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Chemical Composition of Faba Bean (*Vicia faba* L.) Genotypes under Various Water Regimes

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Abstract: A set of thirteen faba bean genotypes were grown at three water regimes (13200, 7600 and 4800 m^3/ha) severe, moderate and normal irrigation during the growing season of the crop, to examine the variability of seed chemical composition. The result showed that faba bean genotypes vary greatly in their chemical composition at various water regimes and proximate analysis showed that faba bean genotypes had low moisture contents that ranged from (7.09 - 7.59%). Carbohydrate contents were fairly high (42.4-47.3%). The highest protein contents under water stress condition and normal irrigation was recorded for Kamlin (36.8 and 39.1%), while the lowest was observed in Sakh1 (35.2 and 37.5%), respectively. Simultaneously, the crude protein content in the seeds varied from 31.8% to 39.7% and consisted of 18 amino acids. Kamlin had the highest (117g/1000g protein) total essential amino acids while $Giza_{402}$ had the lowest (82.2 g/1000g protein). Methionine and cysteine were the limiting amino acids in the faba seeds. The Protein fraction varied among these genotypes, globulin was followed by glutelin, prolamin and albumin respectively. Evidence was found that protein content increased during water stress treatment.

Key words: Faba bean, water regimes, crude protein contents, amino acids, seed chemical composition, carbohydrate

INTRODUCTION

Wide cultivation and spread of faba bean (*Vicia faba* L.) in the temperate and the subtropical regions has ranked it the fourth most important legume crop in the world, next to dry beans, dry peas and chickpea. The crop contributes to human nutrition as a result of its high protein content and other essential nutrients. Faba beans though are less consumed in western countries as human food, it is considered as one of the main sources of cheap protein and energy in Africa, parts of Asia and Latin America, where most people can not afford meat sources of protein (Duc, 1997; Haciseferogullari *et al.*, 2003).

Similarly, faba bean in the Middle East region, is consumed mostly as dried seed while, a little portions is consumed as fresh kernel. The crop is also becoming increasingly important in Saudi diets due to the high lysine content of the seed, which encourages the use of faba bean as a protein supplement for cereals (El-Fiel *et al.*, 2002; Alghamdi, 2003). Meal dishes of faba that can be found in an Arabian home or indigenous restaurants are *Madammis* (stewed beans), *Falafel* (deep fried dought) poured paste *Bissara* and *Nabet* soup (boiled germinated beans).

The nutritional value of faba bean has always been traditionally attributed to its high protein content, which ranges from (27-34%) Duc, 1997; Haciseferogullari *et al.*, 2003) depend on genotypes. Most of these proteins comprise of globulins (79%), albumins (7%) and glutelins (6%) (Hossain and Mortuza, 2006). Legume seeds contain several comparatively minor proteins including trypsin inhibitors, lectins, lipoxygenase and urease, which are relevant to the nutritional quality of the seed.

When spring and winter faba beans were compared, winter beans were found to have slightly higher concentration of protein (Duc *et al.*, 1999), indicating that under water deficit condition, protein content of faba beans tends to increase. Ibrahim and Kandil, 2007 reported for similar result in soybean, where highest values of protein were recorded under 14 day irrigation interval compared with 7 days irrigation interval. Oil content of canola (*Brassica napus* L.) -a popular oil crop- was reduced while protein content increased as a result of drought effect (Sinaki *et al.*, 2007).

Recent genetic and breeding efforts has resulted in an improved faba bean adapted to environmental stresses, high yield protein content and seed free from major anti-nutritional factors (tannins and vicine-convicine) (Duranti and Cristina, 1997), but little effort was done to quantify the effect of water limitation on the protein contents of these new improved lines. The objective of this study was therefore, to asses the effect of three different irrigation water regimes on seed chemical composition of thirteen genotypes of faba beans and selection of promising, high quality genotypes under water stress in the Kingdom of Saudi Arabia.

MATERIALS AND METHODS

The field experiment: A field experiment was conducted in two consecutive growing seasons 2003/2004 and

Table 1: Source of the studied faba bean genotypes and their 100-seed weight

Genotypes	Source	100-seed weight (g)
Reina Blanca	KSU, Legume unit. Riyadh	88.1
$Joff_1$	Joff market	84.4
$Hassawi_1$	Hassa market	91.8
$Hassawi_2$	Hassa market	87.1
$Hassawi_3$	Hassa market	83.5
$Gazeira_1$	KSU Legume unit. Riyadh	89.4
$Gazeira_2$	KSU Legume unit. Riyadh	92.4
Kamlin	KSU Legume unit. Riyadh	84.5
$Giza_2$	KSU Legume unit. Riyadh	81.4
$Giza_{40}$	KSU Legume unit. Riyadh	78.5
$Giza_{402}$	KSU Legume unit. Riyadh	84.4
$Sakha_1$	KSU Legume unit. Riyadh	74.0
Habashi	Jazan	76.2
L.S.D. (0.05)		1.2

2004/2005 at the Agricultural Research and Experimental Station in Dirab, King Saud University, Riyadh. The genotypes were grown in rows, 70cm apart and in holes 30 cm apart, with three seeds in each hole. The size of the subplots was 4×3m consisting of three rows of 3m long each. A gap of 1.5m was left between the main plots as a guard area for water movement. The crop was irrigated every week during the first month. The stress treatments were then imposed during vegetative until late maturity stage. The experiment was laid out in split plots design with three replications. The main plots were allocated to the watering regimes and the subplots to the genotypes.

The volumes of water received by each subplot were measured very accurately through the installation of an irrigation system and through gated pipes having water flow meters. The total amounts of irrigation water received by treatments 1, 2 and 3 were 13200, 7600 and 4800 m^3/ha/season (normal, mild and stress condition) respectively.

Fertilizers were applied at monthly intervals starting two weeks from seedlings emergence at the following rates: 40 kg N/ha as urea, 40 kg P_2O_5/ha as triple superphosphate, 20 kg K_2O/ha as sulphate of potash. The planting materials utilized in this research are local seeds and introduced faba bean genotypes collected from different research programs and farms (Table 1). The seeds were cleaned and the weight of 100 seeds was determined for each genotype and sent to lab for chemical analysis prior to the start of experiment.

Seed composition proximate composition

Seed analysis: after harvest, samples were taken for the determination of crude protein and amino acids contents. The clean seeds samples were ground to pass through a 0.4 mm screen. The moisture content, crude protein, lipids, ash and crude fiber in the samples were determined by the standard methods detailed (AOAC, 2000). The carbohydrate content was determined as the weight difference using moisture, crude protein, lipids and ash content data. Each sample was analyzed in triplicate and the values were then averaged.

Protein fractionation: The protein from defatted meal was extracted, stepwise, by a series of solvents according to the method explained by Landry and Moureaux (1970). The seed meal was extracted successively with water, 5% (w/v) NaCl, 75% (v/v) ethanol and 0.25% (w/v) NaOH. Due to the presence of salts in the seeds themselves, extraction with water removed some salt-soluble proteins that precipitated on dialysis (termed A Globulins). The pellet fractions were air-dried overnight at 4°C and the supernatant fractions were lyophilized; each fraction was re-suspended in sample buffer (10mM Tris-HCl, pH 6.8, 100 mM 2-mercaptoethanol, 2% (w/v) sodium dodecyl sulphate (SDS), 15% (v/v) glycerol and 0.06% (w/v) *m*-cresol purple).

An amino acids determination: Total amino acids except tryptophan and sulphur amino acids were determined as described by Shahidi *et al*., 1992). The samples were freeze-dried and then hydrolyzed in HCl, 6 mol/l at 110°C for 24 hours (Bishnoi and Khetarpual, 1993). The HCl was removed in vacuo and dried samples were reconstituted using lithium citrate buffer at pH 2.2. The hydrolyzed amino acids were then determined using a Beckman 121 MB amino acid analyzer (Beckman Instruments Inc., Palo Alto CA). Correction factors were applied to allow for incomplete liberation of isoleucine (1.06) and valine (1.05). Methionine and cystine were oxidized with performing acid before hydrolysis for 18 hours. For tryptophan, hydrolysis was performed in Ba $(OH)_2$, 1.5 mol/l at 120°C for 18 hrs.

RESULTS AND DISCUSSION

Chemical composition of faba bean seeds: The chemical composition of 13 faba bean genotypes is shown in Table 2. Result revealed that genotypes differed significantly in moisture and value ranged from 7.09 to 7.59%. The results demonstrate the considerable genetic variation for moisture, protein, fat and carbohydrate. The range in protein content extended from 31.8 to 39.7%, for Reina Blanca and $Gazeira_1$ respectively. The fat contents ranged from 1.50 to 2.12% with no significant differences among genotypes. This is in agreement with values obtained by El Tiney *et al.* (1989) and El Sheikh (1998). Carbohydrate ranged from 42.2 to 47.6% which is in harmony with the mean carbohydrate content for raw legumes reported by El Tiney *et al.* (1989), but lower than values obtained by El Sheikh and El Zidany (1997). Statistical analysis revealed significant differences within the genotypes.

Table 2: The chemical analysis of seeds in 13 faba bean genotypes

Genotypes	Moisture %	Protein %	Fat %	Carbohy-drates%
Renia Blanka	7.59	31.8	1.50	45.2
$Joff_1$	7.21	35.7	1.76	42.2
$Hassawi_1$	7.23	32.8	2.06	45.2
$Hassawi_2$	7.30	33.1	2.12	45.6
$Hassawi_3$	7.37	33.8	1.65	44.2
$Gazeira_1$	7.32	39.7	1.70	45.6
$Gazeira_2$	7.27	34.6	1.76	43.8
Kamlin	7.38	37.7	1.73	47.3
$Giza_2$	7.59	35.6	1.94	43.5
$Giza_{40}$	7.43	32.8	1.65	46.3
$Giza_{402}$	7.34	31.9	2.01	44.7
$Sakha_1$	7.31	32.6	1.72	42.4
Habashi	7.09	32.7	1.62	44.7
L.S.D. (0.05)	0.19	0.18	0.70	1.7

Table 3: Protein fractionation in seeds of 13 faba bean genotypes

Genotypes	Albumin %	Globulin %	Prolamin %	Glutelin %
Renia Blanka	1.41	73.6	2.68	13.2
$Joff_1$	2.17	76.3	3.14	14.9
$Hassawi_1$	2.43	70.5	3.54	12.8
$Hassawi_2$	1.95	72.7	3.42	18.4
$Hassawi_3$	2.07	75.8	3.44	15.2
$Gazeira_1$	2.32	73.1	2.29	15.6
$Gazeira_2$	2.90	78.1	3.45	14.9
Kamlin	3.01	74.0	3.57	16.8
$Giza_2$	2.31	70.4	2.28	14.6
$Giza_{40}$	2.21	73.1	2.45	14.4
$Giza_{402}$	2.13	71.5	3.14	13.8
$Sakha_1$	2.24	69.5	2.26	12.0
Habashi	1.46	70.7	1.83	12.6
L.S.D. (0.05)	0.32	1.7	0.76	1.5

Variations in these traits among different genotypes can be attributed to both genetic and environmental factors (Musallam *et al*., 2004).

Fractionation of Seed protein by different solvent: As shown in Table 3, the extraction by water, alcohol and salt-soluble proteins (ablumins and globulins) makes up 81.0% of total protein for $Gazeira_2$ and 72.16% for Habashi. Differences among genotypes are statistically significant ($P<0.05$). Globulin, the predominant fraction for all faba bean genotypes tested, ranged from 78.1% in $Gazeira_1$ and 69.5% in $Sakha_1$. The prolamin fraction was the lowest 1.83% in Habashi and highest 3.57% in Kamlin. These results are comparable with the values found by El-Fiel *et al*., 2002 and Hossain and Mortuza, 2006. In general, globulin constitutes the largest fraction of protein in legume seed.

Glutelins varied from 12.0% in $Hassawi_2$ to 18.4% in $Sakha_1$. These results were different from those found by (Hossain and Mortuza, 2006). The materials in this study can be arbitrarily grouped into low, medium and high protein genotypes, out of which six are low-protein types, four are medium-protein types (relatively high prolamin contents). The remaining three genotypes have relatively the highest protein and prolamin contents, indicating that the increase in protein content in faba bean genotypes is attributable mainly to higher levels of the prolamin fraction.

Table 4: Protein content (%) of 13 faba bean genotypes under three irrigation regimes

	------- m^3/ha -------		
	13200	7600	4800
Collection	------- Protein content % -------		
Reina Blanca	36.5	38.4	39.0
$Joff_1$	35.4	36.6	37.0
$Hassawi_1$	36.8	37.9	38.6
$Hassawi_2$	36.1	37.0	38.0
$Hassawi_3$	35.5	36.9	38.4
$Gazeira_1$	36.5	38.7	38.3
$Gazeira_2$	36.7	38.5	39.0
Kamlin	36.8	38.1	39.1
$Giza_2$	37.0	37.9	38.1
$Giza_{40}$	36.0	37.7	37.9
$Giza_{402}$	36.1	37.0	38.2
$Sakha_1$	35.2	35.8	37.5
Habashi	35.8	36.6	37.3

L.S.D. for genotypes = 0.2, for irrigation regimes = 0.4

Protein content: Data presented in Table 4. indicated no significant differences among genotypes in protein contents of seeds under different levels of water treatment. Significant differences were observed among genotypes when grown under less favorable watering regimes (7600 and 4800 m^3/ha). There is an increasing trend in protein content of seeds with less supply of water, i.e. seeds of 7600 m^3/ha and 4800 m^3/ha water regimes had more protein than those of 13200 m^3/ha treatment. This may be attributed to the fact that plants under the 7600 and 4800 m^3/ha treatments had fewer seeds/plant than those of 13200 m^3/ha high level water irrigation treatment. The allocation of photosynthetic substrates is expected to be high among plants under 13200 m^3/ha compared to plants under stress, the seeds of these plants are also expected to have less protein but higher seed yield. The highest protein contents was recorded for Kamlin seeds and ranged from 36.8-39.1 % low level of irrigation, while the lowest was reported for $Sakh_1$ and ranged from 35.2-37% under normal irrigation. This result was in contrast with (Musallam *et al*., 2004).

Total protein content varied greatly among the faba been genotypes. Despite differences that might be due to genotype and location, values recorded in this study appeared to be somewhat higher than those described elsewhere (Hossain and Mortuza 2006, Musallam *et al*., 2004). It should however, be noticed that protein concentration of the seeds is usually obtained by multiplying the total nitrogen content by a conversion factor of 6.25, which causes an overestimation of protein content due to the contribution of non-protein nitrogen (Mosse, 1990).

Table 5: Albumins (Alb), Globulins (Glo), Glutelins (Glu), Prolamins (Pro) (%) of 13 faba bean genotypes under three irrigation regimes

	13200				7600				4800			
	m^3/ha											
Collection	Alb	Glo	Glu	Pro	Alb	Glo	Glu	Pro	Alb	Glo	Glu	Pro
Rena Blanca	1.89	72.6	13.56	2.45	1.26	65.80	20.58	2.36	1.18	61.6	25.03	2.19
$Joff_1$	2.03	71.50	13.54	2.93	1.94	68.30	16.93	2.83	1.73	60.9	24.74	2.63
$Hassawi_1$	2.17	66.90	12.56	3.37	1.98	57.40	27.32	3.30	1.80	52.2	32.95	3.05
$Hassawi_2$	1.87	69.8	14.32	3.01	1.69	63.01	22.38	2.93	1.54	57.4	28.28	2.78
$Hassawi_3$	1.95	71.41	13.84	2.81	1.80	65.90	19.58	2.72	1.61	58.9	26.95	2.54
$Gazeira_1$	2.11	68.5	18.20	3.19	2.07	65.20	19.62	3.11	1.89	59.5	25.61	3.00
$Gazeira_2$	2.86	77.2	10.45	3.49	2.78	75.10	18.78	3.34	2.62	70.7	23.41	3.27
Kamlin	2.96	72.8	10.54	3.70	2.86	70.40	13.16	3.58	2.69	66.2	27.70	3.41
$Giza_2$	2.07	68.1	22.15	2.68	1.90	58.00	27.50	2.60	1.78	54.3	31.46	2.46
$Giza_{40}$	1.99	67.9	14.84	2.27	1.85	61.20	24.76	2.19	1.73	57.3	28.85	2.12
$Giza_{402}$	1.89	68.5	12.75	2.86	1.74	58.50	27.06	2.70	1.60	53.8	32.10	2.50
$Sakha_1$	1.82	65.4	14.51	2.27	1.67	51.80	34.37	2.16	1.59	54.2	37.01	2.10
Habashi	1.81	67.7	14.13	1.86	1.22	58.60	28.44	1.74	1.13	54.2	32.99	1.68
LSD0.05 -1	0.03	1.6	2.19	ns	0.03	1.60	2.19	ns	0.03	1.6	2.19	ns
LSD0.05 -2	0.32	4.8	4.96	ns	0.32	4.80	4.96	ns	0.32	4.8	4.96	ns

LSD 0.05 -1 for genotypes. LSD 0.05 -2 for irrigation regimes

In general, the crude protein in the seed of the faba bean genotypes varied from 35.2% to 39.1% under three irrigation regimes, where Kamlin had the highest value and $Sakha_1$ had the lowest under normal irrigation. It was observed that protein contents of faba bean changes from 26 to 35% depending on the genotypes and environmental conditions (Duc, 1997). Therefore, based on the recommended average human protein intake of 32-50 g by the National Research Council (1974), the tested faba bean genotypes could contribute significantly to alleviating the problem of protein malnutrition in the developing countries.

Protein fractionation: The sequential extraction of the faba bean seed protein, determined on the basis of differences in solubility allowed for easy separation of the Osborne fractions as follow in Table 5: Albumins, globulins, glutelins and prolamins from the 13 genotypes studied. Globulins represent the major storage proteins in legume seeds, where legume seeds contain two major types of globulins, vicilin- type and legumins. It is evident in this study that glutelins and globulins are the main protein fractions of the seed. Previous researchers (Shastry and John, 1991) have reported albumin and globulin, the major storage plant proteins, as varying between 50% and 75% of the total seed protein content. Such high contents are not usually cited of gluetelin in legume seeds. It should be kept in mind that although the Osborne solubility classes remain useful as working definitions, they do not take into consideration the modern knowledge of protein. Recent molecular and biochemical analyses of seed storage proteins and their genes indicated that these proteins fall into three major groups: Albumins, globulins and prolamins, but the Osborne glutelins considered either of the globulin type (Shewry and Casey, 1999) or of the prolamin type (Shewry and Tatham, 1999).

Results in Table 5. indicated that albumins, globulins, glutelins and prolamins varied significantly among genotypes when grown under a high water supply (13200 m^3/ha). However, significant differences were also observed among genotypes when plants were grown under less favorable watering regimes (7600 and 4800 m^3/ha) except for prolamins. The albumin and globulin fractions increased as the amount of irrigation water increased, reaching their maximum values (2.96% and 77.2% of total extractable protein) respectively, but declined thereafter. The albumin and globulin protein fractions have been reported to have higher levels of lysine (Johnson and Lay, 1974). Thus, the nutritional value of faba bean seeds would be expected to increase due to the increase in the albumin and globulin fractions following heavy irrigation.

Finally, the chemical analysis of faba bean seeds varied according to the genotypes and water regimes. Moreover, less water regimes significantly enhanced protein, amino acid contents, which is in an agreement with (Ibrahim and Kandil, 2007) who found that protein contents increased in response to soil moisture deficit stress, while oil content decreased, it therefore clear that faba bean can serve as a cheap meal to alleviate protein malnutrition, especially for developing countries.

Total amino acids: The amino acid composition of the seeds is shown in Table 6. Kamlin had the highest (117 g/kg protein) total essential amino acids (TEAA) content while $Giza_{402}$ had the lowest (82.2g/kg protein) TEAA. The nutritive value of proteins depends primarily on the capacity to satisfy body needs of nitrogen and essential amino acids (Pellet and Young, 1980) Result of comparison of the amino acid composition in the faba bean genotypes tested with (FAO/WHO, 1985) reference values indicated that the values obtained from this study were higher than the values recommended for pre-school and school children. This implies that the

Table 6: Amino acids content of the studied faba bean seeds (g/kg on an air-dry basis) as affected by watering regimes

Amino acids	Reina blanca	$Joff_1$	$Hassawi_1$	$Hassawi_2$	$Hassawi_3$	$Gazeira_1$	$Gazeira_2$	Kamlin	$Giza_2$	$Giza_{40}$	$Giza_{402}$	Habashi
Essential amino acids												
Arginine	12.5	10.2	9.8	10.6	9.7	12.6	5.5	8.9	8.3	7.9	8.4	8.1
Histidine	2.5	3.5	3.7	3.6	3.5	3.4	3.6	3.8	3.8	3.2	3.2	2.9
Lysine	3.9	4.4	4.8	4.7	5.6	3.1	4.2	5.7	4.7	4.2	4.5	3.8
Phenylalanine	5.9	4.3	6.9	5.6	5.8	6.3	4.3	6.5	5.2	5.0	4.8	4.4
Leucine	5.8	4.5	5.8	5.6	5.7	5.9	7.0	8.1	8.2	6.0	4.5	4.2
Isoleucine	4.6	4.2	4.6	4.7	4.8	4.9	5.6	4.6	3.4	3.0	3.7	4.6
Valine	5.6	4.3	6.2	5.6	4.4	5.1	5.7	4.9	3.8	3.2	2.2	2.6
Methionine	1.2	1.4	0.8	1.1	0.7	0.8	0.8	1.1	1.3	1.2	0.7	1.0
Threonine	3.9	3.1	4.6	5.0	3.9	3.6	3.7	4.1	3.4	2.6	2.7	2.3
Tryptophan	1.3	1.48	1.4	1.7	1.8	1.21	1.26	1.35	1.2	1.46	1.38	1.26
Non-essential amino acids												
Aspartic acid	11.3	8.2	11.6	13.6	10.1	12.4	12.7	13.9	8.4	12.2	8.7	11.3
Glutamic acid	19.8	24.5	25.9	20.9	24.1	24.3	25.5	25.1	19.0	17.9	16.9	17.8
Serine	5.1	4.0	4.3	5.0	5.2	3.1	5.3	6.5	5.0	4.3	4.5	4.4
Proline	4.5	4.1	4.6	4.8	4.3	2.6	6.2	6.9	8.3	3.4	3.1	3.9
Glycine	4.8	5.6	6.7	5.8	6.9	5.4	7.7	5.0	6.0	4.4	4.7	3.7
Alanine	4.8	3.8	4.7	4.5	4.2	5.5	5.6	4.0	4.2	5.3	5.0	4.2
Cystine	1.4	0.7	0.3	0.6	1.2	0.9	0.7	1.7	1.8	0.5	0.4	0.3
Tyrosine	2.3	3.2	3.4	3.6	3.4	3.4	3.8	4.8	2.9	3.3	2.8	2.7
Total of amino acids	101.2	98.7	110.1	107.0	105.3	104.5	109.2	117.0	98.9	89.1	82.2	83.5

evaluated genotypes would be a good source of essential amino acids and could be used for the fortification of cereal-based foods, which are particularly deficient in lysine. Arginine displays a very high increase in essential amino acids. The quality of dietary proteins can be measured in many ways. There is general acceptance that this value is a ratio of the available amino acids in the food or diet compared with the daily requirements.

However, methionine and cysteine were the limiting amino acids in the faba materials evaluated in the present study. The relatively low levels of methionine and cysteine in legumes were also reported by other investigators (Apata and Ologhobo, 1994). However, the high lysine content of the faba bean cultivars is a very important nutritional attribute and probably more important than the total protein content, simply because it makes a significant supplementary protein to cereal-based diets which are known to be deficient in lysine. In faba bean, an increased content of lysine might be advantageous, considering their use in mixed diets with cereals. As a result, there will be more products and a possible increase in demand of this crop in near future.

Conclusion: This faba bean research which was carried out during two planting season in Saudi Arabia with purpose of assessing the effect of three different water regimes on faba bean has shown that thee genetic materials tested can serve as a good resource for protein and other essential nutrients and support any program to alleviate protein malnutrition, especially in the developing countries.

As a result of higher level of protein content with decrease in water supply to the plant, it is obvious that medium level of irrigation will give high protein contents in faba bean with acceptable crop yield thus, water can be logically conserved in the region without any significant yield loss. Further, the best performing cultivars among the genetic materials tested is Kamlin, thus this cultivar is recommended for commercial and extensive faba bean farming in the region as a result of its capacity to give high protein content under various water regime.

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