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Biochemical Screening of Some Groundnut (*Arachis hypogaea* L.) Genotypes for Drought Tolerance

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Abstract: A pot experiment was conducted for screening of ten drought tolerant groundnut (*Arachis hypogaea* L.) genotypes. Ten genotypes were collected from the International Crop Research Institute for Semi Arid Tropics (ICRISAT), India. Plants were grown in Hoagland solution and by adding 4% polyethylene glycol-6000, water stress was imposed. As index of drought tolerance, change in free proline content and stability of nitrate reductase activity in the leaves of water stressed plants was monitored. In the drought tolerant plant, the increase in proline over the control varied from 5.44 to 7.16 fold and the decrease in nitrate reductase activity ranged from 4.97 - 6.69%. On the basis of increase in proline accumulation and degree of stability of nitrate reductase, 3 genotypes were selected as drought tolerant and they were 3203, ICGV-93269 and ICGV-93232.

Key words: Biochemical screening, groundnut, drought

Introduction

Many oil seed crops are being grown in Bangladesh from time immemorial. But the acute shortage of edible oil in the country is increasing every year with increasing population growth. Local production of oil seed was 0.478 million ton in 1996-97 and in the same year, Bangladesh imported 0.516 million ton of edible oil seed at the cost of huge foreign exchange (BBS, 1998).

Groundnut (*Arachis hypogaea* L.) is one of the principal oil seed crop of the world. Groundnut stands first in terms of yield per hectare and fourth in respect of area of production among the oil crops in Bangladesh. It covers about 6.2% of total area and 8.28% of the total production under oil seed crops. The average yield of oil seeds (rape + mustard) is 0.74 t/ha, whereas, the average yield of groundnut is 1.15 t/ha (BBS, 1998).

Unavailability of irrigation is the main constraints of groundnut cultivation. In Bangladesh, groundnut is mainly cultivated in rabi season (Nov-March) when rainfall is minimum. For its cultivation the most suitable soil is sandy to sandy loam in the river banks, beels and haors. The water holding capacity of these types of soil is very poor. That is why the crop generally suffers severe water stress mainly during the reproductive phase resulting in very low productivity of the crop. Introducing drought resistant varieties of groundnut in soils where moisture stress is crucial may be a new device to increase yield.

The accumulation of proline under water stress condition have involvement in the adaptive mechanism (Aspinall and Paleg, 1981). Water stress increased proline content of groundnut leaf (Patil and Patil, 1993; Amaregouda *et al.*, 1994; Ruilian and Gang, 1997; Purushotham *et al.*, 1998). Tyagi *et al.*, (1999) reported that water stress increased proline accumulation 65 fold in lathyrus. Plants producing higher amount of proline in water stress condition may be considered as better drought tolerant. The variation in the accumulation of poline during drought stress could be used for screening the varieties for drought tolerant characteristics (Yoshiba, 1995).

Nitrate reductase activity is considered for screening drought tolerant plants. In the drought susceptible variety, nitrate reductase activity is reduced under water stressed condition (Sharma *et al.*, 1990; Venkateswarlu and Ramesh, 1993). With the above background, a study was undertaken to select some drought tolerant groundnut genotypes based on biochemical assessment.

Materials and Methods

For screening of drought tolerant groundnut genotypes a pot experiment was carried out in the laboratory of the Department of Biochemistry of the Bangladesh Agricultural University (BAU), Mymensingh, during November, 2000 to February, 2001. The plants were grown hydroponically using Hoagland nutrient solution. The concentration of different ingredients of Hoagland solution are mentioned below:

Ingredients	Concentration	Ingredients	Concentration
KNO_3	5 mM	H_3BO_3	0.05 mM
$Ca(NO_3)_2$	5 mM	$MnSO_4.H_2O$	9.00 μM
$MgSO_4.7H_2O$	2 mM	$ZnSO_4.7H_2O$	0.77 μM
KH_2PO_4	1 mM	$CuSO_4.5H_2O$	0.30 μM
Fe-EDTA	0.05 mM	$Na_2MoO_4.2H_2O$	0.10 μM

In this experiment nine genotypes were collected from the International Crop Research Institute for the Semi Arid Tropics (ICRISAT), India and one (3203) from the Department of Genetics and Plant Breeding, BAU. The genotypes collected from ICRISAT were ICGV-93261, ICGV-93269, ICGV-86635, ICGV-92121, ICGV-92109, ICGV-93232, ICGV-93233, ICGV-92118 and ICGV-93255.

Plants were grown in cylindrical shape plastic pots of 16 cm high and 9.5 cm diameter. The cap of each pot contained two circular opening of 1 cm each in diameter. Seven days old seedlings were planted in Hoagland solution. One seedling was planted in one hole of cap of the plastic pot and the other hole was left open for adding nutrient solution. Seedlings were fixed properly in the hole with the help of a small piece of foam. One liter Hoagland solution was taken in each pot. This volume was maintained by adding required amount of Hoagland solution everyday. The pots were placed under sunlight.

When the plants were four weeks old, water stress was imposed. The water stress condition was developed by adding 4% polyethylene glycol-6000 (PEG-6000) with the Hoagland solution. The control plants were grown all along in Hoagland solution. The experiment was laid out in factorial combinations of groundnut genotypes and water stress level following completely randomized design (CRD) with three replications. Seven and fifteen days after imposing the water stress, proline content in the leaves of the individual plants was determined. Generally, 2nd or 3rd leaf descending from the apical bud was collected and immediately subjected to analysis. Each analysis was done in duplicate. Proline content of leaves was determined according to Bates *et al.* (1973). Nitrate reductase (NR) activity of leaves was determined after 20 days of stress imposition. Nitrate reductase (*in vivo*) assays were done according to Stewart and Orebamjo (1979). Data were analysed statistically using ANOVA and DMRT.

Results and Discussion

A general increase in proline accumulation was observed in the stressed plants in relation to those under control, but the magnitude of variation in increments was very wide (Table 1). Imposition of stress for 7 days caused a remarkable high proline content in ICGV-93269 (18.18 mg/100 g leaf), ICGV-93232 (17.43 mg/100 g leaf) and 3203 (18.07 mg/100 g leaf). These increments were 6.09, 6.05 and 7.16 fold higher over the respective control (Fig. 1). The difference of proline accumulation between stress and control plants was also highly significant. After 15 days of stress, the values for prolne content of the same genotypes appeared to be 18.58 mg/100 g leaf, 16.44 mg/100 g leaf and 17.62 mg/100g leaf and those were 6.07, 5.44 and 6.37 fold higher over the respective control (Fig. 1).

There was a decreasing trend in the nitrate reductase activity (Table 2). In the controls, the variation was observed from 0.723 to 2.086 μ mole NO_2^-/g fresh wt/hr having an average value of 1.519 μ mole NO_2^-/g fresh wt/hr. In stressed plants the variation of activity was from 0.412 to 1.976 μ mole NO_2^-/g fresh wt./hr and the average value was 1.240 μ mole NO_2^-/g fresh wt./hr.

The percentage of decreases in NR activity over the control is shown in Fig. 2. In some plants the percentage of decrease in activity was minimum and in others maximum reduction in activity was observed (Fig. 2). Minimum decrease of NR activity (as expressed in percentage) was recorded in the genotype ICGV-92121 (4.50%) followed by ICGV-93269 (4.97%) and ICGV-93233 (5.27%). However, the difference between control and stress plant of the genotypes ICGV 93269, ICGV 92121, ICGV 93232, ICGV 93233, ICGV 92118 and 3203 were insignificant. Although the genotypes ICGV-93269, ICGV-93232 and 3203 were relatively better in accumulating proline in stress condition, the performance of these genotypes may not be considered absolutely superior in respect to NR activity. Nevertheless, the values for percentage of decrease of NR activity were acceptable to consider those genotypes as drought tolerant. The percent of decrease in NR activity of genotypes ICGV-93269, ICGV-93232 and 3203 were 4.97, 6.40 and 6.69% respectively. Some of the genotypes appeared to lose their NR activity to a greater extent and the values were 44.87% (ICGV-9209), 43.02% (ICGV-93261) and 35.69% (ICGV-93255).

It is widely known that water stress induces numerous metabolic alterations in plants. Free proline accumulation is one of the most dramatic stress characteristics. Role of proline has been controversial since the time it was shown as an index of drought resistance. However, a number of reports are in support of this hypothesis. Mehkri *et al.* (1977) Patil and Patil (1993), Koti *et al.* (1994) and Nogueira *et al.* (1998) showed that drought tolerant groundnut genotypes had positive correlation with proline accumulation in the leaves under water stress condition.

In the present investigation, a wide variation in the increase of proline contents in the leaves of different groundnut genotypes under stress was found. The range of variations in proline accumulation was from 5.48 to 18.18 mg/100g (7 days stress) and 6.39 to 18.58 mg/100g (15 days stress) (Table 1). Mehkri *et al.* (1977) suggested that increase in proline content could be used as an indicator at water stress for the selection of groundnut genotypes tolerant to drought. Increase in proline under stress condition could be used as a biochemical tool to select drought tolerant genotype (Patil and Patil, 1993). On the basis of higher proline accumulation and in consideration of manifold increase of the same over the control, the genotypes 3203, ICGV-93269 and ICGV-93232 might be considered as drought tolerant.

Water stress has a great impact on the activity of different enzymes. Fukutoku (1996) reported that the nitrate reductase activity was reduced almost linearly when the osmotic potential was reduced by adding PEG-6000 into nutrient solution. Stability of NR-activity under water stress condition acts as an other index for drought tolerance (Pearson *et al.*, 1987). Saini and Srivastava (1991) studied the effect of water stress on the seedling of groundnut by exposing them in the solution of PEG-6000. They observed that plants retaining the

Table 1: Effect of water stress on free proline accumulation (mg/100g fresh leaf) in leaves of different groundnut genotypes

Genotypes	After 7 days stress			After 15 days stress		
	Control	Stress	Difference	Control	Stress	Difference
ICGV-93261	3.85a-c	9.96b	6.11**	4.13bc	11.77c	7.64**
ICGV-93269	2.98bc	18.18a	15.20**	3.06c	18.58a	15.52**
ICGV-86635	4.90ab	9.46b	4.56**	6.04a	9.71d	3.67**
ICGV-92121	4.90ab	5.48d	0.58NS	5.23ab	6.39f	1.16NS
ICGV-92109	5.14a	6.23d	0.19NS	4.47a-c	6.48f	2.01*
ICGV-93232	2.88bc	17.43a	14.55**	3.02c	16.44b	13.41**
ICGV-93233	3.03bc	8.60bc	5.57**	4.08bc	8.78de	4.70**
ICGV-92118	5.81a	7.18cd	1.37NS	6.05a	9.02d	2.97**
ICGV-93255	4.39a-c	6.32d	1.93*	3.73bc	7.22ef	3.49**
3203	2.52c	18.07a	15.55**	2.76c	17.62ab	14.86**
Mean	4.04	10.60	6.56**	4.25	11.20	6.95**

** = Significant at 1% level, * = Significant at 5% level and NS = not significant

In a column, means followed by a comon letter are not significantly different at the 5% level by DMRT.

Table 2: Effect of water stress on the activity of nitrate reductase (μ mole NO_2^-/g fresh wt/hr) in the leaves of groundnut genotypes

Genotypes	Control	Stress	Difference
ICGV-93261	0.723e	0.412f	0.311**
ICGV-93269	0.744e	0.707e	0.037 NS
ICGV-86635	1.625cd	1.118cd	0.507 **
ICGV-92121	1.915ab	1.829a	0.086 NS
ICGV-92109	1.870b	1.031d	0.839 **
ICGV-93232	1.735bc	1.624b	0.111 NS
ICGV-93233	2.086a	1.976a	0.110 NS
ICGV-92118	1.625cd	1.532b	0.093 NS
ICGV-93255	1.429d	0.919d	0.510 **
3203	1.449d	1.352c	0.097NS
Mean	1.519	1.240	0.279 **

** = Significant at 1% level, * = Significant at 5% level and NS = not significant. In a column, means followed by a common letter are not significantly different at the 5% level by DMRT.

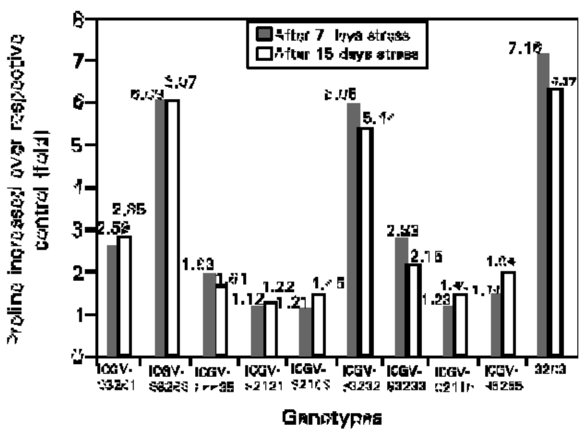

Fig. 1: Increase in free proline accumulation in leaves due to water stress as expressed in fold over the respective control

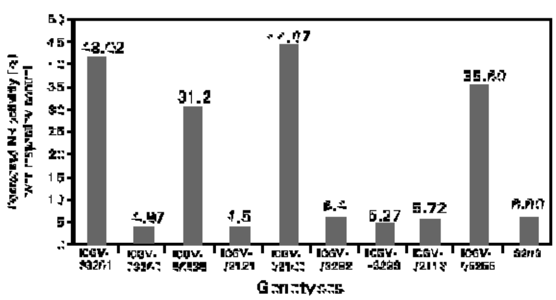

Fig. 2: Decrease in NR activity due to water stress as expressed in percentage compared to respective control

ability to preserve the activity of nitrate reductase in water stress condition could withstand and recover from stress better. In the present study, the groundnut genotypes considered as drought tolerant (ICGV-93269, ICGV-93232 and 3203) were also found to retain the nitrate reductase activity in water stress condition. Other genotypes showed higher percentage of loss of NR activity (Fig. 2).

The genotypes ICGV-93269, ICGV-93232 and 3203 were considered as drought tolerant (on the basis of high proline content in stress condition) showed the decrease in NR activity by 4.97, 6.40 and 6.69% respectively due to stress. Thus performance appeared to be satisfactory for accepting them as drought tolerant. The genotypes ICGV-93255, ICGV-92109 and ICGV-93261 were susceptible to drought as they had lost the NR activity at the rate of 35.69, 44.87 and 43.02% respectively (Fig. 2). However, all the drought-tolerant genotypes selected through biochemical analysis need to be grown in the field for final selection.

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