect of the second spray, and therefore its necessity.

In order to eradicate this parasite, efforts must be coordinated at the international level. To obtain an integrated control, basic research is needed into its growth and development in addition to the research on control by chemical and biological means and through host-plant resistance.

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## REDUCING LOSSES DUE TO INSECTS IN FABA BEANS IN EGYPT

Ezzat Zaki Fam

Stored Products Research, Plant Protection Research Institute, Ministry of Agriculture, Dokki, Cairo, EGYPT.

Faba bean, a major staple food in Egypt, is liable to attack by several insect pests. In surveys conducted in 1981, *Liriomyza* sp. was the most prevalent insect. *Sitona lineatus* infestation was found on 10 percent of fields. Four different species of aphids were identified: *Aphis craccivora* in all areas, *A. fabae* in the Delta area and *A. gossypii* and *Myzus persicae* in the southern region (Diekmann, 1981). The bean weevil, *Bruchus rufimanus*, is an important pest which infests the green pods in the field and later on develops as a storage insect. To reduce the losses caused by the above mentioned species, the following measures are recommended:

### I. Pre-harvest control:

- a) Tamaron (Methamidophos) 60% E.C. at the rate of 2 ml/litre (1.9 litre/ha) is efficient against both the leaf-miner and the aphids. Malathion 57% E.C. at the rate of 2.5 ml/litre can also be used against the aphids.
- b) Since the infestation by *Bruchus rufimanus* is transferred through the seed to subsequent crops, fumigation of the seed before planting is very important.
- c) The use of less susceptible varieties can also help in reducing insect-caused losses.

## II. Post-harvest control:

### I. Preventive measures:

- a) Cleaning and disinfestation of second-hand bags, threshing and sieving equipment and of the storage houses and 'shounas'.
- b) Storage of seeds in places protected from rainfall and water infiltration. In open-air situations, the seed must be stacked on wooden logs and covered with tarpaulins. The storage of seed with high humidity content must be avoided.
- c) Prior fumigation of the seed before dusting or spraying with protectant.
- d) Stacks of seed must be dusted or sprayed every fortnight with a suitable dust or spray to guard against reinfestation.
- e) Periodic inspection of the seed. Lots found to be infested must be fumigated as soon as possible and those found developing heat due to high moisture content should be discarded.

### 2. Chemical control:

a) **Disinfestation of stores and warehouses:** The stores and warehouses must be swept clean and the refuse burned. All cracks in the walls and floors must be plastered before disinfestation is undertaken. Products which can be used to spray walls, floors, roofs, transport vehicles, and threshing and sieving machinery are given in Table 1.

b) **Seed dressing with protectants:** Before dressing, seeds must be free from insects because this practice does not have an effect on internal, developing infestations. Seed protectants to be recommended in Egypt are:

**Katelsousse:** a local mixture of 84% rock phosphate and 16% ground sulphur of 300 mesh fineness. This compound has been successfully used at the rate of 1% by seed weight.

**Pyrethrin:** a mixture of 0.05% pyrethrins, 0.8% piperonyl butoxide and 99.15% inert dust. It can be used at the rate of 0.2% of seed weight.

**Silica gel:** an amorphous substance that can be used at the rate of 0.16% of seed weight.

Malathion: as a dust it can be used at the rate of 8 ppm (800 g of the 1% dust/tonne; in liquid form, at the rate of 14 ml of the 57% formulation in 350 ml of water/tonne of seed.

c) Fumigation: This is one of the most effective methods for treating seeds against storage pests. However, it does not prevent reinfestation. The most widely used fumigants are carbon disulphide at the rate of 200 ml/m<sup>3</sup>; methyl bromide, 16-24 g/m<sup>3</sup> and phosphine, 1.5 tablets/m<sup>3</sup>. With methyl bromide and carbon disulphide the recommended period of exposure of the seed is 24 hours, whereas in the case of phosphine applications, the seed must be exposed for 3 days.

Table 1. Chemicals for control of pests in stores, and on machinery.

Product	Dosage (active ingredient)	How, where, and when to apply
100 ml. light Solar oil + 50 ml water + 5 g soap	diluted in 4 volumes of water	spray walls, floor and ceiling twice in two successive days at the rate of 1 litre/4m <sup>2</sup>
Malathion (57% E.C.) or Actellic (pirimiphos- methyl) (50% E.C.)	1 g/m <sup>2</sup>	mix with water and apply to walls and floor of empty store 2 weeks before storage of grain at the rate of 1 litre/4m <sup>2</sup>

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## TROUGH — APPLICATOR FOR TEST PLOT TREATMENT WITH GRANULAR NEMATOCIDES AND INSECTICIDES

J. Hartwig, M. Hiemer and R.A. Sikora  
*Institut fuer Pflanzenkrankheiten der Rheinischen  
Friedrich-Wilhelms-Universitaet, Nussallee 9, D-5300 Bonn  
1, GERMANY.*

Row application of granular nematicides and insecticides at low dosage levels with currently available equipment is problematical. This is especially true for field trials with rows of less than 3 meters in length. Under these conditions fertilizer, or single - row applicators are often impractical, and primitive techniques utilizing test tubes or plastic containers are highly inaccurate (Matthews, 1979; Taylor and Sasser, 1978).

Field trials with soil - borne pest and disease organisms are further complicated by the large variability in the distribution of organisms within the field. This is especially true for studies concerned with the chemical control of plant parasitic nematodes.

We have developed a trough - applicator for treatment of plots up to 3 meters in length (Fig. 1). The trough can be constructed out of metal, wood or plastic. The exact amount of pesticide to be applied to each row is stored in vials, distributed equally along the trough, and applied by tilting the applicator over the row to be treated.

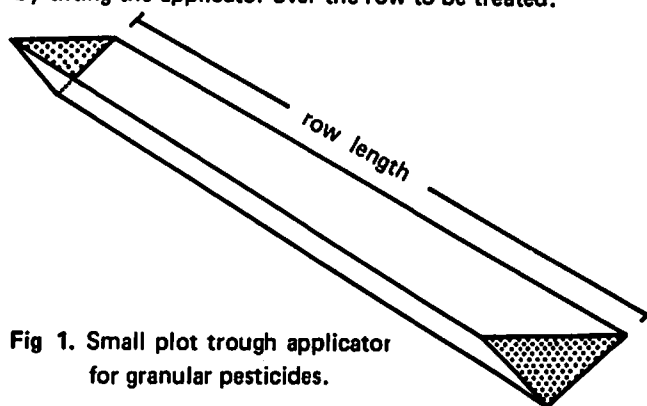


Fig 1. Small plot trough applicator for granular pesticides.

The advantages of this technique are: (1) simplicity and ease in handling, (2) visual control of the distribution of the test material before treatment, and (3) greater precision.

#### References

- Matthews, G.A. (1979). 'Pesticide Application Methods.' Longman Inc. Pub., London, 336 pp.  
Taylor, A.L. and Sasser J.N. (1978) Raleigh, North Carolina

# Seed Quality and Nutrition

## METABOLISM OF VICINE AND CONVICINE IN RAT TISSUES: ABSORPTION AND EXCRETION PATTERNS AND SITES OF HYDROLYSIS.

Ronald R. Marquardt<sup>1</sup> and M. Ihab Hegazy<sup>2</sup>

<sup>1</sup> Department of Animal Science, University of Manitoba, Winnipeg, Manitoba, CANADA R3T 2N2

<sup>2</sup> Institute of Nutrition, 16 Kasr El-Ainy Street, Cairo, EGYPT

### Introduction

Extensive utilisation of faba beans as a protein source for humans is hindered by the presence of factors that induce favism (Belsey, 1973). Vicine and convicine or their aglycones have been implicated as the causative agents of this haemolytic disease. The hypothesis has been put forward that hydrolysis of vicine to divicine and convicine to isouramil occurs by endogenous metabolism (Mager *et al.*, 1980). Frohlich and Marquardt (1983) have recently demonstrated, however, that vicine and convicine were rapidly hydrolysed by digesta from the caeca of the chick but not by other tissue or by digesta samples from other sites in the digestive tract.

The objectives of this study were to establish if the pattern of uptake, turnover, and sites of hydrolysis of vicine and convicine in a mammalian species such as the rat were similar to those of the chicken.

### Materials and Methods

Male weanling rats (Sprague-Dawley) were fed a commercial feed (CF), and water was available *ad libitum*. Vicine and convicine were added as required. The experiment was in four parts. Full experimental details are given in Hegazy and Marquardt (1983).

1. Five groups of rats were fed on a diet of CF, CF plus 1% vicine, CF plus 3% vicine, CF plus 1% convicine, or CF plus 3% convicine, for a period of two days. Faeces and urine were collected during the experimental period, and were analysed for vicine and convicine content.
2. Rats were fed on a diet of CF plus vicine and convicine. Over the 24 hour period following consumption, animals were killed, blood, liver, kidney, and muscle samples taken, and assayed for vicine and convicine.

3. Fresh tissue samples from the liver, kidney, muscle, caecal wall, and large intestine wall were homogenised in a vicine-convicine solution, and incubated at 37°C for up to 2 hours. After incubation, extracts were assayed for vicine and convicine.

Samples of digesta from the stomach, small intestine, caecum, and large intestine were incubated at 37°C with a vicine-convicine solution for up to one hour, after which the extract was assayed for residual vicine and convicine.

4. Fresh human faecal samples were used as above.

### Results and Discussion

Rats receiving the CF plus 1% vicine, 3% vicine, 1% convicine and 3% convicine, respectively, excreted 0.4, 2.6, 0.4 and 1% of total glycoside in the faeces and 1.3, 1.2, 0.5 and 0.3% of total glycoside in the urine. These results demonstrate that only a small percentage of total dietary vicine and convicine are excreted and that urinary excretion of vicine is greater than that of convicine.

The results from the second experiment demonstrated that there was no detectable vicine or convicine in the blood, liver, kidney or muscle tissue of rats during a 24 hr period following the consumption of vicine or convicine. The lower limits of detection of these compounds was 0.5 µg/g tissue which is 50,000 fold lower than their concentrations in the diet. The absence of detectable levels of vicine or convicine in blood and tissue samples, but their presence in the urine, would suggest that they are absorbed by the animal but are rapidly cleared from the body.

The results from the third experiment demonstrated that neither vicine nor convicine were hydrolysed by liver, kidney, muscle, caecal wall or intestinal wall homogenates. This suggests that endogenous enzymes present in animal tissues are not capable of hydrolysing vicine or convicine. The second phase of this experiment (Table 1) demonstrated that digesta extracts when incubated in the presence of vicine and convicine were able to hydrolyse these compounds to their respective aglycones. Digesta from the caecum and large intestine were highly effective in comparison to digesta from the stomach and small intestine. In the fourth experiment it was shown that fresh human faecal samples were also able to rapidly hydrolyse vicine and convicine. Similar results were also obtained in a previous study with avian tissue extracts (Frohlich and Marquardt, 1983). In addition, in chickens, dietary antibiotics not only enhanced the excretion of both vicine and convicine but also reduced the *in vitro* rate of hydrolysis of these compounds.

**Table 1.** *In vitro* hydrolysis of vicine and convicine by digesta from different sections of the gastrointestinal tract of the rat.

Source of digesta	Percent hydrolysis <sup>a</sup>	
	Vicine	Convicine
Stomach	5B	8B
Small intestine	12B	22B
Caecum	94A	100A
Large intestine	93A	100A
SE ±	16	16

<sup>a</sup> Values are expressed as percent of vicine or convicine hydrolyzed *in vitro* during a 1 hr incubation at 37°C. Means in each column not sharing a common letter differ significantly ( $P < 0.01$ ).

The results of these studies would suggest that the catalytic agent is a  $\beta$ -glycosidase-like enzyme that is produced by the anaerobic bacteria in the lower portions of the gastrointestinal tract. The hydrolytic products which are stable in the anaerobic atmosphere of the digesta are presumably absorbed into the blood stream where they undergo decomposition to yield  $H_2O_2$  in a manner similar to that described by Chevion *et al.* (1982).

An overview of hypothetical events that may occur when humans consume faba beans is given below:

1. Consumption of faba beans by humans.
2. Transport of vicine (convicine) to the large intestine.
3. Hydrolysis of vicine (convicine) by bacterial enzyme(s) in an anaerobic media.
4. Transport of hydrolytic products (isouramil and divicine) to the blood.
5. Interaction in the blood of isouramil and divicine with oxygen to form  $H_2O_2$  and oxidised aglycones.
6. Incomplete conversion of  $H_2O_2$  to  $H_2O$  and  $O_2$  in favism susceptible individuals due to a deficiency of NADPH and glutathione.
7. Interaction of excess  $H_2O_2$  with red cell membrane causing irreversible cellular alterations and destruction of red blood cells.

## References

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- Marquardt, R.R. and Frohlich, A.A. (1981). 'Rapid reverse-phase high-performance liquid chromatographic method for the quantitation of vicine, convicine and related compounds.' J. Chrom. 208, 373-379.

## A RAPID HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD FOR THE QUANTITATION OF VICINE, CONVICINE AND RELATED COMPOUNDS

Ronald R. Marquardt and Andrzej A. Frohlich  
Department of Animal Science, University of Manitoba  
Winnipeg, R3T 2N2, CANADA

## Introduction

Vicine and convicine are present in faba beans and have been implicated in the human disease, favism (Mager *et al.*, 1980).

Previous methods for the analysis of vicine and convicine have been nonspecific or require arduous and time-consuming techniques (Marquardt and Frohlich, 1981). Also, none of the previous methods are capable of directly detecting the presence of the unstable hydrolytic products of these compounds (divicine and isouramil) or have not been utilized for the detection of these compounds in animal tissues. The objective of this study was to develop a simple and rapid method for the quantitation of vicine, convicine, their hydrolytic products and dihydroxyphenylalanine (DOPA) in faba beans and animal tissue samples.

## Materials and Methods

The materials and methods were as previously described by us (Marquardt and Frohlich, 1981). In summary, finely ground faba beans or tissue samples were diluted with 100 volumes or less of 5% perchloric acid, mixed for 10 min, centrifuged or filtered and injected into a Beckman high-performance liquid chromatograph (HPLC). The column was prepacked by the manufacturer with ultra-sphere ODS-5 (Beckman) and the effluent was monitored at 280 nm. Vicine and convicine were prepared in pure form in our laboratory (unpublished results).

## Results and Discussion

The chromatogram in Figure 1A shows the separation of a standard mixture of vicine, convicine, DOPA and other related compounds. Free cytosine, which is not present in faba beans, can be employed as an internal standard. DOPA and tyrosine, two ultraviolet absorbing compounds present in faba beans, are resolved into two distinct peaks. The average elution time for all compounds was 8 min, 2 min for vicine, and 2.7 min for convicine.

Studies on the reliability of the procedure demonstrated that the correlation coefficients (*r*) between peak heights or peak areas of the chromatograms and the concentration of all of the standards was greater than 0.99. The lower limits of detection for vicine and convicine were approximately 0.1 µg/ml or 2 ng of glycoside injected into the column. The elution profile of dehulled faba beans (Fig. 1B) demonstrated the presence of two major peaks (vicine and convicine) and two minor peaks, DOPA and tyrosine. The concentration of vicine and convicine was 0.53 and 0.28%, respectively. Treatment of the extract with β-glucosidase caused complete disappearance of the vicine and convicine which suggests that other coeluting compounds are not present in extracts prepared from faba beans.

Vicine and convicine when added to animal tissue extracts could be readily separated from coeluting compounds in blood, liver and kidney but not muscle homogenates. Divicine and isouramil, which are the aglycones of vicine and convicine, are eluted prior to vicine and also strongly absorb UV light at 280 nm.

The method reported here, and by Marquardt and Frohlich (1981), is believed to be the first for the separation of vicine and convicine using HPLC. Two methods have subsequently appeared which in the authors' opinion have certain disadvantages. The procedure reported by Lattanzio *et al.* (1982) requires extraction by refluxing for 2 x 30 min with methanol/water followed by concentration *in vacuo*. This is a very time-consuming step compared to the rather simple extraction procedure described above. The extraction procedure reported by Quemener *et al.* (1982) is simpler than that of Lattanzio *et al.* (1982) but elution times are rather long. The respective elution times for vicine and convicine were approximately 8 and 30 min compared to elution times of only 2 and 2.7 min in the current procedure.

Overall, the use of HPLC methods, particularly the one reported here, offers a convenient and simple procedure for the quantitation of vicine, convicine and related compounds in faba beans, faba bean fractions and animal tissue samples.

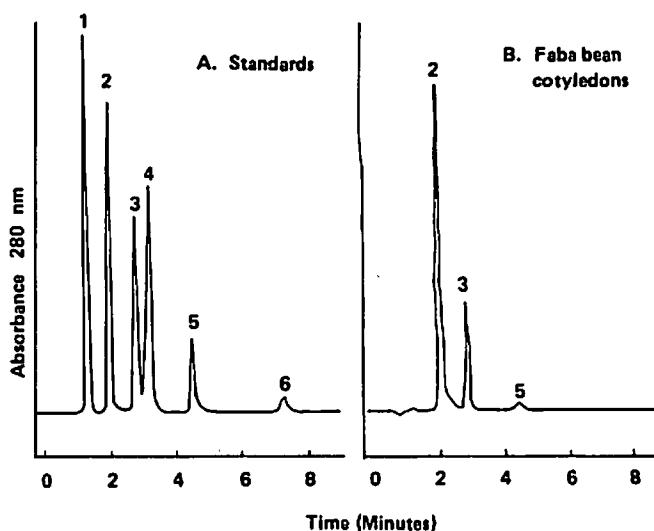


Fig 1. Chromatogram of a standard solution (A) containing: (1) 0.17 mM cytosine; (2) 0.17 mM (51 µg/ml) vicine; (3) 0.17 mM (50 µg/ml) convicine; (4) 0.17 mM uric acid; (5) 0.33 mM DOPA and (6) 0.33 mM tyrosine; and (B) an extract prepared from the cotyledon portion of faba beans (0.4 g/40 ml). Compounds eluted in chromatogram B were vicine (2), convicine (3), and DOPA (5).

## References

- Lattanzio, V., Bianco, V.V. and Lafiandra, D. (1982). 'High-performance reversed-phase liquid chromatography of favism-inducing factor in *Vicia faba* L.' *Experientia* 38, 789-790.
- Mager, J., Chevion, M. and Glaser, G. (1980). 'Favism' In: 'Toxic constituents of plant foodstuffs.' (Liener, I.E., Ed.), 2nd edn., Academic Press, New York, NY.
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## ANNOUNCEMENT

### **Dr. Ali Abdul Aziz Ibrahim**

Dr. Abdul Aziz Ibrahim, a renowned faba bean scientist and a highly respected administrator in the Agricultural Research Services of the Government of Egypt, died in a car accident on July 10, 1983 while driving from Alexandria to Cairo on duty.

Dr. Abdul Aziz joined the Food Legume Improvement Program of the Egyptian Agricultural Research Centre (ARC), Giza, in 1949 immediately upon his graduation from Cairo University. During the tenure of his service as a legume researcher he completed his M.Sc. and Ph.D. degrees from the University of Cairo, working on genetic aspects of faba bean. In recognition of his meritorious services, he was appointed the leader of the Food Legumes Improvement Program at Giza in 1973, Deputy Director of the Field Crops Research Institute, ARC, in 1979 and its Director in 1980. In his capacity as the Director he also headed the Collaborative Agricultural Research Projects on Field Crops between the Government of Egypt and foreign organizations. In 1983 he was appointed as the Deputy Director General of the ARC.

Dr. Abdul Aziz maintained his interest in faba bean research, recognizing the importance of faba bean in the diet of Egyptian people and its role in the Egyptian cropping system. He was, therefore, very intimately involved in the research in the ICARDA/IFAD Nile Valley Research Project on faba bean and served as the Egyptian National Coordinator of the Project from the very beginning. Dr. Abdul Aziz was a great patron of FABIS.

The untimely and tragic demise of Dr. Abdul Aziz has come as a great shock to all faba bean researchers amongst whom he was so popular. May his soul rest in peace.

### **IDRC Grant**

We gratefully acknowledge the very generous grant from IDRC (the International Development Research Centre) for the development of FABIS and to enable the more frequent publication of the FABIS Newsletter.

This grant also includes funding for the development of special bibliographies, and a question and answer service on all aspects of faba bean research, a facility we hope our subscribers will utilise to the fullest extent.

## FABA BEAN WORKSHOP

A workshop on factors affecting cooking properties of faba beans was held at the University of Alexandria, Egypt, on 8 and 9 June, 1983.

Papers were presented by scientists from Egyptian universities and research centers on:

- I Cooking properties
- II Chemical constituents
- III Agronomic variables
- IV Soil characteristics
- V Soil salinity
- VI Storage

with respect to their effects on cooking/nutrition of the crop. Abstracts have been published by the University of Alexandria.

For further information, contact:

Professor Ahmed M. El-Tabey Shehata,  
Dept. of Food Science and Technology,  
University of Alexandria,  
Alexandria,  
EGYPT

## **SECOND INTERNATIONAL VICIA FABA CYTO- GENETICS REVIEW MEETING 9 - 13 APRIL 1984**

Following the success of the meeting held in February 1983, (see FABIS No. 6) a second meeting will be held at Wye College, England, from 9 - 13 April, 1984.

The meeting will have five sessions, covering:

- I Progress and problems in breeding
- II Cytogenetic structures
- III Cytogenetic function
- IV Application of cytogenetics to breeding
- V Plenary meeting to identify research objectives

The Zeiss Company of West Germany will again demonstrate recent advances in optical and electron microscopy.

Those wishing to attend the meeting should contact:

Dr. G.P. Chapman  
Wye College,  
Ashford,  
Kent TN25 5AH  
ENGLAND

Provisional cost for the meeting, all inclusive, is £110.

## News of faba bean scientists

Dr. Farouk Elsayed, a Post-Doctoral Research Fellow in faba bean breeding at ICARDA, left the organisation on April 1, 1983. Dr. Elsayed was working on the ICARDA/IFAD Nile Valley Project. He has been replaced by Dr. Mohamed El-Sherbeeney, from the Agricultural Research Corporation, Giza, Egypt.

**Senior faba bean pathologist:** Dr. Salim Hanounik has taken responsibility for faba bean pathology in ICARDA. Based at the ICARDA sub-site at Lattakia, in north-western Syria, he is responsible for all research work on all aspects of faba bean diseases. Correspondence to Dr. Hanounik should be addressed to him c/o FLIP, ICARDA, P.O.Box 5466, Aleppo, Syria.

**Food Legume Entomologist:** Dr. Cesar Cardona, a Colombian national, has joined ICARDA as the Senior Food Legume Entomologist. Dr. Cardona is based at the Aleppo headquarters of ICARDA.

Dr. M. Frauen has left the Plant Breeding Institute, Gottingen, to join Norddeutsche Pflanzenzucht, a private plant breeding company. His postal address is now: Norddeutsche Pflanzenzucht, Hans-Georg Lembke KG, 2331 Hohenlieth, Post Holtsee, German Federal Republic.

**EEC (European Economic Community) *Vicia faba* Meeting,** Nottingham, England, 14-16 September 1983.

Eighty seven delegates from countries in Europe and Canada met for the presentation of 38 papers on aspects of faba bean research. The proceedings will be printed at a later date.

The last day was devoted to discussion of related topics. Concern was expressed that, unless efforts are concentrated on one or two points which may provide dramatic results, funding for research on faba beans may dry up.

Dr. P. Hebblethwaite reported on faba bean research in Europe.

For further information on this meeting, contact

Dr. P. Hebblethwaite  
School of Agriculture  
Sutton Bonington  
Loughborough  
ENGLAND, LE12 5RD.

## Faba Bean in the Nile Valley

**Report on the First Phase of the ICARDA/IFAD Nile Valley Project**

*M.C. Saxena and R.A. Stewart (eds)*

*Martinus Nijhoff Publishers, The Hague, 1983, 151 pp.*

This book gives a detailed report of the work of the first phase of the ICARDA/IFAD Nile Valley faba bean project. Much of the work described concerns on-farm trials, which involve farmers, extension workers, and national program research scientists from Egypt and Sudan.

The multi-disciplinary nature of the research, bringing together socioeconomic and agricultural researchers, is a major feature of this unique and highly praised project.

This report should prove useful to all scientists, agricultural administrators, and agricultural research organisations interested in faba beans.

## Favism Meeting

A meeting on the subject of favism, a chronic haemolytic blood disorder related to consumption of high levels of faba beans, has been planned by ICARDA for March 1984.

The meeting aims to bring together scientists from different disciplines to review the present state of knowledge of the subject, and to develop an effective research strategy to tackle the problem. It is hoped that the publication resulting from this meeting will be a definitive work on the subject of favism.

For further information please write to: Leader, Food Legume Improvement Program, ICARDA, P.O. Box 5466, Aleppo, SYRIA.

## Subscriptions

The annual subscription for FABIS is now U.S. \$ 10. The various methods of payment are:

- U.S. Dollar draft drawn on a U.K. bank, payable to 'ICARDA'.
- Eurocheque in U.S. Dollars (place must state 'London'), payable to 'ICARDA'.
- International Money Order or Postal order in U.S. Dollars, payable to 'ICARDA'.
- Direct transfer to ICARDA's bank account at Hong-kong and Shanghai Banking Corp., P.O.Box 199, 99, Bishopsgate, London EC2P 2LA, ENGLAND.  
A/C no. 729-195-43.

**Proceedings of the International Faba Bean Conference  
Cairo, March, 1981  
ICARDA, 1982, 147 pp.**

This publication is intended as a supplement to 'Faba Bean Improvement' (Martinus-Nijhoff, 1982) and contains the opening remarks; a summary of the discussions of the papers presented; recommendations of the conference; concluding statements, and a list of the participants.

In addition, six papers on faba bean research and production in Cyprus, Ethiopia, Jordan, Lebanon, Spain, and Turkey are presented.

All those who participated in the Conference will have received a copy of these Proceedings. Others can address their request to:

**FABIS  
Documentation Unit  
ICARDA  
P.O.Box 5466  
Aleppo, SYRIA**

#### **Reprints**

ICARDA has been designated as the world center for information on faba beans, and as such we are trying to assemble a complete collection of papers relevant to this subject.

We would be most grateful if readers who have published papers relating to faba beans would send reprints to:

**FABIS  
Documentation Unit  
ICARDA  
P.O.Box 5466  
Aleppo, SYRIA**

#### **Lentil Experimental News Service (LENS)**

LENS, a scientific newsletter published jointly by the University of Saskatchewan and ICARDA with financial support from IDRC, is designed to improve communication among world lentil researchers.

For your free copy write to:-

**LENS  
Documentation Unit  
ICARDA  
P.O.Box 5466  
Aleppo, SYRIA**

#### **The Faba Bean (*Vicia faba* L.) A Basis for Improvement**

*Edited by Paul D. Hebblethwaite,  
Senior Lecturer in Agronomy,  
University of Nottingham School of Agriculture.*

The aim of this book is to collate and review the substantial amount of research and breeding work that has been carried out world-wide on the faba bean crop. Wherever possible, areas in which further research is needed are highlighted with a view to increasing and stabilising yields.

The Faba Bean - A Basis for Improvement will enable research workers to obtain an up-to-date evaluation of the problems related to *Vicia faba* production on a world scale and will be a useful reference for scientists, advisors and students at all levels.

#### **Contents**

**I - Background, Physiology and Breeding:** Background and history of faba bean production. Classification, origin, breeding methods and objectives. Pollination. Developmental physiology. Reproductive physiology of *Vicia faba* L. Chemical constituents and biochemistry. The influence of growth regulators on development and yield of *Vicia faba* L. Cytogenetics. Water relations and irrigation response. Nitrogen fixation. **II - Husbandry:** Beans in crop rotations. The husbandry of establishment and maintenance. **III - Pests:** Aphid pests. Nematode pests of *Vicia faba* L. Pests of *Vicia faba* other than aphids and nematodes. **IV - Diseases:** Root diseases of *Vicia faba* L. Viruses and virus-like diseases of *Vicia faba* L. Shoot diseases caused by fungal pathogens. Parasitic diseases in *Vicia faba* L. with special reference to broomrape (*Orobancha crenata* Forsk). **V - Harvesting, Drying, Storage and Utilization:** Grain and whole-crop harvesting, drying and storage. Utilization of *Vicia faba* L.

Butterworths, 1983, 624 pp.; 234 x 165 mm; ISBN: 0 408 10695 6; £55.00.

Available from: Butterworths, Borough Green, Sevenoaks, Kent TN15 8PH, England.

#### **Twice a year**

The FABIS Newsletter is now scheduled to appear twice a year, in June and December. As such we are in need of both General Articles and Short Communications, and we hope that the flow of material from our subscribers will be maintained or increased.

### Third International Symposium on Parasitic Weeds

ICARDA, Aleppo, Syria, May 1984.

"Parasitic Weeds" is the theme of a four-day symposium to be held on 7 - 10 May with the collaboration of ICARDA under the auspices of the International Parasitic Seed Plant Research Group. The purpose of this symposium is to provide a forum for the interchange of data, techniques, and research goals in all aspects of parasitic vascular plants. The following topics will be covered: major parasitic groups (*Striga*, *Orobancha*, *Cuscuta*, mistletoes) and their biology and control, as well as basic research in physiology, biochemistry, structure, ecology, etc.

The symposium participants will visit field trials to view *Orobancha* infestations and any other parasitic species.

For more information, contact: Mr. C. Parker, ARC Weed Research Organization, Yarnton, Oxford, UK, OX5 1PF

#### FABIS Co-ordinating Committee Members

EGYPT	Dr. Abdalla Nassib Food Legume Section, Field Crops Institute, Agricultural Research Center, Giza.
SUDAN	Dr. Farouk A. Salih Agricultural Research Corporation, Shambat Research Station, P.O. Box 30, Khartoum North
SYRIA	Dr. Mohan Saxena Food Legume Improvement Program, ICARDA, P.O. Box 5466, Aleppo.
JAPAN	Dr. Kiyoshi Kogure Faculty of Agriculture, Kagawa University, 2393 Ikenobe, Miki-tyo, Kagawa-ken.
CANADA	Dr. Claude Bernier Department of Plant Science, University of Manitoba, Winnipeg, Manitoba R3T 2N2

#### BRAZIL

Dr. Homer Aidar  
National Center for Research  
on Rice and Beans,  
BR-153, km. 4 - Goiania/Anapolis,  
Caixa Postal 179,  
74.000 - Goiania, Goias.

#### FRANCE

Dr. J. Picard  
Station d'Amelioration des Plantes,  
INRA,  
B.P. 1540,  
21034 Dijon Cedex.

#### ITALY

Dr. Ciro de Pace  
Istituto di Miglioramento Genetico  
della Plante Agrarie,  
Universita di Bari,  
Via Amendola 165,  
70126 Bari.

#### SPAIN

Dr. J.I. Cubero  
Escuela Technica Superior de  
Ingenieros Agronomos,  
Departamento di Genetica,  
Apartado 246,  
Cordoba.

#### U.K.

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

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#### FABIS Production Team

Philip Kemp, Senior Information Scientist  
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Hassan Khairallah and Abdul Rahman Hawa, Art Work

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## Contributors' Style Guide

### Policy

The aim of the newsletter is to publish quickly the results of recent research. Articles should normally be confined to a single subject, be of good quality and of primary interest to research, extension and production workers, administrators and policy makers. Articles for publishing in the newsletter should not be submitted to or published in any other journal.

### Editing

Articles will be edited to preserve uniform style but substantial editing will be referred to the author for his approval; occasionally, papers may be returned for revision.

### Disclaimers

The views expressed and the results presented in the newsletter are those of the author(s) and not the responsibility of ICARDA.

Similarly, the use of trade names does not constitute endorsement of or discrimination against any product by ICARDA.

### Language

The Newsletter will be published in English but ICARDA will endeavour to translate articles submitted in Arabic and French.

### Manuscript

Articles should be typed double spaced on one side of the page only. The original and two other legible copies should be submitted. The contributor should include his name and initials, title, program or department, institute and postal address and telex number if available. Photographs, figures, tables etc. should be either 8.5 cm wide (single column) or 17.5 cm wide (double column including space). Figures and diagrams should be drawn in India ink; send original artwork, not photocopies. Define in footnotes or legends any unusual abbreviations or symbols used in a figure or table.

Units of measurement are to be in the metric system, e.g., t/ha, kg, g, m, km, ml (= milliliter), m<sup>2</sup>.

The numbers one to nine should be written as words except in combination with units of measure; all other numbers should be written as numerals, e.g., Nine plants, 10 leaves, 9 g, ninth, 10th, 0700 hr.

### Examples of common expressions and abbreviations

3g, 18 mm, 300 m<sup>2</sup>, 4 Mar 1983; 27% ; 50 five-day old plants; 1.6 million; 23 µg; 5°C; 1980/81 season; 1981-82; Fig., No.; FAO, USA. *Fertilizers*: 1 kg N or P<sub>2</sub>O<sub>5</sub> or K<sub>2</sub>O/ha.

Mon, Tues, Wed, Thurs, Fri, Sat, Sun; Jan, Feb, Mar, Apr, May, June, July, Aug, Sept, Oct, Nov, Dec. versus = vs, least significant difference = LSD, standard error = SE±, coefficient(s) of variation = CV(s).

*Probability*: Use asterisks to denote probability \* =  $P < 0.05$ ; \*\* =  $P < 0.01$ ; \*\*\* =  $P < 0.001$ .

*Botanical*. Include the authority name at the first mention of scientific names. Cultivar(s) = cv(s), variety = var(s), species = sp./spp., subspecies = subsp., subgenus = subg., forma = f., forma specialis = f.sp.

### References

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