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EFFECT OF DROUGHT STRESS ON BIO-CHEMICAL CHANGE AND CELL MEMBRANE STABILITY OF SOYBEAN GENOTYPES

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Abstract

An experiment was conducted in a venylhouse at the environmental stress site of Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur during September to December 2012 to study the effect of drought stress on proline content, soluble sugar content, chlorophyll content and cell membrane stability of soybean genotypes. Four studied genotypes *viz*., Shohag, BARI Soybean-6 and BD2331 (relatively stress tolerant) and BGM2026 (susceptible) were tested against two water regimes such as water stress and non-stress. Results indicated that due to drought stress there was an increase in proline content and soluble sugar content and decrease in chlorophyll a content, chlorophyll b content, total chlorophyll content, chlorophyll a/b ratio and cell membrane stability. Proline and soluble sugar showed more content in tolerant genotype than in susceptible ones. Chlorophyll reduction was most significant and cell membrane stability was found minimal in susceptible genotypes. From the result, genotype BGM2026 which recorded the lowest proline, soluble sugar content and highest chlorophyll reduction and cell membrane injury was considered as drought susceptible. The variety/genotype of soybean such as BARI Soybean-6, Shohag and BD2331 were more drought stress tolerant and better mechanisms of drought tolerance.

Introduction

Plant growth is accomplished through cell division, cell enlargement and differentiation involving genetical, physiological, ecological morphological and biochemical events and their complex interaction. The quality and quantity of plant growth depends on these events, which are affected by water deficit (Farooq *et al*., 2009). The biochemical changes in plant due to water stress led to acclimate to the situation followed by severe functional damage and the loss of plant parts (Chaves *et al*., 2002). Plants are known to have different mechanisms to adjust water stress conditions. Mechanism of drought tolerance, especially at low plant water status, involve processes at the cellular level, the most important being osmotic adjustment and protection of the membrane system (Mullet and Whitsitt, 1996). An important adjustment under drought stress is to maintain cell turgidity (Ku *et al*., 2013). To maintain cell turgidity under stress, osmotic adjustment is a common mechanism which involves active accumulation of

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solutes in cells (Ku *et al*., 2013) and of these solutes, proline is widely distributed in plants and it accumulates in larger amounts than other amino acids in drought-stressed plants (Ashraf, 2004). Proline accumulation is believed to play adaptive roles in plant stress tolerance (Verbruggen and Hermans, 2008). Soluble sugars are also considered to play an important role in osmotic adjustment in plants and are widely regarded as adaptive response to water stress conditions (Kameli and Loesel, 1993). Structural integrity of cellular membranes is also important for survival under severe dry periods, or in situations where random droughts occur (Martinez *et al*., 2004). It is generally accepted that the maintenance of integrity and stability of membranes under water stress is a major component of drought tolerance in plants (Bajji *et al*., 2002). The degree of cell membrane stability is considered to be one of the best physiological indicators of drought stress tolerance and can be used to screening drought- tolerant genotypes (Kocheva *et al*., 2004). One of the most important changes under stress is the decrease in the total chlorophyll content (Sarker *et al*., 1999). Ommen *et al*. (1999) reported that leaf chlorophyll content decreased as a result of drought stress. Plant water stress can affect the ability of the plant to produce chlorophylls, thus affecting leaf greenness (Sandoval-Villa *et al*., 2002). Hence, the present experiment was undertaken to analyze the drought induced change in bio-chemicales like proline, soluble sugar, chlorophyll content and cell membrane stability of some selected soybean genotypes.

Materials and Method

A pot experiment in a vinyl house was conducted at the Bangabandhu Sheikh Mujibur Rahman Agricultural University, Salna, Gazipur during September to December 2012. Three relatively water stress tolerant variety/line (Shohag, BARI Soybean-6 and BD2331) and one susceptible (BGM-2026) genotypes (selected from the previous experiment) were tested against two water regimes (water stress and non-stress) at vegetative and pod development stages. There were eight treatment combinations. The pot were arranged in a completely randomized design under factorial arrangement with four replications. Seeds of tolerant genotypes and susceptible genotypes were sown in plastic pots (24 cm internal diameter and 30 cm height). The soil of the pot was filled with mixture of soil and cow dung at a ratio of 4:1. Pot contained 12.0 kg of soil which was equivalent to 9 kg oven dry soil and holds about 28% moisture at field capacity (FC). Soil used in the pot was sandy loam. Fertilizer rates of 70 mg N, 35 mg P, 180 mg K and 20 mg S pot^{-1} in the form of urea, triple super phosphate, muriate of potash and gypsum was added and well mixed with the soil before pouring into the pots. Six seeds pot^{-1} were sown on 3 September, 2012. After seedling establishment two uniform and healthy plants pot^{-1} were allowed to grow. Two watering treatments of the plants viz. drought stress i.e. water stress (50% water of the FC) and non-stress i.e. control (80% water of FC) were applied at 21 days after emergence (DAE) and maintained throughout the growing season. Weeding and spraying were done as normal management practices for all the treatments.

To estimate proline accumulation, samples were collected from top third fully expanded young trifoliate leaves at pod development stage of soybean genotypes. The collected leaf samples were immediately kept in an ice-bag and brought to the laboratory. Proline was determined by Ninhydrin method (Troll and Lindsley, 1955). The soluble sugar content of soybean leaves was also estimated at pod development stage. Leaf samples were collected from different soybean plants of the respective treatments. Dried and grounded samples were used to estimate soluble sugar by following the method of Yoshida *et al*. (1976).

Chlorophylls were estimated at vegetative, flowering and pod development stages on fresh weight basis extracting the leaf samples with 80% acetone by using Double Beam Spectrophotometer (Model 200-20, Hitachi, Japan). Different chlorophylls were estimated using the following equations (Witham *et al*., 1986).

Chlorophyll a (mg/g tissue) = [12.7(D663) – 2.69(D645)] x V/(1000 x W)
Chlorophyll b (mg/g tissue) = [22.9(D645) – 4.68(D663)] x V/(1000 x W)
Total Chlorophyll (mg/g tissue) = [20.2(D645) + 8.02(D663] x V/(1000 x W)

Where, D = optical density reading of the chlorophyll extract at the specific wavelength
V = final volume of the 80% acetone-chlorophyll extract
W = fresh weight in gram of the tissue extracted

For cell membrane stability measurement leaf samples were collected from plants at pod development stage. Cell membrane stability of leaf tissues was calculated as the percentage injury using the following equation (Blum and Ebercon, 1981):

$$\text{Percent injury} = 1 - \left[\frac{\left(1 - \frac{T_1}{T_2}\right)}{\left(1 - \frac{C_1}{C_2}\right)} \right] \times 100$$

Where,

T_1= first conductivity measurement of desiccation treatment,
T_2= second conductivity measurement of desiccation treatment,
C_1= first conductivity measurement of control, and
C_2= second conductivity measurement of control.

The data were analyzed with MSTATC statistical package program. The difference between the treatments means were compared by Least Significant Difference (LSD) test (Gomez and Gomez, 1984). Functional relationships between proline content and injury index of soybean genotypes as affected by water stress were established through correlation and regression analyses by using Excel program.

Results and discussion

Proline accumulation

In water stress condition free proline increased markedly in all the genotypes under studied (Fig 1). Similar results was also observed by Ashraf and Iram (2005) in *Phaseolus vulgaris*. Accumulation of proline under stress in many plant species has been correlated with stress tolerance, and its concentration usually higher in stress tolerant than in stress sensitive plants (Silvente *et al*., 2012). Significant differences in proline content were observed among genotypes under water stress (Fig 1). The highest accumulation of proline due to water stress was observed in genotype BARI Soybean-6 (131.5%) followed by Shohag (124.8%) and the lowest accumulation in BGM2026 (54.3%). There were no significant differences among the genotypes under non-stress conditions. A similar finding was observed by Stoyanov (2005) who reported that tolerant cultivar showed the highest accumulation of proline in bean plants. Proline accumulation is a common physiological response to many plants in response to drought stress (Mafakheri *et al*., 2010). Irigoyen *et al*. (1992) reported a relationship between turgor and proline accumulation which could be of useful as a possible drought-injury sensor so selection of new drought tolerant genotypes based on high proline accumulation can be used as a parameter for selection of stress tolerance (Jaleel *et al*., 2007). Proline is one of the most water soluble amino acids and it is supposed to play a significant role in osmotic adjustment with regard to reduction of osmotic potential due to a net accumulation of solutes (Molaei *et al*., 2012). Vendruscolo *et al*. (2007) found that proline is involved in tolerance mechanisms against oxidative stress and this was the main strategy of plant to avoid detrimental effects of water stress.

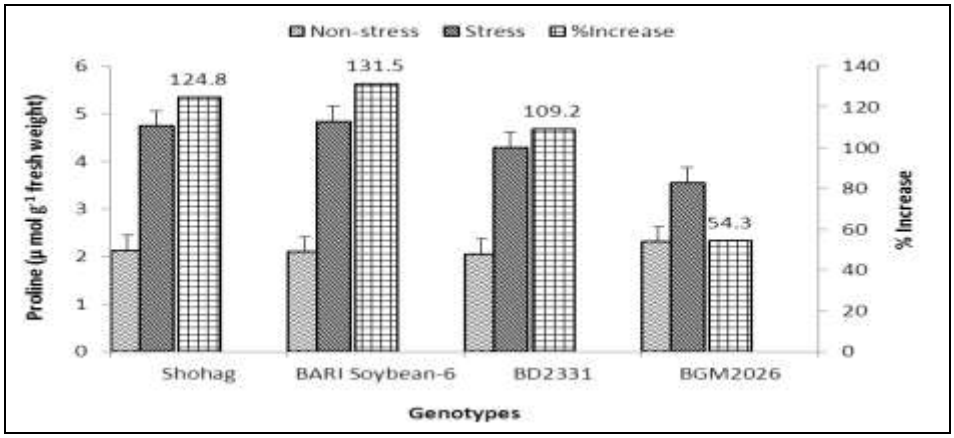

Fig. 1. Proline accumulation in soybean leaf at pod development stage under non-stress and water stress conditions. (Vertical bar represent LSD value at 5% level of significance).

Soluble sugar accumulation

Soluble carbohydrates have a role in osmotic regulations and conservation mechanism (Martin *et al*., 1993). Total soluble sugar contents in all the genotypes were significantly higher under water stress condition than that under non-stress (Table 1). Sarker *et al*. (1999) also obtained similar results. The highest soluble sugar accumulation was observed in BARI Soybean-6 followed by Shohag, and the lowest in BGM2026 under water stress condition. The total soluble sugar increased due to water stress were 21.8, 25.2, 13.9 and 8.4 mg g^{-1} dry matter in Shohag, BARI Soybean-6, BD2331 and BGM2026, respectively which was 44, 47, 28 and 17% higher respectively over non-stress. Kundu and Paul (1997) reported that total soluble sugar content was significantly higher at 46 and 67% in the non-irrigated plants of *Brassica campestris* at flowering and pod-filling stages, respectively. As an osmotic agent, the increased sugar, induced by water stress, was significantly correlated to osmotic adjustment and turgor maintenance (Sa'nchez *et al*., 1998). Another possible function of soluble sugar accumulation under water stress (unrelated to their osmotic contribution) is to form reserve assimilates for seed filling (Sa'nchez *et al*., 1998).

Table 1. Soluble sugar accumulation in soybean genotypes under non-stress and water stress conditions at pod development stage

Genotypes		Soluble sugar accumulation (mg g^{-1} dry matter)		Percentage increase in soluble sugar over non-stress
		Non-stress	Water stress	
Shohag		49.3	71.1	44
BARI Soybean-6		53.4	78.6	47
BD2331		48.8	62.7	28
BGM2026		50.2	58.6	17
$LSD_{(0.05)}$	S	3.41		
	G	8.09		
	SxG	11.45		
CV(%)		11.07		

S=Stress, G=Genotype

Leaf chlorophylls

Water stress significantly affected biosynthesis of leaf chlorophylls as well as chlorophyll a/b ratio in all the genotypes at different growth stages (Tables 2, 3, and 4). Total chlorophyll was significantly decreased by water stress at three growth stages. The chlorophyll a/b ratio was not changed at the vegetative stage though significantly decreased at flowering and pod development stages due to

water stress (Tables 2, 3, and 4). Total chlorophyll content of all the genotypes was higher under non-stress environment at the three stages studied, while water stress caused a reduction in total chlorophyll contents and chlorophyll a/b ratio. Similar results were reported by Makbul *et al*. (2011) in soybean under drought stress conditions. A reduction of chlorophyll formation due to water stress was also reported by Sarker *et al.* (1999). Chlorophyll a, chlorophyll b and a/b ratio were higher in BARI Soybean-6 at all three growth stages under stress and non-stress conditions. Lowest Chlorophyll a and b content and a/b ratio was obtained in BGM2026 under water stress condition. Reduction percent of chlorophyll a and b was the lowest in BARI Soybean-6 and the highest in BGM2026 irrespective of growth stages. The ratio of chlorophyll a/b decreased due to water stress. Decreased in chlorophyll a/b ratio indicated that chlorophyll b is not more sensitive to drought than chlorophyll a (Mafakheri *et al*., 2010). Water stress decreased chlorophyll a (chl a) more than chlorophyll b (chl b) and thus decreased the ratio. The genotypes BGM2026 had the most affected chlorophyll a/b ratio compared to other genotypes. Sairam and Siravastava (2002) reported that chlorophyll content of resistant and sensitive cultivars to drought stress reduced but resistant cultivar had high chlorophyll content. The decreased in total chlorophyll content may have resulted from a decrease in leaf water status in the soybean (Makbul *et al*., 2011).

Table 2. Chlorophyll a, chlorophyll b and chlorophyll a/b ratio in soybean leaves at vegetative stage under two water regimes

Genotypes	Chlorophyll a (mg g^{-1} leaf tissue)			Chlorophyll b (mg g^{-1} leaf tissue)			Chlorophyll a/b ratio	
	Non-stress	Water stress	% reduction	Non-stress	Water stress	% reduction	Non-stress	Water stress
Shohag	2.19	1.95	11	0.93	0.83	10	2.35	2.34
BARI Soybean 6	2.46	2.21	10	0.97	0.89	8	2.53	2.48
BD2331	2.14	1.86	13	0.93	0.82	11	2.30	2.26
BGM2026	2.30	1.79	22	0.96	0.80	17	2.39	2.23
$LSD_{(0.05)}$ S	0.15			0.04			NS	
G	0.16			0.03			NS	
SxG	NS			NS			NS	
CV(%)	6.11			3.12			4.17	

S=Stress, G=Genotypes, NS=Not significant.

Table 3. Chlorophyll a, chlorophyll b and chlorophyll a/b ratio in soybean leaves at flowering stage under two water regimes

Genotypes	Chlorophyll a (mg g^{-1}leaf tissue)			Chlorophyll b (mg g^{-1}leaf tissue)			Chlorophyll a/b ratio	
	Non-stress	Water stress	% reduction	Non-stress	Water stress	% reduction	Non-stress	Water stress
Shohag	2.45	2.08	16	1.19	1.02	14	2.05	2.00
BARI Soybean-6	2.67	2.26	15	1.27	1.10	13	2.10	2.05
BD2331	2.24	1.43	18	1.11	0.93	16	2.01	1.96
BGM2026	2.50	1.63	34	1.25	0.86	31	2.00	1.90
$LSD_{(0.05)}$ S	0.31			0.01			0.04	
G	0.32			0.12			0.039	
SxG	NS			NS			NS	
CV(%)	12.02			8.83			3.88	

S=Stress, G=Genotypes, NS=not significant.

Table 4. Chlorophyll a, chlorophyll b and chlorophyll a/b ratio in soybean leaves at pod development stage under two water regimes

Genotypes	Chlorophyll a (mg g^{-1}leaf tissue)			Chlorophyll b (mg g^{-1}leaf tissue)			Chlorophyll a/b ratio	
	Non-stress	Water stress	% reduction	Non-stress	Water stress	% reduction	Non-stress	Water stress
Shohag	2.22	1.73	22	1.11	0.92	17	1.00	1.88
BARI Soybean 6	2.50	2.00	20	1.16	0.98	15	2.14	2.04
BD2331	2.06	1.56	24	1.08	0.88	18	1.90	1.77
BGM2026	2.42	1.30	46	1.15	0.76	34	2.10	1.70
$LSD_{(0.05)}$ S	0.16			0.11			0.10	
G	0.14			0.07			0.07	
Sx G	0.20			0.11			0.11	
CV(%)	6.06			5.95			3.38	

S=Stress, G=Genotypes

Membrane thermo-stability

Membrane thermo-stability was determined to estimate the percentage of injury of soybean genotypes under water stress condition. According to Blum and Ebercon (1981), injury index signifies the degree of membrane damage due to

stress. Cell membrane thermo-stability as evaluated by the relative electrolyte leakage at 44^0C temperature differed among the soybean genotypes under both non-stress and water stress conditions (Table 5). Under non-stress condition all the four genotypes showed identical injury index but under stress condition the genotypes differed significantly for injury index. The genotype BGM2026 presented the greatest membrane damage and the genotype BARI Soybean-6 had the least damage followed by Shohag and BD2331. The injury index was 31.4% higher in BARI Soybean-6 whereas, it was 55.65% higher in BGM2026 in water stress condition than non-stress. Increased injury index was also observed due to water stress in soybean (Sarkar, 1993). The results indicated that the degree of membrane damage was higher due to water stress. The genotype Shohag and BARI Soybean-6 exhibited the lower rate of injury than BGM2026 to cell membranes that means genotype BGM2026 was found to be more injurious than Shohag and BARI Soybean-6. Variety Shohag and BARI Soybean-6 appeared to be relatively tolerant to water stress in terms of membrane stability.

Table 5. Percentage injury of soybean genotypes leaf at pod development stage under non-stress and water stress conditions

Genotypes	Percentage injury of leaf		Increase in injury % in stressed leaf at pod development stage
	Non-stress	Water stress	
Shohag	28.08	37.54	33.68
BARI Soybean-6	27.79	36.51	31.4
BD2331	28.13	38.39	36.47
BGM2026	27.83	43.32	55.65
$LSD_{(0.05)}$ S	4.38		
G	3.49		
G x S	4.94		
CV(%)	8.73		

S=Stress, G=Genotypes

A negetive relationship existed between injury percent and proline content of soybean genotypes in both the water regimes where R^2 were 95 and 0.29 for water stress and non-stress conditions, respectively (Fig. 2). The negative relationship indicated that the increase in proline content decreased the injury percent. Higher R^2 value as found in stress condition than non-stress condition further indicated a stronger relationship between injury index and proline content in the stress condition than control condition. There was a significant and negative correlation between proline accumulation and cell membrane injury (for non-stress r= -0.54 and for stress r=-0.97). Synthesis of proline and proteins, which have been implicated to have a role in protecting cellular structures during

dehydration, enables plant to survive under a condition of cellular water deficits (Molaei *et al*., 2012).

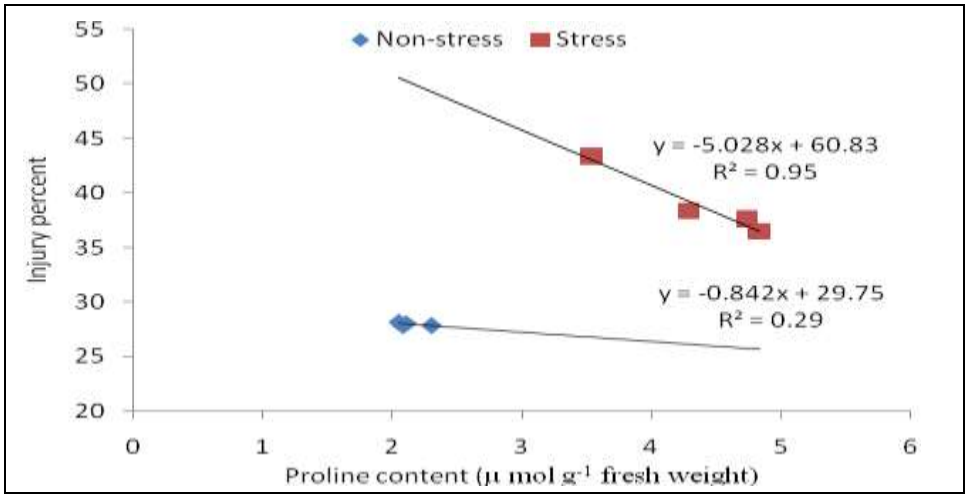

Fig. 2. Relationship between proline content and injury index of soybean genotypes leaf at pod development stage

Conclusion

All biochemical parameters and cell membrane stability of drought tolerant and drought sensitive genotypes showed similar pattern to drought stress. The tolerant genotypes accumulate more proline and soluble sugar than sensitive one. Drought stress decreased chlorophyll a, b and a/b concentration. Cell membrane stability was also higher in tolerant genotype. The variety/genotype Shohag, BARI Soybean-6 and BD2331 is considered as drought tolerant because of their higher proline, soluble sugar accumulation, chlorophyll content and cell membrane stability.

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