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Effect of nutritional status of faba bean (*Vicia faba* L.) on protein solubility profiles

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Abstract

A field experiment was carried out to study the effect of mycorrhizal inoculation. phosphorus and sulphur fertilization, under two water regimes, on protein solubility fractions of faba bean. The major protein fraction of faba bean was the globulin fraction that ranged from 69.5 to 78.1%. Phosphorus treatment significantly ($P \leqslant 0.05$) increased the globulin fraction of faba bean seeds in the wet treatment but significantly ($P \leqslant 0.05$) decreased it in the dry treatment. All fertiliser treatments significantly ($P \leqslant 0.05$) increased the albumin fraction in the wet treatment, whereas only treatments of mycorrhiza, mycorrhiza + sulphur and mycorrhiza + phosphorus + sulphur significantly increased it in the dry treatment compared to control. With the exception of the treatment mycorrhiza + sulphur, all other fertilisation treatments showed prolamin contents of faba bean seeds, similar to that of the control in the wet treatment. The G_3-glutelin fraction of faba bean ranged from 8.9 to 14.4%. Treatments with sulphur, mycorrhiza and mycorrhiza + sulphur significantly increased the G_3-glutelin fraction, whereas phosphorus and mycorrhiza + phosphorus treatments significantly decreased it. The insoluble protein (residue) of faba bean ranged from 1.8 to 3.4%. Generally, fertiliser treatments significantly increased the insoluble protein in the wet treatment. With the exception of treatments mycorrhiza and mycorrhiza + sulphur, all other fertiliser treatments significantly increased the insoluble protein content in the dry treatment. Water regime significantly ($P \leqslant 0.05$) affected the protein fractions. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: Faba bean; Fertilizers; Nutritional status; Protein solubility profiles; Water regime

1. Introduction

Faba bean (*Vicia faba* L.) is an important cash crop in the Sudan. The crop contributes to human nutrition and it is the staple food for many people in the Sudan, because of its high protein content as well as other essential nutrients. The high lysine content has encouraged the use of faba bean as a protein supplement for cereals. Fertiliser application significantly increases the protein content of faba bean (Elsheikh, El Tinay & Fadul, 1999), chickpea (El Hadi & Elsheikh, 1999) and fenugreek (Abdelgani, Elsheikh & Mukhtar, 1999). The effects of chemical, organic and biological fertilisers are found to vary with the type of the crop, the cultivar used and the concentration of the fertilizer, as well as environmental factors (Elsheikh & Elzidany, 1997; Elsheikh & Mohamedzein, 1998).

The major portion of protein in beans was in the form of globulins, followed by glutelins and lesser amounts of albumins and prolamins (Nikokoyris & Kandylis, 1997). Albumins can act as enzymes present in the seed, which are utilised in the germination process. This fraction is richer in methionine and cysteine than the globulin, at least in peas and faba bean (Bailey & Boulter, 1972). Prolamin contains major polypeptides with average molecular weights of 2500 and 21 800 daltons (Misra, Jambunathan, Mertz, Golover, Barbsa & McWhinter, 1972). Paulis (1982) reported that prolamins are deficient in lysine and tryptophan. and other essential amino acids with higher levels of leucine, proline and glutamic acid than other fractions. Glutelins are associated with lower molecular weight proteins through non-covalent bonding; they consist mainly of two categories of polypeptides linked by disulphide bonds. G_1-glutelin has an amino acid composition somewhat similar to prolamin but with higher levels of glycine, methionine, histidine and proline and lower levels of aspartic acid, leucine, isoleucine. G_1-glutelin contains major polypeptides of molecular weight 26 000, 23 000 and 18 000 daltons (Misra et al., 1972). The insoluble protein (residue) consists mainly of proteins from previously defined

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groups, becoming insoluble due to interaction with lipids, carbohydrates, or polyphenols via oxidation (Landry & Moureaux, 1981). The objective of the present study was to investigate the effect of faba bean nutritional status on protein profiles.

2. Materials and methods

2.1. Field experiment

A field experiment was conducted, during the 1997/1998 season, in the Demonstration Farm of the Faculty of Agriculture at Shambat (Latitude 15° 40′ N. longitude 32° 32′ E). The land was prepared by disc plough, followed by ridging and the spacings between ridges and holes were 70 and 20 cm, respectively. The size of the subplot was 4×4 m consisting of 5 ridges of 3 m length. Between the main plots, one meter was left as a guard area for water control. Sowing of faba bean cultivar "Shambat 75" was done at a rate of two seeds/hole. The crop was irrigated every week during the first month. The experiment was arranged in split-plot design with four replications. The main plots were allotted to the watering intervals:

(a) Wet = plants were irrigated every week, and
(b) Dry = plants were irrigated every two weeks, and the subplots to the following treatments:

1. Untreated control plants
2. Plants inoculated with vesicular arbuscular mycorrhiza (VAM; *Glomus* sp.), as previously described by Mahadi and Atabani (1992).
3. Plants fertilized by 200 kg/ha P_2O_5 superphosphate (at sowing).
4. Plants fertilized with 50 kg/ha sulphur (at sowing).
5. Plants treated with both mycorrihza and phosphorus (treatments 2 and 4).
6. Plants treated with both mycorrhiza and sulphur (treatments 2 and 4).
7. Plants treated with both phosphorus and sulphur (treatments 3 and 4).
8. Plants treated with mycorrhiza, phosphorus and sulphur (treatments 2, 3 and 4).

The seeds were carefully cleaned then ground to pass through a 0.4-mm screen, for analysis, on dry weight basis.

2.2. Protein fractionation

The nitrogen from defatted meal was extracted, stepwise, by a series of solvents according to the Landry and Moureaux (1970) procedure. Defatted samples (3.5 g) were kept in suspension with 35 ml of extractant by magnetic stirring in 50 ml centrifuge tubes. The duration (min) and number of extractions with each solvent are shown in Table 1.

Fraction I contained salt-soluble protein globulins, fraction II contained water-soluble protein albumins, fraction III contained alcohol-soluble protein prolamins. fraction IV contained G_1-glutelins, fraction V contained G_2-glutelins and fraction VI contained G_3-glutelins. The solid material was isolated by centrifugation at 3000 *g* for each solvent; supernatants were combined to give total extract. The nitrogen content of each of these six fractions was determined by the micro-Kjeldahl method. The residue left after extraction was also analyzed for nitrogen content.

2.3. Statistical analysis

Each sample was analyzed in triplicate and the figures were then averaged. Data were assessed by analysis of variance. The Duncan multiple range test was used to separate means. Significance was accepted at $P \leqslant 0.05$.

3. Results and discussion

3.1. Albumin and globulin fractions

The major protein fraction of faba bean was the globulin fraction that ranged from 69.5 to 78.1% (Table 2). Phosphorus treatment significantly ($P \leqslant 0.05$) increased the globulin fraction of faba bean seeds in the wet treatment and significantly ($P \leqslant 0.05$) decreased it in the dry treatment. Sulphur treatment significantly increased the globulin fraction in the dry treatment but significantly decreased it in the wet treatment. Mycorrhiza treatment showed similar results for the globulin fraction to that of the control of the dry treatment but significantly reduced it in the wet treatment. The mycorrhiza + phosphorus + sulphur treatment significantly decreased the globulin fraction whereas mycorrhiza + phosphorus showed similar results to that of the control in both wet and dry treatments. Mycorrhiza + sulphur and phosphorus + sulphur treatments showed similar results for the globulin content to that of the control of the dry treatment but were significantly lower than the control of the wet treatment. The general pattern indicated that water treatment is associated with higher globulin contents. Globulins are storage proteins used during germination, and form discrete bodies bound to cell membranes. They are rich in aspartic and glutamic acids, leucine and arginine, together with basic amino acids (Bailey & Boulter, 1972).

The albumin fraction of faba bean ranged from 1.4 to 3.4% (Table 2). All fertiliser treatments significantly ($P \leqslant 0.05$) increased the albumin fraction in the wet treatment, whereas only treatments mycorrhiza, mycorrhiza + sulphur and mycorrhiza + phosphorus + sulphur

Table 1
Protein extraction procedure

Step	Extractant	Time of extraction (min)	Fraction groups	Protein
1	NaCl, 0.5M (4°C)	60, 30, 30	I	Globulins
2	Water (4°C)	15, 15	II	Albumins
3	EtOH, 60% (20° C) and then at (60° C) 2-Pr-OH 55% (20° C)	30, 30, 30 60, 30, 15	III	Prolamins
4	EtOH, 60% +2ME 0.6%(v/v)(20°C) 2-PrOH 55% + 2ME 0.6% (v/v) (20°C)	30, 30 30, 30	IV	G_1- glutelins
5	NaCl, 0.5M, pH 10 + 2ME 0.6%(v/v) (20° C)	60, 30, 30	V	G_2-glutelins
6	Na DodSO4 0.5% (w/v) pH 10 + 2 ME 0.6% (v/v) (20° C)	60, 30, 15	VI	G_3-glutelins
7				Insoluble protein

significantly increased it in the dry treatment compared to control. Treatments: sulphur, phosphorus and mycorrhiza + phosphorus were similar to the control, whereas phosphorus + sulphur significantly decreased it in the dry treatment. However, mycorrhiza + sulphur treatment, in both dry and wet samples, resulted in significantly higher albumin content compared to other fertiliser treatments. It appears that water stress has a positive relationship towards the albumin content. The results in this investigation are in agreement with those of Deshphande and Nielsen (1987) who found that the water-soluble plus salt-soluble proteins of legumes ranged from 63 to 83% of the total protein.

3.2. Prolamin fraction

The prolamin fraction of faba bean ranged from 2.1 to 4.1% (Table 2). With the exception of the treatment mycorrhiza + sulphur, all other fertilisation treatments showed prolamin contents of faba bean seeds similar to that of the control in the wet treatment. The mycorrhiza + sulphur treatment significantly ($P \leqslant 0.05$) increased prolamin content in wet samples, but in dry samples it was significantly decreased compared to control. It appears that water stress has a positive relationship to the prolamin content. Prolamin contains major polypeptides with average molecular weights of 2500 and 21 800 daltons (Misra et al. 1972). Moreover, Paulis (1982) reported that prolamins contain higher levels of leucine, proline and glutamic acid than other fractions.

3.3. G_1-glutelin fraction

The G_1-glutelin fraction of faba bean ranged from 0.9 to 2.2% (Table 2). Phosphorus + sulphur and mycorrhiza + phosphorus treatments significantly ($P \leqslant 0.05$) decreased the G_1-glutelin content of faba bean seeds compared to the control of the wet treatment; whereas all other fertiliser treatments showed similar G_1-glutelin contents to that of the control of the wet treatment (Table 2). Fertiliser treatments gave similar G_1-glutelin contents to that of the control in the dry treatment except the phosphorus + sulphur treatment, which significantly increased the G_1-glutelin fraction; whereas mycorrhiza treatment significantly reduced it in dry treatment (Table 2). It appears that water stress has a positive relationship to G_1-glutelin content. Glutelins are associated with lower molecular weight proteins through non-covalent bonding; they consist mainly of two categories of polypeptides linked by disulphide bonds. G_1-glutelin has an amino acid composition somewhat similar to prolamin but with higher levels of glycine. methionine, histidine and proline and lower levels of aspartic acid, leucine and isoleucine.

3.4. G_2-glutelin fraction

The G_2-glutelin fraction of faba bean ranged from 1.9 to 6.1% (Table 2). Phosphorus treatment significantly ($P \leqslant 0.05$) decreased the G_2-glutelin fraction while treatments of mycorrhiza + phosphorus and mycorrhiza + phosphorus + sulphur significantly ($P \leqslant 0.05$) increased it compared to the control of the wet treatment (Table 2). With the exception of the treatment mycorrhiza + phosphorus, all other fertiliser treatments showed lower G_2-glutelin contents than that of the control of the dry treatment (Table 2). The general pattern indicated that water treatment was associated with higher G_2-glutelin contents. The amount of G_2- glutelins, isolated at step 5 depends on conditions used earlier to extract salt-soluble and alcohol-soluble proteins (Landry & Moureaux, 1981). Nugdalla and El Tinay (1997) found that the G_2-glutelin of nine cowpea cultivars ranged from 1.4 to 2.9%.

Table 2
Effect of mycorrhiza. phosphorus and sulphur fertilisation on protein solubility profiles irrigated every week (wet treatment) or every 2 weeks (dry treatment)[a]

Treatments	Total protein (%)		Globulin (%)		Albumin (%)		Prolamin (%)		G_1-glutelin (%)		G_2-glutelin (%)		G_3-glutelin (%)		Insoluble protein (%)		Total protein recovered	
	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry
Control	31.8	31.9	75.8b	73.6cd	1.41e	2.17cde	2.68cde	3.53ab	1.19ab	1.57b	3.69bc	4.61a	10.2e	11.52bc	2.07d	1.93cd	97.0	98.9
Sulphur	33.1	32.7	73.1d	76.3ab	2.43c	1.95e	2.73cde	405a	1.28a	1.78b	3.38c	1.92g	12.9b	09.16d	2.13cd	2.77a	98.0	97.9
Phosphors	32.8	32.4	78.1a	70.5f	2.07d	2.32cd	2.14e	3.42ab	1.14ab	1.77b	2.40d	3.86bcd	8.87f	12.0b	2.30c	2.19b	97.0	96.0
Mycorhiza	35.7	33.4	72.7d	72.7de	2.09d	3.19ab	2.62de	3.86ab	1.26c	1.14c	3.98b	2.28fg	14.4a	13.8a	2.62b	1.78d	100	98.8
Phosphorus + sulphur	34.6	32.1	74.0d	72.3def	3.01b	1.48f	2.64cde	3.78ab	0.87c	2.16a	3.50c	4.16b	10.2e	11.4bc	2.77b	2.71a	96.9	98.0
Mycorrhiza + sulphur	39.7	32.2	70.4 ef	75.4bc	3.37a	3.32a	3.44ab	2.12c	1.00bc	1.61b	3.77bc	3.44de	11.6c	11.5bc	2.27cd	2.13bc	95.9	99.5
Mycorrhiza + phosphorus	33.8	32.6	76.65ab	73.1de	2.11d	2.13de	2.45de	3.29b	0.88	1.63b	5.68a	4.84a	08.96f	11.0c	2.27cd	2.56a	99.0	98.6
Mycorrhiza + phosphorus + sulphur	32.8	32.4	69.5f	71.5ef	2.24cd	2.96b	3.01bcd	3.57ab	1.00bc	1.62b	6.12a	3.69cde	10.8de	11.7bc	3.38a	2.57a	99.0	97.5
Mean	33.4	32.5	73.4a	73.2b	2.34b	2.44a	2.71b	3.45a	1.08b	1.67a	4.05a	3.60b	11.0b	11.5a	2.48a	2.33b	97.0	98.2

[a] Means followed by the same letter are not significantly different at $P \leqslant 0.05$.

3.5. G_3-glutelin fraction

The G_3-glutelin fraction of faba bean ranged from 8.9 to 14.4% (Table 2). Treatments of sulphur, mycorrhiza and mycorrhiza + sulphur significantly increased the G_3-glutelin fraction whereas phosphorus and mycorrhiza + phosphorus treatments significantly decreased it. All other fertiliser treatments were insignificantly different from the control of the wet treatment (Table 2). Mycorrhiza treatment significantly ($P \leqslant 0.05$) increased the G_3-glutelin fraction of faba bean seeds, whereas sulphur treatment significantly reduced it, compared to the control of the dry treatment. All other fertiliser treatments gave similar values to that of the control of dry treatment (Table 2). It appears that water stress has a positive relationship towards G_3-glutelin content.

3.6. Insoluble protein

The insoluble protein (residue) of faba bean ranged from 1.8 to 3.4% (Table 2). Generally, fertiliser treatments significantly increased the insoluble protein in the wet treatment. Sulphur, mycorrhiza + sulphur or mycorrhiza + phosphorus treatments were similar to the control (Table 2). With exception of treatments mycorrhiza and mycorrhiza + sulphur all other fertiliser treatments significantly increased the insoluble protein content in the dry treatment (Table 2). The general pattern indicated that water treatment is associated with higher insoluble protein contents. The insoluble protein (residue) consists mainly of proteins from previously-defined groups, becoming insoluble due to interactions with lipids, carbohydrates, or polyphenols via oxidation (Landry & Moureaux, 1981).

4. Conclusion

Fertiliser treatment of faba bean resulted in improved protein content which was more pronounced in the wet treatment fertilised with mycorrhiza + sulphur. However, this fertilisation treatment was associated with significantly lower globulin content compared to the control. This was counteracted by significantly higher albumin content. The general pattern indicated that water stress had a negative effect on the globulin fraction but a positive effect on the albumin fraction.

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