Qatar Univ. Sci. J. (1997), 17(2): 271-279

Changes in carbohydrate and nitrogen fractions during germination of fenugreek (*Trigonella foenum-graecum* L.) seeds presoaked in GA_3, growing under different osmotic potentials

By

Alhadi, F. A., Yasseen, B. T*. and Al-Dubaie, A. S.

Department of Biology, Faculty of Science,

Sana'a University, Yemen

التغيرات في المكونات الكربوهيدراتية والنتروجينية خلال إنبات بذور الحلبة المنقوعة في حامض الجبريليك والنامية في مستويات مختلفة من الجهد الأزموري

فاطمة أحمد الحدي، بسام طه ياسين*، عبد الرحمن سعيد الدبعي

قسم علوم الحياة ـ كلية العلوم ـ جامعة صنعاء ـ اليمن

درست التغيرات في المكونات الكربوهيدراتية والنتروجينية خلال إنبات بذور الحلبة (*Trigonella - foenum - graecum* L.) تحت ظروف الإجهاد المائي ومعاملة حامض الجبريليك. وكانت العلاقة خطية بين اختزال النسبة المئوية للإنبات وهبوط الجهد الأزموزي لوسط البذور، لتصل تلك النسبة إلى أوطأ قيمة عند -٠,٨ ميجا باسكال. تراكم السكروز كثيراً والذي كان له مساهمة رئيسية في المحتوى الكلي للكربوهيدرات، بينما اختزل محتوى السكريات الأحادية لدرجة كبيرة ولم تُظهر السكريات المتعددة نمطاً متناغماً في التغيرات نتيجة للإجهاد المائي. وتوحي استجابة المكونات النتروجينية إلى تباين النتروجين الإجمالي حيث زاد لدرجة كبيرة عند -٠,١ و -٠,٣ ميجا باسكال وتلى ذلك هبوط حاد عند -٠,٥ و -٠,٨ ميجا باسكال. ذات السلوك قد وجد في النتروجين الذائب الكلي، بينما تغير البروتين فقط لدرجة طفيفة، وتراكم البرولين بكميات كبيرة بنقص الجهد الأزموزي لوسط البذور.

وبالرغم من أنه ليس لمعاملة البذور بحامض الجبريليك أي تأثير تحفيزي على إنبات البذور تحت ظروف الإجهاد المائي، فإنه قد سبب تغيرات كبيرة في مكونات الكربوهيدرات في البادرات النامية، فقد اختزلت السكريات الأحادية وتراكم السكروز وتحللت السكريات المتعددة مقارنة مع معاملة الماء. ولم تُظهر المكونات النتروجينية استجابات واضحة بمعاملة حامض الجبريليك.

وقد نوقشت الأدوار الأيضية المحتملة لتلك المكونات في البادرات النامية تحت ظروف الإجهاد المائي.

Key - Words : Carbohydrate, Fenugreek, Gibbrellic acid, Nitrogen, Proline, Water Stress.

ABSTRACT

Changes in carbohydrate and nitrogen fractions during seed germination of fenugreek under water stress and GA_3 treatment were investigated. The germination percentage was reduced with the decrease in the osmotic potential of seed media, reaching its lower value at -0.8 MPa. Sucrose was accumulated considerably which could have had a major contribution in the total carbohydrate, while monosaccharides showed a substantial reduction and polysaccharides demonstrated no consistent pattern of changes due to water stress. The response of total nitrogen was variable; it increased considerably in -0.1 and -0.3 MPa osmotic potential, then dropped sharply at -0.5 and -0.8 MPa. Similar behavior was found in TSN(Total Soluble Nitrogen), whereas protein-N changed only slightly and proline was accumulated substantially by decreasing the osmotic potential of the seed media.

Although GA_3 application had no any promotive influence on seed germination under water stress, it caused considerable changes in carbohydrate fractions in the growing seedlings. For example, monosaccharides were reduced, sucrose was accumulated and polysaccharides were degraded in hormone treatment as compared to water treatment. Nitrogen constituents, on the other hand, showed no clear responses to GA_3 treatment.

The possible metabolic roles of all these fractions in the growing seedlings due to water stress were discussed.

* The present address: Department of Biological Sciences, Faculty of Science, Qatar University, Doha, Qatar.

INTRODUCTION

It has been long recognized that osmotic stress induced by drought or salinity causes series of metabolic changes during the various stages of growth and development of crop plants. The reduction in the water potential of the plant environment leads to disturbances in the physiological and biochemical activities, thereby exerting deleterious alterations in the rate of germination, vegetative growth and productivity [1,2]. During the period between seed germination and the seedling growth stage, plants are dependent on the reserve substances that are stored in the seed. Carbohydrates and nitrogenous compounds have been implicated in various roles in the metabolic and physiological responses of plants to water stress [3]. However, the relative contribution of these compounds to those responses were varied between species and cultivars [4,5]. Hormone imbalance in plant tissues exposed to water stress or salinity, on the other hand, has been considered as one of the drastic disturbances acting on the physiological and biochemical processes [6]; thus, increasing the concentration of endogenous growth promoters is essential in returning the metabolic activities to their normal levels under these conditions [7]. GA_3 at certain concentrations has been shown to be beneficial for the seed germination of many crops especially those exposed to osmotic stress [8,9]. The stimulatory effect of GA on seed germination can be considered a consequence of changing the metabolic processes in the embryonic axis of germinating seeds [6,10]. However, other investigators have reported that GA_3 slightly retarded the germination percent in some plants growing under water stress [11,12].

The objective of this work was to study the effect of water stress and GA_3 treatment on the germination of fenugreek seeds as well as the physiological and biochemical activities involved during the seedling stage . Fenugreek plant has been cultivated in many countries of the Middle East for a long period of time. Seeds of this crop store various compounds such as proteins , carbohydrates, fats, etc., and have been used as a nutritional flavor and spice as well as in medicinal purposes [13]. The responses of this crop to drought or salinity at various stages of growth and development is not well understood. Thus, the present study can be considered as an attempt to evaluate the changes in carbohydrate and nitrogen fractions during seed germination under water stress. Also, the study included the possible roles of GA_3 treatment on these changes.

MATERIALS AND METHODS

Plant material and germination experiments:

Seeds of fenugreek (*Trigonella foenum-graecum* L.) were obtained from the main crop market in Sana'a, and 98 to 100% of these seed were viable. The seeds were surface sterilized in a sublimate formaldehyde solution for 15 minutes, then washed thoroughly with sterile distilled water. The seeds were then soaked for 2 hours either in distilled water or in $3x10^{-5}M$ GA_3 (BDH) aqueous solution, in darkness, at room temperature [14]. The treated seeds were left to air dry for 24 hours before being used .

Batches of thirty seeds of uniform size from each treatment were germinated on cellulose paper in sterilized petri-dishes of 11 cm in diameter , and wetted with 30 cm^3 of different osmotic potentials, 0, -0.1 -0.3, -0.5, and -0.8 MPa of mannitol. The osmotic potentials of mannitol solutions were calculated according to Van't Hoff relation.

Each treatment was replicated three times and the petri dishes were incubated in the dark for 5 days at room temperature (20-26°C). The dishes were opened daily to allow gas exchange. Seeds were considered germinated when the radicles had emerged from the testa, and the number of germinated seeds were counted daily for each replicate. On the fifth day, the final percentage of germination was determined.

Biochemical analysis:

After five days of germination, seedlings were oven dried and ground from which a known weight was extracted with 5 cm^3 of borate buffer, left for 24 hours , then centrifuged and filtered. The filtrate was used for the determination of the direct reducing value (DRV), total reducing value (TRV) and total soluble nitrogen(TSN), while the residue was dried at 80°C for the determination of polysaccharides and protein-N.

Estimation of DRV was carried out by evaporation of 0.1 cm^3 of cleared borate buffer extract until dry and then mixed with 1 cm^3 of modified Nelson solution [15]. The mixture was maintained on a boiling water bath for 15

minutes, then cooled rapidly and 1 cm^3 of arsinomolybdate was added [16]. The developed color was measured spectrophotometrically at 700 nm. TRV was determined by the same method after hydrolysis with invertase (EC.3.2.1.26 BDH). Sucrose was calculated (in terms of glucose) from the equation:

$$\text{Sucrose} = (\text{TRV-DRV}) * 0.95$$

TSN was determined after the digestion of 1 cm^3 of borate buffer extract using 50% sulphuric acid followed by 2-3 drops of 35% perchloric acid for completion of oxidation until the whole mixture turned pale green in color. A known aliquot was neutralized and its ammonia content was determined by Borthelot reaction[17,18].

NO_3-N was estimated in the borate buffer extract according to the method of Paech and Tracey [19], while NH_2-N was determined in the same extract according to the procedure described by Russel [20].

Polysaccharides were determined after transferring a definite weight of the plant residue into 10 cm^3 of NH_4Cl, and hydrolysis was accomplished by heating the mixture in a boiling water-bath for at least 2 hours. The extract was neutralized and the reducing power was estimated [16,21]. Protein-N was estimated after dissolving a known weight of dry residue in a definite volume of 4% NaOH and kept standing for 24 hours at 25°C before centrifugation. Then, the procedure of Lowry et al. [22] followed using folin-phenol reagent.

Proline analysis: Proline analysis was followed daily during the germination period (5 days) in the whole seedlings. The method descried by Bates et al. [23] was adopted and the proline concentration was calculated on fresh weight bases.

The experiments were designed as factorial experiments with complete randomized design, and analysis of variance was made for all the data using Windows/ Excel version (3.0) IBM computer program.

RESULTS

Germination percentage:

It was evident that decreasing the osmotic potential of the seed media caused a delay in the initiation of germination, and the effect of water stress was highly significant in reducing the germination percentage (Table 1). The reduction in the germination percentage was linear with the decrease in the osmotic potential of the germination media. However, such reduction was more pronounced in seeds exposed to osmotic potential of -0.8 MPa, since the germination percentage was reduced to about 6% of the control. GA_3, on the other hand, did not improve the germination of fenugreek seeds under different conditions of osmotic stress.

Changes in carbohydrate fractions:

There was an obvious increase in the soluble sugars (TRV) with decreasing osmotic potential of the seed media (Fig.1). This accumulation came mainly from sucrose since the effect of water stress was highly significant ($P<0.001$) in promoting its accumulation. DRV, on the other hand, was reduced progressively with the decreasing osmotic potential of mannitol solutions giving lowest value at -0.8 MPa. It should be noted that the accumulation of sucrose at -0.8 MPa osmotic potential was less than that of -0.3 and -0.5 MPa. Polysaccharides showed no consistent pattern of changes with stress during germination. It seemed that the degradation of polysaccharides was more pronounced in seedlings exposed to -0.3 and -0.5 MPa as compared to other osmotic potentials. The lowest osmotic potential (-0.8 MPa) might have had little effect on the polysaccharide degradation.

Presoaking seeds in GA_3 solution, on the other hand, caused considerable reduction in DRV and a significant accumulation ($P<0.01$) of sucrose in the growing seedlings under different levels of osmotic potential as compared to the water treated seeds. Thus, it seems that sucrose made a major contribution to TRV due to GA_3 treatment. Moreover, GA_3 might have induced the degradation of polysaccharides in the germinating seeds which could further contribute to the remarkable accumulation of soluble sugars.

Examination of the results of the total carbohydrate contents revealed that their accumulation was progressive as the external osmotic potential decreased. Such accumulation was accompanied by a sharp reduction in DRV. Thus, the substantial formation of sucrose might have had a major contribution in the considerable accumulation of the total carbohydrates.

Changes in nitrogen fractions:

The total-N showed no consistent pattern of changes with reducing the osmotic potentials of the seed media, since it increased markedly in -0.1 and -0.3 MPa osmotic potentials, then dropped sharply at -0.5 and -0.8 MPa. This pattern of changes was almost similar to that found in TSN (Fig.2) and its fractions (Table 2). The high concentrations of NH_4-N and other soluble nitrogen found in the germinating seeds might have contributed greatly to the total soluble nitrogen (TSN). Moreover, protein-N showed slight changes with water stress; however, at low osmotic potentials (-0.5 and -0.8 MPa), its content was reduced slightly. GA_3 treatment, on the other hand, had a highly significant ($P<0.001$) effect on the total nitrogen and its fractions (Fig.2). It seems that hormone application increased the content of protein-N and TSN, as well as some of its fractions (NH_4-N and NH_2-N).

Proline started to accumulate in the germinating seeds from the first day of the experiment, reaching its maximum concentration on the fourth day. However, the data of Table 3 showed proline concentration of the fifth day. Also, water stress seemed to accelerate proline accumulation while GA_3 treatment had no consistent effect during the period of germination .

DISCUSSION

This investigation confirmed reports that water stress caused considerable reduction in the seed germination of many crops[24], and GA_3 treatment did not promote the germination more than the water did in presoaked seeds at different osmotic potentials [11,12].

The outcome of the study was that the decreasing of the osmotic potentials of seed media altered the total amounts of carbohydrates and nitrogen as well as their fractions. The great increase in the total carbohydrate was mostly accompanied by a reduction in monosaccharides (DRV) and marked accumulation of sucrose, especially at low osmotic potentials. The possible reason for sucrose accumulation may have come from converting the carbon skeletons of the nitrogenous compounds to carbohydrate ones [25] as well as from the considerable degradation of polysaccharides especially at -0.1 to -0.5 MPa osmotic potentials. The increase in the soluble sugars could help water-stressed plants to cope with the water deficiency by their contribution to the process of osmotic adjustment [26]. The reduction of the monosaccharides in stressed plants, on the other hand, could be attributed to the utilization of these sugars as respiratory substrates [27]. It is interesting to observe that polysaccharide value remained higher at -0.8 MPa, which clearly indicates that the degradation of these compounds was lower than it was at all other levels of osmotic potential. It is very likely that the great inhibition of the activity of hydrolytic enzymes under this level of water stress could be a reason for such a result [6, 28].

The data also confirmed that TSN and its fractions showed a substantial reduction with water stress, thereby reducing the total nitrogen in the germinating seeds. In support of this conclusion, the protein content of seedlings was almost maintained at the same level at most osmotic potentials [29]. Thus, the nitrogen lost at low osmotic potentials (-0.5 and -0.8 MPa) can be explained by (1) converting some of the carbon skeletons of soluble nitrogen compounds to organic acids by a series of biochemical reactions leading to sucrose [25], (2) the alteration of both structure [30] and functions [31] of plasma membranes changing the physical and chemical properties [32] thereby causing a leakage of some solutes to the seed media under these conditions [33, 34]. Moreover, the reduction in the fractions of soluble nitrogen at low osmotic potentials can be explained by the inhibition of proteolytic activity which might lead to a limited supply of nitrogenous compounds from the stored protein material in the embryo and endosperm [35]. The large variations in the protein levels due to water stress that have been noticed in various studies were dependent upon the experimental materials and conditions [36].

The data also demonstrated that proline was accumulated considerably in the growing fenugreek seedlings due to water stress. Proline may play several physiological and biochemical roles such as osmoregulation, turgor maintenance and hydration, protection of the activity of some enzymes, energy source and conservation of some amino groups and sink for soluble nitrogen [4].

Presoaking fenugreek seeds in GA_3 solution caused remarkable changes in all fractions of carbohydrate and nitrogen of the seedlings under all levels of osmotic potentials. These changes included the reduction in DRV

which can be explained as monosaccharides that were further metabolized by accelerating the respiration process [37], giving lower values as compared to the water treatment. In contrast, sucrose formation seemed to be accelerated by GA_3 treatment [38] by stimulating the starch degradation and diverting the intermediate compounds such as triose phosphate away starch storage to build up sucrose [25]. The accumulation of soluble sugars due to GA_3 treatment can be explained either by further degradation of polysaccharides or by stimulating the activity of the enzyme sucrose-phosphate synthase. Thus, further investigation is needed to elucidate the effect of GA_3 on a-amylase and invertase [39] as well as sucrose-phosphate synthase in the germinating seeds of fenugreek plant.

REFERENCES

[1] Yasseen, B.T. 1983. An analysis of the effects of salinity on leaf growth in Mexican wheats Ph.D Thesis. The University of Leeds. UK.

[2] Yasseen, B.T. and Al-Omary, S.S. 1994. An analysis of the effects of water stress on leaf growth and yield of three barley cultivars. Irrig. Sci. 14:157-162.

[3] Timpa, J.D., Burke, J.J., Quisenberry, J.E. and Wendt, C.W. 1986. Effects of water stress on the organic acid and carbohydrate composition of cotton plants. Plant Physiol. 82:724-728.

[4] Paleg, L. G. and Aspinall, D. 1981. The physiology and biochemistry of drought resistance in plants. Academic Press. New York.

[5] Gorham, J., Wyn Jones, R.G. and McDonnell, E.1985. Some mechanisms of salt tolerance in crop plants. Plant & Soil. 89:15-40.

[6] Yasseen, B.T. 1992. Physiology of water stress in plants. The University of Mosul. University Press. Iraq.

[7] Yasseen, B.T. 1977. Effects of some salts and growth regulators on germination and vegetative growth of safflower (*Carthamus tinctorius* L.) M Sc. Thesis. Baghdad University, Iraq.

[8] Khan, A., Goss, J.A. and Smith, D.E. 1957. Effect of gibberellin on germination of lettuce seeds. Science. 125:645-646.

[9] Banyal, S. and Rai, V.K. 1983. Reversal of osmotic stress effects by gibberellic acid in *Brassica campestris*. Recovery of hypocotyl growth, protein and RNA levels in the presence of GA. Physiol. Plant. 59: 111-114.

[10] Spyropoulos, C.G. 1986. Osmoregulation, growth and sucrose accumulation in germinated *Trigonella foenum-graecum* (fenugreek) seeds treated with polyethylene glycol. Physiol.Plant. 68:129-135.

[11] Darra, B.L., Seth, S.P., Singh, H. and Mendiratta, R.S. 1973. Effect of hormone-directed presoaking on emergence and growth of osmotically stressed wheat (*Triticum aestivum* L.) seeds. Agron.J. 65:292-295.

[12] Heikal, M.M., Shaddad, M.A. and Ahmed, A.H. 1982. Effect of water stress and gibberellic acid on germination of flax, sesame and onion seeds. Biologia Plant.(PRAHA), 24:124-129.

[13] Duke, J.A. and Ayensu, E.S. 1985. Medicinal plants of China. Vol. I Reference publication Inc. Press. Algonac, Michigan. pp 345 .

[14] Dadoura, S.S. 1990. Physiological studies on seeds treated with a growth regulator grown at different levels of osmotic potential. M.Sc. Thesis. Cairo Univ. Egypt.

[15] Naguib, M.I. 1964. Effect of serine on the carbohydrate and nitrogen metabolism during the germination of cotton seeds. Ind. J. Exp. Biol. 2: 149-152.

[16] Nelson, N. 1944. A photometric adaptation of Somogi method for the determination of glucose. J. Biol. Chem. 153:375-380.

[17] Fawcett, J.K. and Scott, J.E. 1960. A rapid and precise method for the determination of urea. J. Clin. Pathol. 13:156-159.

[18] Chaney, A.L. and Marbach, E.P. 1962. Modified reagents for determination of urea and ammonia. Clin. Chem. 8:130-132.

[19] Paech, K. and Tracey, M.V. 1956. Modern methods of plant analysis. Springer-Verlag, Berlin.

[20] Russel, J.A. 1944. Colourimetric detection of amino-nitrogen. J. Biol. Chem. 56:467-468.

[21] Naguib, M.I. 1963. Colourimetric estimation of plant polysaccharides. Zucker. 16:15-18.

[22] Lowry, O.H., Rosenbrough, J., Fan, A.C. and Randal, R.J. 1951. Protein measurement with folin-phenol reagent. J. Biol. Chem. 193:265-275.

[23] Bates, L.S., Wadleigh, R.P. and Teare, I.D. 1973. Rapid determination of free proline for water stress studies. Plant and Soil. 39:205-207.

[24] Yasseen, B.T. and Al-Maamari, B.K.S. 1995. Further evaluation of the resistance of Black barley to water stress: Preliminary assessment for selecting drought resistant barley. J.Agron.& Crop Science.174:9-19.

[25] Taiz, L. and Zeiger, E. 1991. Plant Physiology. The Benjamin Cummings Publicity Company, Inc. Redwood City, California, USA.

[26] Munns, R. and Weir, R. 1981. Contribution of sugars to osmotic adjustment in elongation and expanded zones of wheat leaves during moderate water deficits of two light levels. Aust. J. Plant Physiol. 8:15-18.

[27] Stocker, O. 1960. Physiological and morphological changes in plants due to water deficiency. In: Plant-Water Conditions. UNESCO. 15:63-104.

[28] Levitt, J. 1980. Responses of plants to environmental stresses. Vol.II. Academic Press, New York.

[29] Todd, G.W. and Basler, E. 1965. Fate of various protoplasmic constituents in droughted wheat plants. Phyton. 22:79-85.

[30] Pearce, R.S. and Beckett, A.1987. Cell shape in leaves of drought stressed barley examined by low temperature scanning electron microscopy. Ann. Bot. 59:191-195.

[31] Hubac, C., Guerrier, D., Ferran, J. and Tremolieres, A. 1989. Changes of leaf lipid composition during water stress in two genotypes of *Lupinus albus* resistant or susceptible to drought. Plant Physiol. Biochem. 27:737-744.

[32] Navari-Izzo, F., Quartacci, M.F., Melfi, D. and Izzo, R. 1993. Lipid composition of plasma membranes isolated from sunflower seedlings grown under water stress. Physiol. Plant. 87:508-514.

[33] Wilkins, M.B 1984. Advanced plant physiology. Pitman Publishing Limited. London.

[34] Jurjees, J.A., Al-Maamari, B.K.S. and Yasseen, B.T. 1996. Cell membrane stability and ion leakage in leaves of barley(*Hordeum* spp.) in response to water stress. Mesopotamia J. Agric. 28:7-10.

[35] Sheoran, I.S. and Garg, O.P. 1978. Effect of salinity on the activities of RNase, DNase and protease during germination and early seedling growth of mung bean. Physiol.Plant. 44: 171-174.

[36] Singh, G. and Rai, V.K. 1982. Responses of two differentially sensitive *Cicer arietinum* L. cultivars to water stress:protein content and drought resistance. Biol. Plant. (PRAHA). 24:7-12.

[37] Romanowvska, E., Parys, E. and Poskuta, J. 1984. The effect of light quality and gibberellic acid (GA_3) on photosynthesis and respiration rates of pea seedlings. Photosynthesis Research. 5:205-214.

[38] Miyamoto, K. and Kamisaka, S. 1988. Growth and osmoregulation in *Pisum sativum* subhooks as affected by gibberellic acid and cotyledon excision. Physiol.Plant. 74:669-674.

[39] Kaufman, P.B., Ghosheh, N.S., Lacroix, J.D., Soni, S.L.and Ikuma, H. 1973. Regulation of invertase levels in *Avena* stem segments by gibberellic acid, sucrose, glucose and fructose. Plant Physiol. 52:221-228.

Table1. The germination percentage of fenugreek seeds presoaked in water or GA_3 and exposed to different osmotic potentials of mannitol for five days.

Osmotic Potential (MPa)	H_2O	GA_3	Mean
Control (0)	100.0	100.0	100.0
- 0.1	93.3	93.3	93.3
- 0.3	66.7	70.0	68.4
- 0.5	46.7	43.3	45.0
- 0.8	6.7	6.7	6.7
Mean	62.7	62.7	

GA_3 : n. s.
Stress : $P < 0.001$
Interaction : n. s.

Table 2. Soluble nitrogen fractions (mg g^{-1} DW) of fenugreek seeds after five days exposure to different osmotic potentials of mannitol (seeds were presoaked in water or GA_3 solution).

Osmotic Potential (MPa)	NO_3-N			NH_2-N			NH_4-N			Other soluble-N		
	H_2O	GA_3	Mean	H_2O	GA_3	Mean	H_2O	GA_3	Mean	H_2O	GA_3	Mean
Control (0)	0.66	0.60	0.63	2.03	2.30	2.17	3.26	5.55	4.41	3.34	7.10	5.22
- 0.1	0.66	0.53	0.60	2.06	2.42	2.24	5.88	6.03	5.96	11.92	5.59	8.76
- 0.3	0.53	0.45	0.49	1.96	2.25	2.11	5.62	5.70	5.66	6.48	8.01	7.25
- 0.5	0.49	0.46	0.48	1.75	1.65	1.70	1.50	2.79	2.15	2.85	4.40	3.63
- 0.8	0.36	0.44	0.40	0.42	0.49	0.46	0.25	0.44	0.35	1.93	1.35	1.64
Mean	0.54	0.50		1.64	1.82		3.30	4.10		5.30	5.29	
GA_3	: n. s.			: $P < 0.001$			: $P < 0.001$			: n. s.		
Stress	: $P < 0.001$			: $P < 0.001$			: $P < 0.001$			: $P < 0.001$		
Interaction	: n. s.			: $P < 0.01$			: $P < 0.001$			: $P < 0.001$		

Table 3. Proline concentration (micromole g^{-1} FW) of the germinating seeds of fenugreek under different osmotic potentials (seeds were presoaked in water or GA_3 solution).

Osmotic Potential (MPa)	H_2O	GA_3	Mean
Control (0)	0.92	1.20	1.06
- 0.1	1.13	1.32	1.23
- 0.3	1.61	1.57	1.59
- 0.5	3.13	2.31	2.72
- 0.8	0.70	0.74	0.72
Mean	1.52	1.43	
GA_3	: $P < 0.01$		
Stress	: $P < 0.001$		
Interaction	: $P < 0.001$		

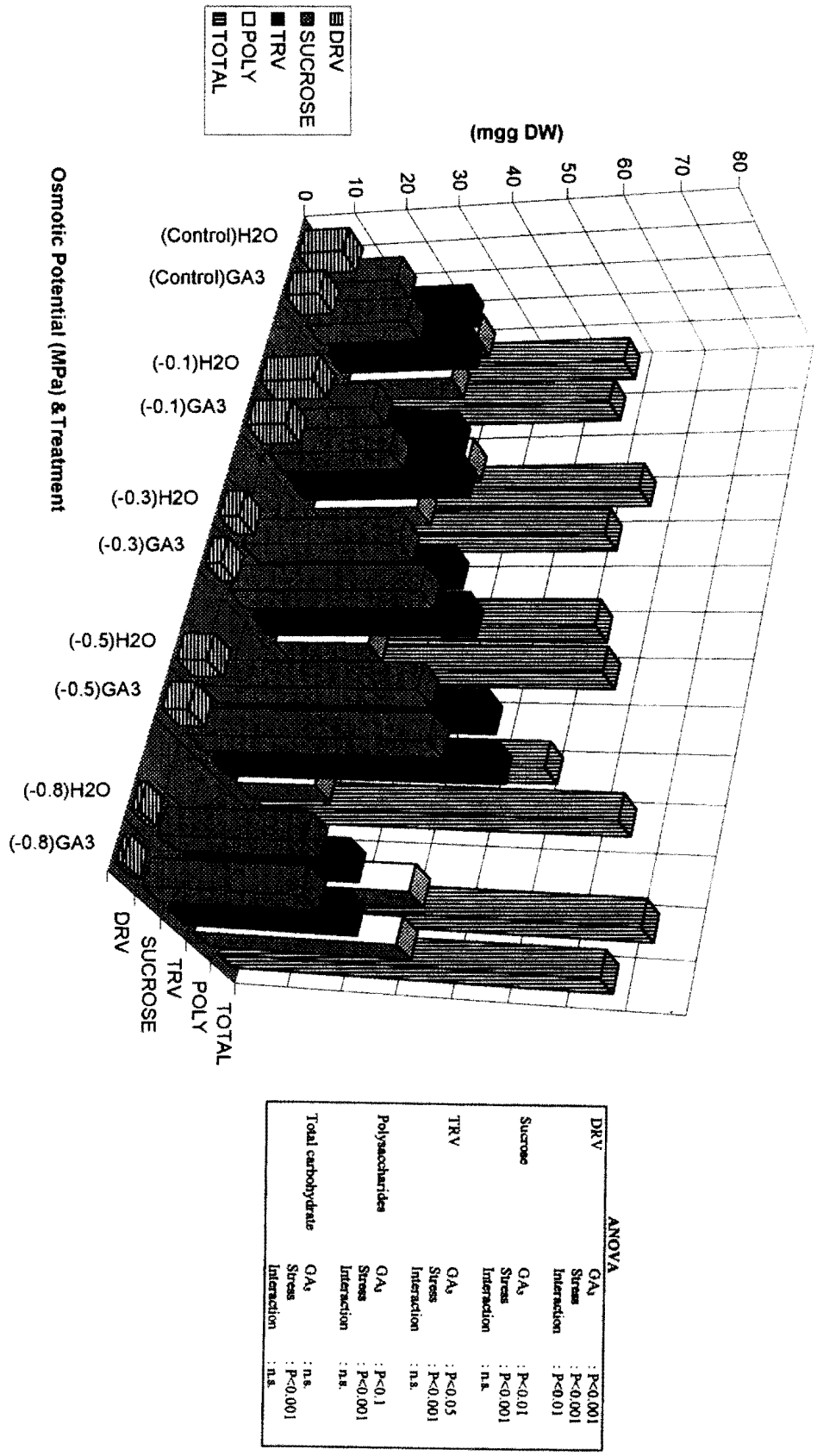

Fig. (1): Carbohydrate fractions of fenugreek germinating seeds after five days exposure to different osmotic potentials of mannitol. (seeds were presoaked in water or GA_3 solution).

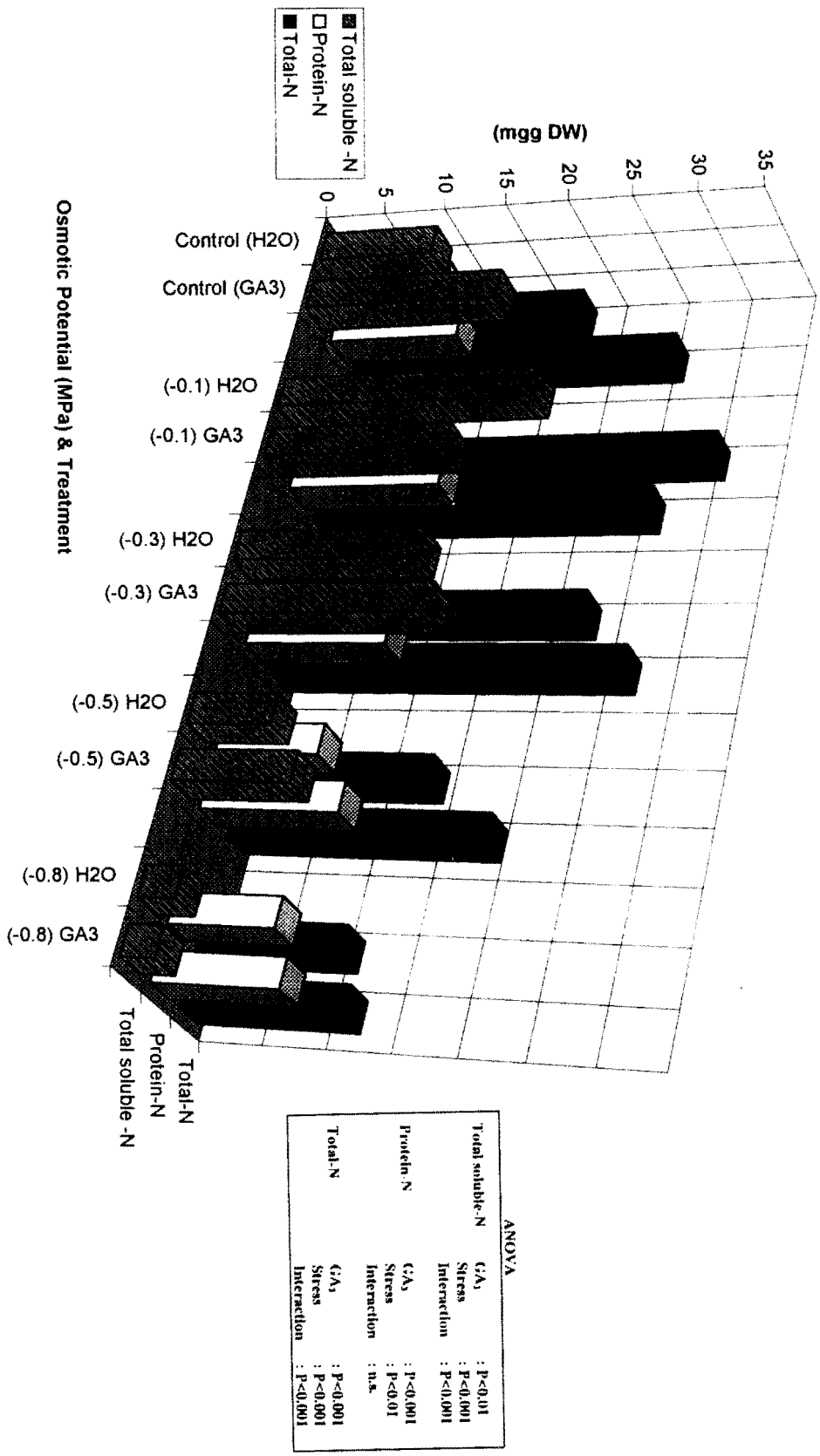

Fig. (2): Nitrogen fractions of fenugreek germinating seeds after five days exposure to different osmotic potentials of mannitol. (seeds were presoaked in water or GA_3 solution.)