

EFFECT OF WATERLOGGING ON SOME BIOCHEMICAL PARAMETERS DURING EARLY GROWTH STAGES OF MAIZE

RAJESH KUMAR RAI¹, J.P. SRIVASTAVA*² AND J.P. SHAHI³

¹Horticulture and Agro-Forestry Research Programme, Plandu, Post-Rajaulatu, Ranchi 834010. ²Department of Plant Physiology,

³Department of Genetics and Plant Breeding, Institute of Agricultural Science, Banaras Hindu University, Varanasi, 221005, India.

A pot culture experiment was conducted in *kharif* with ten lines of maize (*Zea mays* L.). After 20 days of growth, plants were subjected to waterlogging. Changes in biochemical characters were recorded in 2 cm root apices after 48 and 96 hours of waterlogging. On an average, soluble sugar content initially increased but after 96 hours of waterlogging it decreased. Proline content also followed a similar trend. Free amino acids content increased under waterlogging and the amount further increased with increased waterlogging duration. Soluble protein content initially increased but later on declined after 96 hours of waterlogging. On the other hand alcohol dehydrogenase (ADH) activity increased after 48 hours of waterlogging. Changes in the levels of these traits followed no definite trend in resistant and susceptible lines. However, the resistant lines, viz. Pant 7377 and Pant 7379 registered lesser increase in ADH activity as compared to susceptible lines.

Key words : Alcohol dehydrogenase, amino acids, anoxia, maize, proline, soluble sugars, waterlogging.

Crop productivity is adversely affected by biotic and abiotic stresses. Although all higher plants require access to free water, excess water in the root zone may be even more lethal because it blocks the transfer of oxygen and other gases between the soil and the atmosphere. Plant roots become oxygen deficient because of slow transfer of dissolved oxygen in the water filled pore spaces of the soil under waterlogging (Drew 1997).

Maize is highly sensitive to waterlogging. Some of the symptoms of waterlogging include chlorosis of leaves, decreased stem elongation often accompanied by adventitious root formation and increased susceptibility to pathogens. Waterlogging has been found to decrease sucrose, glucose and starch contents, sucrose to starch ratio, and acid phosphatase activity in leaves (Sinha *et al.* 1995). It is reported that due to waterlogging, total sugar content in maize root, and transport of sugars from shoot to root is decreased (Waters *et al.* 1991). During starvation proteolysis gives rise to an increase in free amino acids like cystine, glutamic acid, arginine, tyrosine and tryptophan (Kemble and MacPherson 1954). It is

reported that under anaerobic condition new proteins are synthesised in maize, mainly the enzymes of sugar metabolism and fermentation (Drew 1997). However, because of dissociation of polyribosomes under anoxia, there is a rapid decrease in protein synthesis (Sachs *et al.* 1980). Accumulation of proline is a widespread plant response to environmental stresses (Yancey *et al.* 1982, Srivastava 1998). Proline acts as an osmoticum under stress condition. Because of its zwitterionic and highly hydrophilic characteristics, proline also acts as a "compatible solute" (Samaras *et al.* 1995). Effect of waterlogging on proline metabolism in plants is not fully understood.

Anaerobiosis of the root environment of vascular plants can be achieved either by flooding soil or by flushing oxygen free gas through culture solution. Such treatments usually result in increased activities of ADH and pyruvate decarboxylase in the root tissues (Wignarajan *et al.* 1976). Activity of ADH in plant roots may depend on the oxygen supply to the roots. In flooding sensitive species, the level of ADH activity in the root tissues has

* Corresponding author

been found to be high particularly at low oxygen supply in maize (Greenway 1970). The present investigation was under taken to investigate the effect of water logging at early growth stages on biochemical parameters in maize genotypes differing in waterlogging resistance.

Experiments were conducted during *kharif* 1999 in the Department of Plant Physiology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi. Ten lines of maize, viz. CML-15, CML-39, CML-42, CM-400, CM-300, Pant-7372, Pant-7374, Pant-7376, Pant-7377 and Pant-7379, procured from Department of Genetics and Plant Breeding, Institute of Agricultural Science, B.H.U., Varanasi, were used for the study. The lines Pant-7374 and Pant-7372 were identified as susceptible and Pant-7377 and Pant 7379 as resistant lines, while other lines as moderately resistant to waterlogging. Seeds were sown in plastic bags (diameter 15 cm) filled with 1.5 kg fine garden soil. Soil was irrigated with tap water and five seeds of each line were sown in 10 replications. After 7 days of germination, 3 healthy seedlings were retained in each bag. Seedlings were maintained on normal supply of water. After 20 days of growth five bags of each line were put in a pit containing water so that roots were completely submerged. The water level was maintained regularly by adding tap water in such a way that nearly 2 to 3 cm standing water column remained over the soil surface of plastic bags. The remaining five replications of each line were maintained at normal supply of water and these are termed as control plants. Samples from root apices (2 cm) of plants were drawn just before start of waterlogging (zero hour after waterlogging), 48 hours and 96 hours after waterlogging to study various parameters. Data were analyzed using completely randomized design (CRD).

Alcohol dehydrogenase (ADH) activity was assayed by the method as described by Hanson and Jacobsen (1984). The protein content, free amino acids, ethanol soluble sugars, and proline contents were determined by the methods as described by Lowry *et al.* (1951), Yemm and Cocking (1955), Dubois *et al.* (1951) and Bates *et al.* (1973), respectively.

After 36 hours of waterlogging plants started yellowing of lower leaves. Visually wilting was recorded in waterlogged plants. In the present investigation, 48 hours after waterlogging, on an average, root ethanol

soluble sugar content decreased, but again increased after 96 hours (Table 1). Initial reduction in soluble sugar content under waterlogged situation might be due to reduced translocation from shoot. Marginal increase in soluble sugar content at 96 hours after waterlogging might be due to increased degradation of root stored starch. Such observations have been observed by Lorenzo *et al.* (1995) and Tianfeng *et al.* (1995) where total sugar content in maize roots decreased under waterlogged conditions. After 48 hours of waterlogging there was significant increase (3 folds) in the proline content in root tissues (Table 1) but after 96 hours of waterlogging it reduced drastically. In the present investigation it was observed that, on an average, free amino acid content increased after 48 hours of waterlogging and the amount further increased at 96 hours.

Water logging for a period of 48 hours increased soluble protein content in root tissues (Table 1). It is well documented that under anaerobic condition new proteins are synthesized in roots of maize seedlings (Sachs *et al.* 1980). The observed increased protein synthesis after 48 hours of waterlogging might be due to increased synthesis of specific proteins and its further reduction after 96 hours of waterlogging may be by reduction in protein synthesis as it is reported that dissociation of polyribosomes and rapid decrease in protein synthesis occurs in maize, pea, rice and soybean under anoxia (Sayed 1998).

During waterlogging, due to anaerobic environment in the root zone, anaerobic respiration was increased (Wignarajan *et al.* 1976). It was also reported that waterlogging resistant species show increase in ADH activity in roots under waterlogging (Crawford 1967). However, contrasting results were also reported (MacManmon and Craford 1971). In the present investigation ADH activity increased with increased waterlogging duration but resistant lines, viz., Pant 7377 and Pant 7379 registered lesser increment in ADH activity (Table 1). John and Greenway (1976) also reported similar results in ADH activity under such condition. The investigation further indicated that waterlogging induced ADH activity but induction was less in resistant lines. This may cause less production of ethanol under waterlogging in resistant lines resulting in less tissue damage.

EFFECT OF WATERLOGGING ON MAIZE

Table 1. Changes in parameters in different lines of maize germplasm under different durations of waterlogging.

Lines	PARAMETERS														
	Soluble sugar (mg g ⁻¹ fw of root)			Soluble proline (µg g ⁻¹ fw of root)			Free amino acids (mg g ⁻¹ fw of root)			Soluble protein (mg g ⁻¹ fw of root)			Alcohol dehydrogenase Activity (Δ OD g ⁻¹ fw min ⁻¹)		
	Hrs after water logging			Hrs after water logging			Hrs after water logging			Hrs after water logging			Hrs after water logging		
	0	48	96	0	48	96	0	48	96	0	48	96	0	48	96
CML-15	12.21	12.13	14.77	8.08	42.24	6.51	1.28	1.78	1.56	18.32	25.12	14.80	0.270	0.483	0.618
CML-39	20.51	10.68	7.84	4.95	20.33	4.95	1.52	1.39	1.46	13.01	22.02	13.78	0.345	0.210	0.239
CML-42	19.26	9.26	19.89	3.12	23.46	5.99	1.17	1.55	1.60	19.04	22.68	15.99	0.259	0.204	0.450
CM-400	10.34	11.87	19.27	24.77	18.51	3.91	0.87	1.36	1.26	16.47	23.04	17.30	0.172	0.247	0.502
CM-300	15.79	12.67	18.52	6.77	17.73	4.95	1.27	1.15	1.43	18.38	23.69	18.14	0.252	0.309	0.586
Pant-7372	11.70	15.57	15.00	7.56	18.98	14.18	1.69	1.59	2.02	18.50	25.96	16.58	0.315	0.357	0.759
Pant-7374	9.49	12.10	16.14	4.69	15.12	4.42	1.52	1.40	1.62	17.72	23.63	16.41	0.169	0.247	0.740
Pant-7376	14.83	16.42	18.07	5.21	19.55	5.73	1.33	1.39	1.43	18.20	21.24	17.30	0.238	0.112	0.589
Pant-7377	19.55	9.20	13.01	4.43	14.33	2.86	1.50	1.26	1.64	17.78	21.90	17.25	0.286	0.124	0.591
Pant-7379	12.73	16.08	9.54	1.56	13.81	4.95	1.20	1.28	1.60	15.75	24.70	18.14	0.114	0.339	0.297
Mean	14.64	12.70	15.21	7.11	20.41	5.85	1.34	1.42	1.56	17.32	23.40	16.57	0.242	0.263	0.537
C.D. at 1%															
Line	NS			5.330			0.249			5.572			0.169		
Stage	3.478			5.125			0.239			2.471			0.162		
Line x Stage	5.337			7.865			NS			NS			0.249		

The present investigation indicated that under waterlogging, there was significant reduction in soluble sugar content of root, while proline content initially increased significantly and decreased thereafter. Free amino acid content increased marginally, while soluble protein content initially increased, then declined. Alcohol dehydrogenase activity increased steadily with increase in waterlogging duration. No definite trend was observed with the change in the levels of these substances in resistant, moderately resistant and susceptible maize lines. However, resistant lines registered lesser increment in ADH activity. Poor carbohydrate supply to roots under waterlogging might be the main reason for poor root growth, water and mineral absorption. Genotypes with high root sugar content and low ADH activity under waterlogged condition might perform better under such situation.

ACKNOWLEDGEMENT

Authors are thankful to Dr. R.K. Singh, Department of Genetics and Plant Breeding for providing necessary facilities.

REFERENCES

Bates, L.S., Waldren, R.P. and Teare, I.D. (1973). Rapid determination of free proline for water stress studies. *Plant and Soil* **39**: 205-207.

Crawford, R.M.M. (1967). Alcohol dehydrogenase activity in relation to flooding tolerance in roots. *J. Exp. Bot.* **18**: 458-462.

Drew, M.C. (1997). Oxygen deficiency and root metabolism : Injury and acclimation under hypoxia and anoxia. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **48**: 223-250.

Dubois, M., Giles, K.A., Hamilton, T.K., Robeos, P.A. and Smith, F. (1951). Colorimetric methods for determination of sugars and related substances. *Anal. Chem.* **28**: 350-356.

Greenway, H. (1970). Effect of slowly permeating osmoticum on metabolism of vacuolated and non vacuolated tissues. *Plant Physiol.* **46**: 254-260.

Hanson, A.D. and Jacobsen, J.V. (1984). Control of lactate dehydrogenase, lactate glycolysis and amylase by oxygen deficiency in barley aleurone layers. *Plant Physiol.* **75**: 566-572.

John, C.D. and Greenway, H. (1976). Alcoholic fermentation and activity of some enzymes in rice roots under anaerobiosis. *Aust. J. Plant Physiol.* **3**: 325-330.

- Kemble, A.R. and MacPherson H.T. (1954). Liberation of amino acids in perennial raya grass during wilting. *Biochem. J.* **58**: 46-59.
- Lorenzo, G., Pierdomenico, P. and Amdeo, A. (1995). Effect of anoxia on carbohydrate metabolism in rice seedlings. *Plant Physiol.* **108**: 737-741.
- Lowry, O.H., Roseborough, H.J., Farr, A.K. and Randall, R.J. (1951). Protein measurement with folin phenol reagent. *J. Biol. Chem.* **193**: 265-275.
- McManmon, M. and Craford, R.M.M. (1971). A metabolic theory of flooding tolerance : The significance of enzyme distribution and behavior. *New Phytol.* **38**: 190-194.
- Sachs, M.M., Freeling, M. and Okimoto, R. (1980). The anaerobic proteins of maize. *Cell* **20**: 761-767.
- Samaras, Y., Bressan, R.A., Csonka, L.N., Gracia-Rios, M.G., Paino, D., Urzo, M. and Rhodes, D. (1995). Proline accumulation during drought and salinity. In : N. Smirnov (ed.), *Environment and Plant Metabolism : Flexibility and Acclimation*, pp. 161-187. BIOS Scientific Publishers, Oxford.U.K.
- Sayed, S.A. (1998). Impacts of boron application on maize plants growing under flooded and unflooded conditions. *Biol. Plant.* **41**: 101-110.
- Sinha, B.K., Haque, H. and Pandey, O.P. (1995). Metabolic modification in *Zea mays* during waterlogging. *Plant Physiol. Biochem.* **22**: 173-177.
- Srivastava, J.P. (1998). Influence of salinity stress on crop plants. In : A. Hemantranjan (ed.), *Advances in Plant Physiology*. Vol. I, pp. 381-396. *India. Scientific Publishers*, Jodhpur.
- Tianfeng, C.H.U., Jigging, Z.H.U., Mingshi, Xu and Honghui, G.U. (1995). The effects of soil wetness and submergence on early growth of maize. *Acta. Ag. Zhegiagensis.* **7**: 286-288.
- Waters, I., Morrell, S., Greenway, H. and Colmer, T.D. (1991). Effect of anoxia on wheat seedling II. Influence of oxygen supply prior to anoxia, alcoholic fermentation and sugar levels, *J. Exp. Bot.* **42**: 1437-1447.
- Wignarajan, K. and Greenway, H. and John, C.D. (1976). Effect of anaerobiosis on activities of alcohol dehydrogenase and pyruvate decarboxylase in roots of *Zea mays*. *New Phytol.* **77**: 575-584.
- Yancey, P.H., Clark, M.E., Hand, S.C., Browlus R.D. and Somero, G.N. (1982). Living with water stress : Evaluation of osmolyte systems. *Science* **217**: 1214-1222.
- Yemm, E.W. and Cocking, E.C. (1955). The determination of amino acids with ninhydrin. *Analyst.* **80**: 209-230.