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SHORT COMMUNICATION

SALINITY INDUCED COMPOSITIONAL CHANGES IN GERMINATING SEEDS OF MUSTARD GENOTYPES

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Mustard genotypes Panchali and Bhavani (tolerant), and TS-46 and PT-303 (susceptible) were subjected to varied levels of salinity stress (control, 4.0, 8.0, 12.0 dS m^{-1}) to investigate the biochemical basis of salt tolerance during germination. Increase in salt stress resulted in decrease in sugars and phospholipids, and increase in malondialdehyde and phenol content in all genotypes. However, tolerant genotypes showed higher content of sugars, lipids, phenols and lower MDA content as compared to susceptible genotypes.

Key words **: Malondialdehyde, mustard, phenol, phospholipid, salinity, sugar.**

Mustard (*Brassica campestris* L. var. toria) is an important oilseed crop, grown as catch crop on residual soil moisture in post-rainy season, which tends to increase salt concentration in the soil solution. Among the abiotic stress factors, salinity is important yield reducer, which delays germination at lower levels and response may vary with the type of salinity and species or genotypes (Levitt 1980). Thus, exploitation of genetic variability in cultivated species offer the possibility of developing salt tolerant crops (Epstien *et al.* 1980). The mechanisms, which impart salt tolerance to some plants and sensitivity to others, however have not been fully worked out in oilseed crops. Hence, the present investigation was undertaken to find out the biochemical basis of salinity stress tolerance in rapeseed genotypes during germination.

Seeds of mustard (*Brassica campestris* L. var. toria) genotypes were screened for their relative salinity tolerance (Singh *et al.* 2001a). Genotypes Bhawani and Panchali were identified as tolerant, whereas PT-303 and TS-46 were found to be susceptible. Seeds of above four genotypes were surface sterilized with 0.1% $HgCl_2$ solution for one minute and then thoroughly washed with distilled water. The salt solution was prepared by taking NaCl : $CaCl_2$: Na_2SO_4 in the ratio of 7 : 2 : 1 and electrical conductivity of different salinity levels were adjusted on a direct reading conductivity meter (Systronics, Model-303). The seeds were germinated at different salinity levels, viz. control, 4.0, 8.0 and 12.0 dS m^{-1} in sterilized germination boxes lined with blotting papers and kept in a BOD incubator at 25 ± 2^0C. The experiment was terminated on 8th day. Eight-day-old seedlings were subjected to various analyses. Quantitative estimation of total lipid, soluble sugar, phospholipid, malondialdehyde (MDA) and total phenols were done by the procedures described by Miller (1959), Jayaraman (1981), Halliwell and Gutteridge (1989) and Farkas and Kiraly (1962), respectively.

All the four genotypes showed a decrease in lipid content with increase in salinity stress as compared with control (Table 1). At 12 dS m^{-1} stress level, Panchali and Bhawani had lower lipid content, 7.21 and 5.15 mg, respectively as compared to susceptible genotypes, viz. TS-46 (15.43 mg) and PT-303 (10.52 mg). The higher reduction of lipid in tolerant genotypes might be due to its more break down. This is in agreement with the findings of Munshi *et al.* (1986).

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Table 1. Influence of salinity levels on total lipid and sugar content of rapeseed genotypes.

Genotypes	Salinity level (dS m^{-1})	Total Lipid (mg g^{-1} dry wt.)	Reducing Sugar (mg g^{-1} dry wt.)	Total Sugar (mg g^{-1} dry wt.)
		Tolerant		
Panchali	0.0	41.14	11.20	36.23
	4.0	24.05 (-41.59)	10.40 (-7.14)	35.00 (-2.77)
	8.0	16.02 (-61.05)	9.40 (-16.07)	32.00 (-11.11)
	12.0	7.21 (-82.47)	8.00 (-28.57)	30.00 (-16.66)
Bhawani	0.0	38.21	11.00	39.33
	4.0	21.12 (-44.72)	10.40 (-5.45)	34.16 (-2.56)
	8.0	13.39 (-64.95)	9.60 (-12.72)	31.83 (-7.64)
	12.0	5.15 (-86.52)	7.60 (-30.90)	26.00 (-10.25)
		Susceptible		
TS-46	0.0	31.01	8.5	26.28
	4.0	30.17 (-3.07)	7.60 (-10.58)	25.00 (-3.84)
	8.0	26.26 (-15.53)	7.20 (-10.00)	22.00 (-15.38)
	12.0	15.43 (-50.36)	4.40 (-45.00)	20.00 (-23.07)
PT-303	0.0	33.17	9.40	25.61
	4.0	31.21 (-5.90)	8.00 (-14.89)	22.00 (-12.00)
	8.0	20.19 (-39.13)	7.20 (-23.40)	18.00 (-28.00)
	12.0	10.52 (-68.28)	5.60 (-40.42)	15.00 (-40.00)
C. D. at 5%				
Stress (S)		0.92	0.83	1.12
Genotype (G)		0.92	0.83	1.12
S x G		1.85	1.67	2.24

Figures in parentheses indicate per cent decrease (-) over control.

Table 2. Influence of salinity levels on phospholipid, malondialdehyde and phenols of rapeseed genotypes.

Genotypes	Salinity level ($dS\ m^{-1}$)	Phospholipid ($mg\ g^{-1}$ dry wt.)	Malondialdehyde ($\mu mol\ g^{-1}$ fr. wt.)	Total Phenols ($mg\ g^{-1}$ dry wt.)
		Tolerant		
Panchali	0.0	29.65	0.0028	4.95
	4.0	23.22 (-21.68)	0.0031 (+10.71)	7.07 (+42.82)
	8.0	13.18 (-55.54)	0.0034 (+21.42)	10.12 (+104.44)
	12.0	8.13 (-72.14)	0.0038 (+35.71)	11.01 (+112.42)
Bhawani	0.0	33.45	0.0024	5.57
	4.0	21.23 (-36.53)	0.0028 (+16.60)	6.87 (+23.33)
	8.0	15.16 (-54.67)	0.0031 (+29.16)	9.86 (+77.01)
	12.0	7.22 (-78.41)	0.0036 (+50.00)	10.37 (+87.17)
		Susceptible		
TS-46	0.0	18.66	0.0018	4.11
	4.0	10.34 (-44.58)	0.0029 (+61.11)	4.55 (+10.76)
	8.0	4.42 (-76.31)	0.0041 (+127.77)	6.43 (+40.55)
	12.0	2.82 (-84.88)	0.0067 (+272.22)	8.02 (+95.13)
PT-303	0.0	20.15	0.0015	4.89
	4.0	9.98 (-50.47)	0.0025 (+66.66)	5.47 (+11.86)
	8.0	6.54 (-67.54)	0.0035 (+133.33)	6.87 (+40.49)
	12.0	4.34 (-78.46)	0.0060 (300.00)	8.14 (+66.40)
C. D. at 5%				
Stress (S)		1.46	0.00017	1.05
Genotype (G)		1.46	0.00017	1.05
S x G		2.93	0.0040	2.84

Figures in parentheses indicate per cent increase (+) or decrease (-) over control.

Reducing sugar content in 8-day-old seedlings showed a decreasing trend with rise in salinity stress from control to the maximum (12.0 dS m^{-1}). At maximum salinity level, higher reducing sugar content was observed in Panchali (8.00 mg) and Bhawani (7.60 mg) than TS-46 (4.40 mg) and PT-303 (5.60 mg). All the four genotypes showed the same trend for total sugar content (Table 1). Lower values of total sugar were recorded in TS-46 (20.00 mg) and PT-303 (15.00 mg) in comparison to tolerant genotypes Panchali (30.00 mg) and Bhawani (26.00 mg), respectively at 12.0 dS m^{-1}. Salt stress induced reduction in sugar content has been reported by Singh and Singh (1995).

The phospholipid content of all the four genotypes exhibited gradual reduction with the increasing salinity stress (Table 2). However, susceptible genotypes showed lower values, viz., TS-46 (2.82 mg) and PT-303 (4.34 mg) in comparison to tolerant genotypes, viz. Panchali (8.13 mg) and Bhavani (7.22 mg) at 12.0 dS m^{-1}. Phospholipid metabolism is also involved in plant responses to salinity stress (Katagiri *et al.* 2001). Phospholipids are major constituent of all the biological membrane. It is suggested that salt induced membrane injury may be due to changes in the membrane lipid composition and protein or both (Levitt 1980).

Free radicals and other active derivatives of oxygen (ROS) are inevitable product of natural redox reaction in various cellular compartments under aboitic stress conditions. The free radical mediated peroxidation of unsaturated fatty acids, also known as lipid peroxidation, is a chain reaction occurring mainly in biomembranes (Ursini *et al.* 1991). Increasing trend of MDA content, a measure of lipid peroxidation, was noticed in all the genotypes under study with increasing salinity stress (Table 2). At 12.0 dS m^{-1}, the values were lower in tolerant ones, viz. Panchali (0.0038 µmol) and Bhawani (0.0036 µmol) in comparison to susceptible ones, viz. TS-46 (0.0067 µmol) and PT-303 (0.0060 µmol). Stress induced increase in malondialdehyde has also been reported by Kasturi Bai *et al.* (2001).

Our findings (Table 2) indicates that the total phenols increased in all the genotypes under reference with rise in salinity levels, and the tolerant genotypes maintained a higher level of phenols at all the stress levels. Salinity induced increase in phenol content have been reported by Latha *et al.* (1989) and Singh *et al.* (2001b). Phenol constitute a part of cellular solutes and provide a reducing environment to the system (Das *et al.* 1990). Levitt (1980) reported that salt stress exerts its effect through membrane peroxidation, which indicates that oxygen free radicals are formed during stress. Thus, higher phenol accumulation in tolerant genotypes could be a cellular adaptive mechanism for scavenging oxygen free radicals and preventing sub-cellular damages during stress. Higher sugar and phenol contents, and lower malondialdehyde content might prove useful for screening or improving mustard genotypes to withstand salinity.

REFERENCES

Das, N., Misra, M. and Misra, A. N. (1990). Sodium chloride salt stress induced metabolic changes in callus cultures of pearl millet (*Pennisetum americanum* L.) : Free solute accumulation. *J. Plant Physiol.* **137** : 244-246.

Epstein, E., Norlyn, J.D., Rush, D.W., Kingsbury, R.W., Kelly, D.W., Kunningham, G.A. and Wrona, A.F. (1980). Saline culture of crops : A genetic approach. *Science* **210** : 399-404.

Farkas, G.L. and Kiraly, Z. (1962). Role of phenolic compounds in the physiology of plant disease and disease resistance. *Phytopathology* **44** : 105-150.

Helliwel, B. and Gutteridge, J.M.C. (1989). Free Radical in Biology and Medicine (2nd Edn.). Oxford, Claredo Press.

Jayaraman, J. (1981). Laboratory Manual in Biochemistry, Wiley Eastern Ltd., New Delhi.

Kasturi Bai, K.V., Rajagopal, V. and Ratnambal, M.J. (2001). Relationship between leaf water potential and peroxidation of cell wall lipids in coconut. *J. Plant Biol.* **28** : 161-164.

Katagiri, T., Takahashi, S. and Shinozaki, K. (2001). Involvement of a novel *Arabidopsis* phospholipase, D., At PLDdelta, in dehydration - inducible accumulation of phosphatidic acid in stress signalling. *Plant J.* **26** : 595-605.

Latha, V.M., Satakopan, V.N. and Jayasree, H. (1989). Salinity induced changes in phenol and ascorbic acid content in groundnut (*Arachis hypogaea*) leaves. *Curr. Sci.* **58** : 151-152.

Levitt, J. (1980). Responses of Plants to Environmental Stresses. Academic Press, New Delhi.

Miller, G.L. (1959). Use of dinitrosalicylic acid reagent for the determination of reducing sugars. *Analy. Chem.* **31** : 426-428.

Munshi, S.K., Bhatia, N., Dhillon, K.S. and Sukhija, P.S. (1986). Effect of moisture and salt stress on oil filling in Brassica seeds. *Proc. Indian National Sci. Acad.* **52** : 755-759.

Singh, A.K., Ranjan, R.D. and Pandey, A. (2001a). Germination study in rapeseed (*Brassica campestris* L. var. toria) under salt stress. *J. Appl. Biol.* **11** : 23-26.

Singh, A.K., Singh, R.A. and Sharma, S.G. (2001b). Salt stress induced changes in certain organic metabolites during seedling growth of chickpea. *Legume Res.* **24** : 11-15.

Singh, M. and Singh, S. (1995). Sodicity induced changes in saccharides, free proline and protein content of different pea genotypes. *Indian J. Plant Physiol.* **38** : 109-113.

Ursini, F., Maiorino, M. and Sevanian, A. (1991). Membrane hydroperoxide. In: H. Sies (ed.), Oxidative Stress Oxidant and Antioxidants, pp. 319-336. Academic Press, New York.