

## Amino Acid Metabolism in Berseem Cultivars During Salinity Stress

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### Abstract

Salt tolerant (Wardan) and salt susceptible (BL-10) cultivars of berseem were evaluated for amino acids in relation to imposed salinity stress. The results revealed that total amino acids decreased significantly with increasing levels of salinity except proline and glycine. Wardan had a higher concentration of glycine, cystine, leucine, lysine, glutamic acid and proline compared to BL-10. The contents of arginine, threonine, cystine, leucine, lysine and glutamic acid decreased progressively with increasing levels of salinity but increased with advancement of plant age. Greater accumulation of arginine and threonine is registered in BL-10 as compared to Wardan.

**Key words :** Berseem, Salinity, Agriculture field, Amino acids, Metabolism.

Berseem (*Trifolium alexandrinum* L.) is an annual legume chiefly used as a forage crop, for animal fodder. Salt affecting soil is a world wide problem, while high concentrations of have detrimental effect on plant growth, excessive amounts cause death of growing plants. Such soils are poor in fertility with low available nutrients, high osmotic pressure, poor air, low water movement and less microbial activity. Of various solutes found in cell sap of mesophytes, organic solutes such as amino acids are major components, which play important role in osmoregulating in relation to salinity stress. Osmoregulation is an important process governing salt tolerance in plants because it reduces the cell osmotic potential to a level which provides high turgor potential for maintaining growth. The osmotic effects on plant growth are related to water availability or soil water potential. An increase in salinity is manifested by reduction in the soil water potential. The aim of present investigation was to determine the physiological and biochemical factors associated with salt tolerance and the role of amino acids in amelioration in the two cultivars differing in degree of salt tolerance.

### Methods

The seeds of berseem varieties (*Trifolium alexandrinum* L. cv Wardan and *Trifolium*

*alexandrinum* L. cv BL-10), were collected from IGRI, Jhansi (UP) and PAU, Ludhiana (Punjab), simultaneously. The field experiment was conducted in 2005-2006 at Talampur (Moradabad) to evaluate the effect of saline water irrigations on amino acids spectrum. The plots of 1 × 1 m area of separate salinity were made with each other by polythene sheets of 0.20 mm thickness, up to a depth of 25 cm to stop leaching of different salts. Saline solutions of 4, 8, 12, 16, 20 and 24 dS/m were prepared by mixing salts (NaCl, NaHCO<sub>3</sub>, Na<sub>2</sub>SO<sub>4</sub> and CaCl<sub>2</sub>) in tube well water by using method (Richards 1954). Control sets were treated with tube well water. The seeds were surface sterilized with 0.1% HgCl<sub>2</sub> for one minute to exclude the possibility of any kind of fungal infection. The experiment was carried in randomized block design method, nearly three gram seeds were sown in respective plot at equal distance. First irrigation was carried at the next day of sowing. Ten liter saline water was used for irrigation in each plot. Second, third, fourth and fifth irrigations were carried at 60, 90, 120, 150 and 180 days after sowing (DAS). The samples per plant were taken randomly in respective plots. For each observation, the three replicates were maintained. The observations were recorded at 60, 90 and 120 DAS. The plant samples were kept in hot air oven at 60 C for 48 hours. Dry samples were subjected to analysis of amino acids by using the procedure of (Block et al. 1958).

**Table 1.** Effect of salinity on amino acids ( $\mu\text{g}/100$  mg dry weight) in leaves of berseem cv Wardan at different durations. C – Control sets, M–Mean value.

Amino acids	Duration in days on DAS															
	60								90							
	Salinity (dS/m)								Salinity (dS/m)							
	C	4	8	12	16	20	24	M	C	4	8	12	16	20	24	M
1. Arginine	7.1	6.9	6.2	5.5	4.5	3.5	2.5	5.1	7.5	7.0	6.5	6.0	4.9	4.0	3.0	5.5
2. Glycine	3.3	4.2	4.3	4.5	5.5	5.9	6.0	4.8	3.4	5.3	5.7	5.8	6.0	6.5	7.4	5.7
3. Threonine	5.6	5.5	5.4	5.2	4.9	4.8	4.7	5.1	6.5	6.0	5.9	5.6	5.3	5.1	5.0	5.6
4. Cystine	6.2	6.0	5.8	5.6	5.4	5.0	4.5	5.5	7.5	7.1	6.8	6.2	5.9	5.4	5.0	6.2
5. Leucine	5.9	5.2	4.7	4.2	4.0	3.8	3.4	4.4	6.5	6.2	6.0	5.5	5.1	5.0	4.7	5.5
6. Lysine	4.8	4.5	4.1	3.9	3.5	3.1	2.8	3.8	5.5	5.1	5.0	4.2	4.1	3.8	3.2	4.4
7. Glutamic acid	5.1	5.0	4.2	4.1	3.8	3.2	2.9	4.0	6.0	5.8	5.2	5.1	4.7	4.1	3.8	4.9
8. Proline	2.7	2.9	3.1	3.4	3.6	3.9	4.0	3.3	2.9	3.2	3.5	4.1	4.2	4.8	4.9	3.9
Mean	5.0	5.0	4.7	4.5	4.4	4.1	3.8	4.5	5.7	5.7	5.5	5.3	5.0	4.8	4.6	5.2
CD at 5%																
Salinity (S)	0.014								0.029							
Amino acids (AA)	0.025								0.031							
S $\times$ AA	0.003								0.005							

**Table 1.** Continued.

Amino acids	Duration in days on DAS									
	120									
	Salinity (dS/m)									
	C	4	8	12	16	20	24	M		
1. Arginine	7.9	7.4	6.9	6.5	5.1	4.5	4.0	6.0		
2. Glycine	5.0	5.8	6.0	6.4	6.5	6.9	7.9	6.3		
3. Threonine	7.0	6.8	6.2	5.9	5.5	5.4	5.1	5.9		
4. Cystine	8.1	7.8	7.1	6.5	6.0	5.8	5.2	6.6		
5. Leucine	7.2	7.0	6.9	6.2	5.9	5.5	5.1	6.2		
6. Lysine	6.2	6.1	5.9	5.4	5.1	4.8	4.2	5.3		
7. Glutamic acid	6.5	6.4	6.1	5.9	5.4	5.0	4.6	5.7		
8. Proline	3.1	3.8	4.0	4.5	5.0	5.1	5.5	4.4		
Mean CD at 5%	6.3	6.1	6.1	5.9	5.5	5.3	5.2	5.8		
Salinity (S)	0.035									
Amino acids (AA)	0.029									
S $\times$ AA	0.001									

### Results and Discussion

In salt stressed plants, amino acids accumulate at the expense of structural and functional proteins, the balance between amino acids and protein is changed due to disturbed metabolism of plants, which results in the form of suppressed growth. Data on the salt induced effects on amino acids of berseem at various growth stages (60, 90 and 120 DAS) are presented in Tables 1 and 2. Total amino acid content showed a declining trend both tolerant and susceptible genotypes. The findings are in consonance with earlier reports (Singh et al. 2008). The salinity has differential effects on amino acids spectrum and in

the present study, eight amino acids (arginine, glycine, threonine, cystine, leucine, lysine, glutamic acid and proline) have been identified under salinity stresses. In general, the concentrations of two amino acids i.e. glycine and proline had increased, while arginine, threonine, cystine, leucine, lysine and glutamic acid decreased with increasing levels of salt stresses (4 to 24 dS/m). The increase in amino acid content showed a differential response in both genotypes of berseem. The contents of arginine and threonine were higher in BL-10, while the contents of glycine, cystine, leucine, lysine, glutamic acid and proline were higher in Wardan at all growth stages. It indicates

**Table 2.** Effect of salinity on amino acids ( $\mu\text{g}/100$  mg dry weight) in leaves of berseem cv BL-10 at different durations. C – Control sets, M – Mean value.

Amino acids	Duration in days on DAS															
	60								90							
	Salinity (dS/m)								Salinity (dS/m)							
	C	4	8	12	16	20	24	M	C	4	8	12	16	20	24	M
1. Arginine	7.8	7.2	6.4	5.6	4.8	3.7	2.8	5.4	8.0	7.6	7.2	7.0	6.5	6.0	3.2	6.5
2. Glycine	5.5	5.2	5.0	4.0	3.9	3.5	2.7	4.2	7.2	6.3	5.9	5.7	5.5	5.1	3.0	5.5
3. Threonine	6.6	6.5	6.4	6.2	5.9	5.8	5.6	6.1	7.5	7.2	6.9	6.6	6.3	6.1	5.8	6.6
4. Cystine	6.0	5.8	5.7	5.5	5.1	4.8	4.1	5.2	7.4	7.0	6.5	6.0	3.5	5.2	4.7	5.1
5. Leucine	5.8	5.1	4.6	4.0	3.9	3.5	3.0	4.2	6.2	6.0	5.9	5.4	5.0	4.8	4.0	5.3
6. Lysine	4.7	4.4	3.8	3.6	3.2	2.9	2.5	3.5	5.4	4.9	4.9	4.0	3.9	3.5	2.9	4.1
7. Glutamic acid	4.9	4.8	4.0	3.9	3.6	3.1	2.5	3.8	5.6	5.4	5.0	4.7	4.3	4.0	3.2	4.6
8. Proline	3.9	3.6	3.3	3.0	2.8	2.5	2.4	3.0	4.8	4.6	4.0	3.9	3.2	3.0	2.6	3.7
Mean CD at 5%	5.6	5.3	4.9	4.4	4.1	3.7	3.2	4.4	6.5	6.1	5.7	5.4	5.0	4.7	3.6	5.2
Salinity (S)	0.017								0.027							
Amino acids (AA)	0.024								0.034							
S $\times$ AA	0.004								0.009							

**Table 2.** Continued.

Amino acids	Duration in days on DAS							
	120							
	Salinity (dS/m)							
	C	4	8	12	16	20	24	M
1. Arginine	8.5	8.0	7.8	7.4	7.0	6.5	4.3	7.0
2. Glycine	7.8	6.8	6.2	6.0	5.9	5.4	4.8	6.1
3. Threonine	8.2	7.8	7.3	6.8	6.6	6.4	6.0	7.0
4. Cystine	8.0	7.7	6.9	6.1	5.8	5.3	4.9	6.3
5. Leucine	7.0	6.9	6.7	6.1	5.7	5.3	4.8	3.0
6. Lysine	6.0	5.9	5.8	5.1	4.8	4.5	3.7	5.1
7. Glutamic acid	6.0	5.9	5.5	5.1	4.8	4.3	3.9	5.0
8. Proline	5.3	5.0	4.8	4.5	4.0	3.8	2.6	4.2
Mean CD at 5%	7.0	6.7	6.3	5.8	5.5	5.2	4.3	5.8
Salinity (S)	0.040							
Amino acids (AA)	0.031							
S $\times$ AA	0.001							

that the amount of amino acids showed differential behavior under salt stress in two genotypes. The present findings also confirm the reports of Parihar and Bajjal 1982. Characteristic changes in levels of free amino acids were observed in the leaves of plant exposed to salt stress. The most obvious of them was an increase in free proline ascribed to proline, the role of a cytoplasmatic osmoticum counterbalancing the effect of salts and play a significant role in salt tolerance of the variety. The accumulation of proline and glycine may be due to shift of organic acid to amino acid synthesis and it is due to inhibition of malic dehydrogenase activity and stimulation of transamina-

tion reaction under saline conditions.

Hence, salinity inhibits the NR enzyme activity which regulates the structural and functional units of the plant. Loss of nitrogen during salt stress is avoided by forming and storing amino acids. The incorporation of amino acids in protein synthesis is also suppressed due to salts. As a result, accumulation of some amino acids takes place, which leads to toxicity in plant cells and this toxicity may be due to formation or synthesis of toxic substances. Therefore, a comparative assessment of the present results for the two genotypes of berseem shows that Warden variety is tolerant to salt stress and can be recom-

mended for cultivation in saline areas.

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