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Antioxidative Activities and Metabolomic Profiling of Common Indian Wheat Cultivars for Determining Resistance against Elevated Salinity Stress

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Abstract—*Nine different varieties of wheat (Triticum aestivum L.), Mohan Wonder (MW), Kedar (KD), Gayetri (GY), Gandhari (GN), Kaweri (KW), PBW 343, UP 2752, Sonalika (SO) and a Local variety (LV) were screened for resistance against salinity stress on the basis of antioxidative activities and metabolic profiling. One month old seedlings of all the cultivars were subjected to salinity stress in vitro containing a gradient of 50mM, 100mM and 200mM solution in replicates. It was observed that the relative water content (RWC) decreased in all the cultivars with increase in salt concentration. RWC levels aggravated up to 200mM after 72h which also affected the membrane stability index (MSI) hinting to the disintegration of membrane structures at higher salt concentration. Increase in total antioxidative activities in the cultivars such as KD, GN, KW, UP 2752 and PBW 343 was observed which conferred better tolerance to these cultivars against salinity stress. However, cultivars MW, GY, SO and LV showed decreased level of tolerance. Activities of key metabolites like Phenol, ascorbate and carotenoid were enhanced with increase in the concentration and duration of salt stress for all varieties. Antioxidative enzymes like super oxide dismutase (SOD), peroxidase (POX), glutathione reductase (GR) and catalase (CAT) in cultivars MW, GY, LV and SO decreased whereas activities of these enzymes was high in other varieties. Apart from this, higher H_2O_2 accumulation and higher lipid peroxidation activities as well as higher accumulation of stress metabolites such as proline, total carbohydrates were also recorded in the leaf of salt stressed plants of MW, GY, and LV and SO. HPLC analysis revealed that the accumulation of ferulic acid, salicylic acid, chlorogenic acid and caffeic acid were significantly higher in cultivars like GN and LV. In MW, GY, LV and SO, Na^+ content in the leaves and root increased following stress whereas K^+ content increased significantly during the initial phase of salt stress but later with prolonged stress, the content of K^+ declined. The result of present study shows that wheat cultivars KD, GN, KW KD showed the highest tolerance to salinity stress followed by PBW 343, UP 2752 and MW, GY and SO showed the least tolerance to salinity stress.*

Keywords*: Triticum aestivum, salinity stress, antioxidants, metabolites.*

1. INTRODUCTION

Diverse environmental stresses like salinity, drought and temperature are major limiting factors in plant productivity and abiotic stress; particularly salinity and drought are major stresses that cause crop losses worldwide (Bartels and Sunkar, 2005; Vinocur and Altman, 2005). Sustaining productivity under water limited conditions and saving irrigation water are the two most important aspects that need to be addressed immediately in agriculture is to feed the burgeoning population of the country. Rain fed agro–ecosystem has a distinct place in Indian Agriculture, occupying 67% of the cultivated area, contributing 44% of the food grains and supporting 40% of the human and 65% of the livestock population (Venkareswarlu, 2005). In India, water deficit stress limits crop production in about 67% of the net sown area, while 7mha of agriculture land suffers from soil salinity. The percentage of drought affected land areas more than doubled from the 1970s to the early 2000s in the world (Isendahl and Schmidt, 2006). One third of the world's agricultural land is damaged, and approximately 5% of 1.5 Bha of cultivated land is affected by salt (Tabatabaei, 2006). Nagaraja *et al* (2010) reports the impact of drought on agriculture and the challenges being faced by the farmers of South India. Among those stresses that limit plant growth and development, drought is a major factor and is closely related to salt stress (Song *et al.*, 2008). Investigating the physiological and biochemical changes that occur during water stress may help in understanding the effect of water stress. Salt stress is one of the major threats to crop productivity worldwide including India, since about 20–27% of world irrigated land is affected by salinity stress (Ghassami *et al.*, 1995). It is estimated that increased salinization of aerable land will have devastating global effects, resulting in

30% land loss within next 25 years, and up to 50% by the year 2050 (Wang *et al.*, 2003). The problem of soil salinity is further increasing because of the use of poor quality water for irrigation and poor drainage. Therefore, it poses serious problem to food security in developing countries like India due to high rate of population growth and stagnation or declining of crop productivity in high productivity areas (Abdin *et al.*, 2000). Salinity inhibition of plant growth is the result of osmotic and ionic effects and the different plant species have developed different mechanisms to cope with these effects (Munns, 2002).The knowledge acquired regarding the growth and survival of plants under natural conditions could be used as a tool for screening of plant species for afforestation of saline lands. Wheat is a major staple food crop for more than one third of the world population and is the main staple food of Asia (Shirazi *et al.*, 2001). Wheat is essential nourishment for more than 1/3 of the world population and crop yield will be considerably influenced in the perspective of global climate change and limitation of water resources in the environment (Chaves and Oliveira, 2004). Various approaches have been taken to study the effect of drought and salinity stress on wheat varieties. Selection of wheat genotypes/cultivars with better adaptation to water stress should increase the productivity in rainfed areas (Rajaram, 2001). Food insecurity that has increased in recent times owing to competing claims for land, water, labor, energy, and capital, has created more pressure to improve production per unit of land (Godfray *et al.* 2011; Varshney *et al.* 2011). Yamaguchi and Blumwald (2005) states that agricultural productivity is severely affected by soil salinity because salt levels that are harmful to plant growth affect large terrestrial areas of the world. The impact of water and salinity stress in the days following germination, that challenges seedling survival, is almost certainly one of the major limitations for the establishment of species in many habitats. Further work in seedling stage, i.e., fifteen to one month old plant is expected to give significant results.

The present study investigates the effect of salt stress on morphological, physiological and biochemical aspects of nine wheat varieties which differ in their relative tolerance. The results obtained will lead to a better understanding of plants' responses under stress conditions and can be of value in programs conducted to breed salt tolerant crop varieties and these attributes can also be introduced in species of interest through genetic engineering and molecular breeding programmes.

2. MATERIALS AND METHODS

2.1. *Plant material*: Seeds of nine varieties of wheat (*Triticum aestivum* L.) – Mohan Wonder (MW), Kedar (KD), Gayetri (GY), Gandhari (GN), Kaweri (KW), Sonalika (SO), PBW 343, UP 2752, Local variety (LV) were selected for experimental purposes. The seeds were allowed to germinate in the petri plates for one week and then the seedlings were transferred to earthen pots of 12" height and 8" diameter containing sandy loam soil which was mixed with farmyard manure in the proportion of 2:1 by weight (Fig. 2). Plants were maintained in growth chamber at a temperature of 20–25^{0}C, RH 65–70%, 16 h photoperiod and irradiance of 400 μmol m^{-2} s^{-1}.

2.2. *Salt treatment*: To impart salinity stress on the tested nine wheat varieties, one month old plants were treated with sodium chloride (NaCl) solution in water of three different concentrations, i.e. 50mM, 100mM and 200mM sampling was done on 1st and 3rd day of salt stress in each case. For the control (0mM) set, one set of plants from each variety was kept separately and watered regularly. The sampling of the control set was done on the 0 day of treatment when the plants were one month old.

2.3. *Determination of Relative water content (RWC)*: Relative water content (RWC) of leaves was determined as described by Farooqui *et al*. (2000), calculated by the following formula as RWC (%) = (Fresh weight – Dry weight)/ (Fully turgid weight–Dry weight) X 100.

2.4. *Determination of cell membrane stability index (CMS)*: Membrane thermo stability was tested by cell membrane stability (CMS) test following the method of Premchandra *et al*. (1990) as modified by Sairam (1994). The MSI was calculated as Membrane stability index (MSI) = [1 – (C1/ C2)] × 100.

2.5. *Determination of lipid peroxidation*: Peroxidation of lipid was measured as accumulation of malondialdehyde (MDA) which was determined by the thiobarbituric acid reaction. The concentration of MDA was calculated using an extinction coefficient of 155 mmol^{-1} cm^{-1}(Heath and Packer, 1968).

2.6. *Extraction and estimation of phenols*: The total phenol content was extracted from the leaf tissues by following the methodology as given by Mahadevan and Sridhar (1982). The content of total phenol from the leaf tissue was estimated using the method of Bray and Thorpe (1954). The estimation of total phenol was done in dark conditions in the laboratory at normal room temperature.

2.7. *HPLC analysis of phenols*: Phenol extraction and preparation of the sample for HPLC was done by the method described by Pari & Latha (2004) in the dark. Standards for total phenols (1mg/ mL) such as ferulic acid, salicylic acid, chlorogenic acid and caffeic acid were prepared in the same way for HPLC. For the analysis of total phenols in HPLC a method followed by Pari *et al* (2007) was used. For the HPLC finger print analysis of phenolic compounds present in extracts a Shimadzu system (Shimadzu Corp., Kyoto, Japan) was used, a flow rate of 1 mL/min, and gradient elution of HPLC grade of acetonitrile–water–acetic acid (5:93:2, v/v/v) [solvent A] and of acetonitrile–water–acetic acid (40:58:2, v/v/v) [solvent B], a 0– 50 min solvent B from 0 to 100%; and injection volume of 20 μL were applied; whereas the separation of compounds was monitored at 280 nm.

2.8. *Extraction and estimation of antioxidative enzymes*: For the extraction of the enzymes from the leaf sample, 0.5g of leaves from control and stressed wheat seedlings were homogenized in 5 mL of ice–cold 50 mM sodium phosphate buffer, pH 6.8, containing 1% (w/v) polyvinylpolypyrrolidone (PVPP) using liquid nitrogen in a pre–chilled mortar and pestle. The homogenate was then centrifuged at 10,000 rpm for 20 min at –4°C. The supernatant was taken out and used directly as crude extract for enzyme assays. Assay of enzyme activities was done by the method of Chance and Machly (1955) for CAT, Chakraborty *et al*. (1993) for POX, Asada and Takahashi (1987) for APOX, Lee and Lee (2000) for GR and by the method of Dhindsa *et al*. (1981) with some modification for SOD.

2.9. Determination of H_2O_2 accumulation: H_2O_2 levels in the leaves were estimated according to Jena and Choudhuri (1981).The intensity of the yellow colour was measured at 410 nm in the spectrophotometer and H_2O_2 levels were calculated using extinction coefficient 0.28 μmol^{-1} cm^{-1}.

2.10. Microscopic detection of H_2O_2: *In situ* detection of H_2O_2 was carried out following the method of Thordal–Christensen *et al*. (1997) with minor modifications using diaminobenzidine. H_2O_2 was visualized as reddish–brown colour at the site of diaminobenzidine polymerization. Diaminobenzidine polymerizes instantly and locally at sites of peroxidase activity into a reddish–brown polymer.

2.11. **Extraction and quantification of non–enzymatic antioxidants:** Carotenoids were extracted and estimated following the method described by Lichtenthaler (1987) and ascorbate was extracted and estimated following the method described by Mukherjee and Choudhuri (1983).

2.12. **Estimation of total antioxidant activity:** The total antioxidant activity was measured by the method described by Blois (1958).

2.13. **Estimation of $Na^+$and K^+ content:** Na^+ and K^+ content were quantified by flame–photometer (Chemi Line, CL – 411) and expressed as mg g^{-1} dry weight.

3. RESULTS

3.1. Morphological Effect: The plants under salt stress did not show wilting symptoms in all the three concentration of salt, i.e. 50mM, 100mM and 200mM on the first day, but later during the 3rd day wilting symptoms were visible in all cases. The wilting symptoms were much more pronounced in case of LV, SO, MW and GY than the other five varieties in all the concentration of salt with the highest rate of wilting being more in the salt concentration of 200mM during the 3rd day along with yellowing of leaves. In the case of KW, KD, GN the rate of wilting, yellowing of leaf and damage due to salt stress was lowest followed by UP 2752 and PBW 343.

3.2. RWC: RWC in the leaf decreased significantly with induction of salinity stress. RWC in case of KD, GN, PBW 343, UP 2752 and KW showed a lesser decrease during 200mM of salt stress for 3rd day with respect to their control set (0d; 0mM of salt) than in case of MW, GY, LV and SO where increase in the concentration and the duration of salt stress resulted in a greater decline in RWC (Fig. 1).

***3.3. Tolerance index**:* The value of tolerance index in wheat varieties showed a slightly higher value for tolerance index during salt stress, however, in general, the tolerance index during stress decreased in salt stress in all the wheat varieties. GN showed significantly the highest tolerance index (Table 1).

Table 1: Stress tolerance index of nine wheat varieties during salt stress

Varieties/Stress	Tolerance index
MW	– 32.67 ± 2.1
GY	– 35.45 ± 1.2
KD	– 12.19 ± 1.2
GN	– 11.01 ± 1.0
KW	– 11.99 ± 3.4
LV	– 41.02 ± 2.8
UP 2752	– 16.32 ± 4.0
PBW 343	– 18.25 ± 3.7
SO	– 28.66 ± 1.9

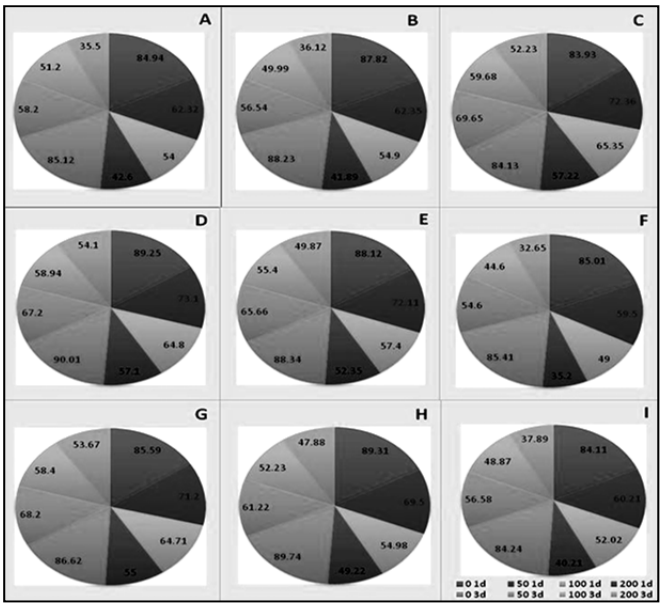


Fig. 1: Relative water content of nine varieties of wheat subjected to salt (NaCl) stress treatments: A– MW, B– GY, C– KD, D– GN, E– KW,F– LV,G– UP 2752, H– PBW 343, I– SO. Results are expressed as the mean of three replicates (10 plants each). 0, 50, 100, 200 corresponds to the concentration of salt (NaCl) in mM and 1d, 3d corresponds to the days of salt treatment

3.4. Cell Membrane Stability: The CMS index was lower during the third day of salt treatment and lowest at the salt

concentration of 200mM in general in all the nine varieties of wheat. LV, GY, SO and MW showed lower value for membrane stability index and the lowest value was observed in LV following salinity stress treatments (Fig. 2).

3.5. Lipid peroxidation of membranes: MDA content following salt stress increased significantly in all the nine varieties with the increase in salt concentration and the duration of salt stress with the highest value observed during the 3rd day of salt stress of 200mM (Fig. 3).

3.6. Phenol contents: The content of total phenol increased with increase in the severity of the stress in all varieties. During salt stress with the increase in the concentration of salt for the 1st and 3rd day the total phenol content increased only in case of KD, GN, KW and UP 2752 with a little lower accumulation during the salt concentration of 100mM in all the days of stress (Fig. 4).

3.7. HPLC profiles: The profile of total phenols in HPLC in the leaf of LV and GN was studied during salinity stress in order to determine and identify the source of the phenols in the leaves. It was evident that the content of total phenols during salinity stress enhanced in case of all the wheat varieties in our study with the highest content recorded in case of GN and the least in case of LV following salt. One of the most prominent peaks observed during the analysis in GN and LV was identified as ferulic acid, followed by vanillic acid, cinnamic acid, chlorogenic acid and also salicylic acid (Fig. 5).

3.8. Changes in anti–oxidative enzymes: Activity of antioxidative enzymes like POX, CAT, APOX, GR and SOD (Fig. 6) following salt stress i.e. salinity stress showed a difference in their activities during different concentration and stages of salinity stress. The activity of all the antioxidative enzymes seemed to be correlated with the each other during the stress response of the plant.

3.9. Changes in H_2O_2 accumulation in the leaves: **D**uring salt stress the accumulation of H_2O_2 continued to increase in all the varieties with increasing concentration of salt and the duration of salt stress however, in case of KW, GN, KD, PBW 343 and UP 2752 the accumulation of H_2O_2 decreased whereas in LV, SO, GY and MW the content of H_2O_2 continued to increase (Table 2).

3.10. Microscopic localization of H_2O_2 following DAB staining: During microscopic studies of the leaf tissues in DAB staining test for the detection of H_2O_2, dark–brown spots were observed as big and small patches at the site of DAB polymerization. The leaf of SO, LV, GY and MW showed more darkly stained DAB–sites in the tissues than in the leaves from the other five varieties with respect to their control during salt stress (Fig. 7).

3.11. Variations in levels of non–enzymatic antioxidants, carotenoids and ascorbate: Carotenoid content (Table 4) showed a general increase in its accumulation with the increase in the concentration and duration of salt stress in all the varieties but with prolonged days of salt stress and increasing concentration of salt the accumulation declined at 100mM concentration in case of MW, GY, LV and SO and at 200mM concentration in case of KD, GN, KW, UP 2752 and PBW 343. Accumulation of ascorbic acid increased at all periods of salt stress and enhanced with increasing concentration of salt in all the varieties (Table 4).

3.12. Total antioxidative activities: The percent inhibition of DPPH i.e. the total antioxidant activity in the leaf of salt stressed plants increased with the increase in the concentration and duration of stress but decreased at the concentration of 100mM in case of MW, LV, SO and GY (Table 3).

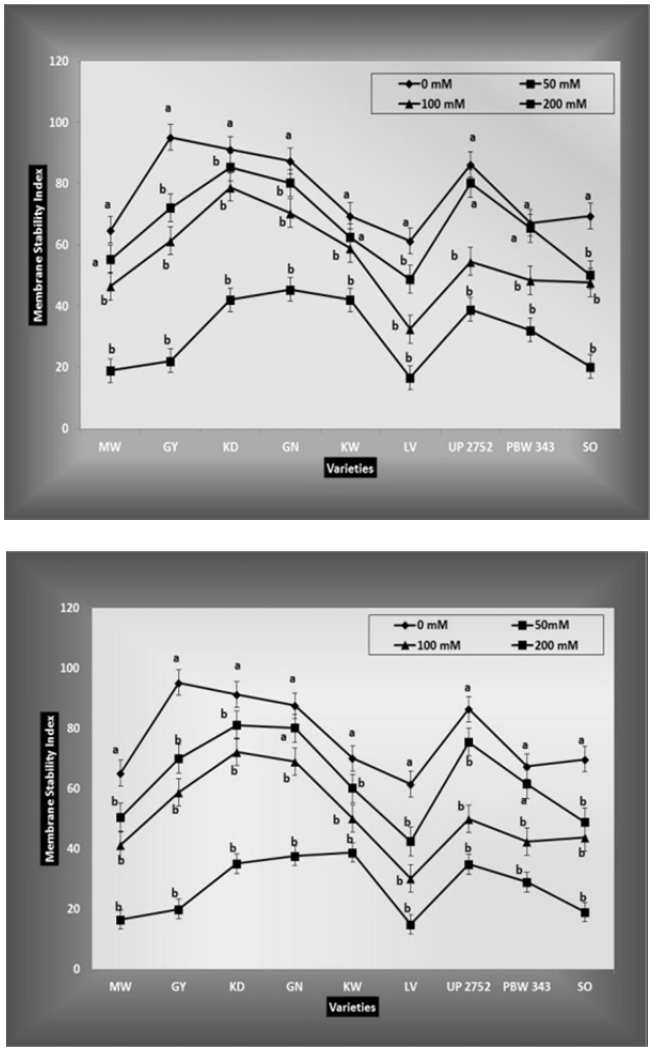


Fig. 2: Cell membrane stability in nine wheat varieties subjected to salt (NaCl) stress treatments for 1 day (A) and 3 days (B) expressed in terms of membrane stability index with K=0.946, Cell constant=1, solution condition=84µS, coefficient–1, 25°C. Bars represent SE. Different letters indicate significant differences with respect to control (p≤0.01). 0mM, 50mM, 100mM, 200mM corresponds to the concentration of salt (NaCl)

In KD, GN, KW, UP 2752 and PBW 343 the total antioxidative activity was significantly high even at 200mM concentration and the highest value for the content of DPPH were observe in these varieties.

3.13. Na^+ and K^+ content: Na^+ and K^+ content in salt stress increased significantly with the onset of stress treatments. Following salt stress treatments the content of Na^+ in case of roots was much higher than that of leaf in all varieties whereas K^+ content was higher in the leaf than the roots during the stress (Table 5).

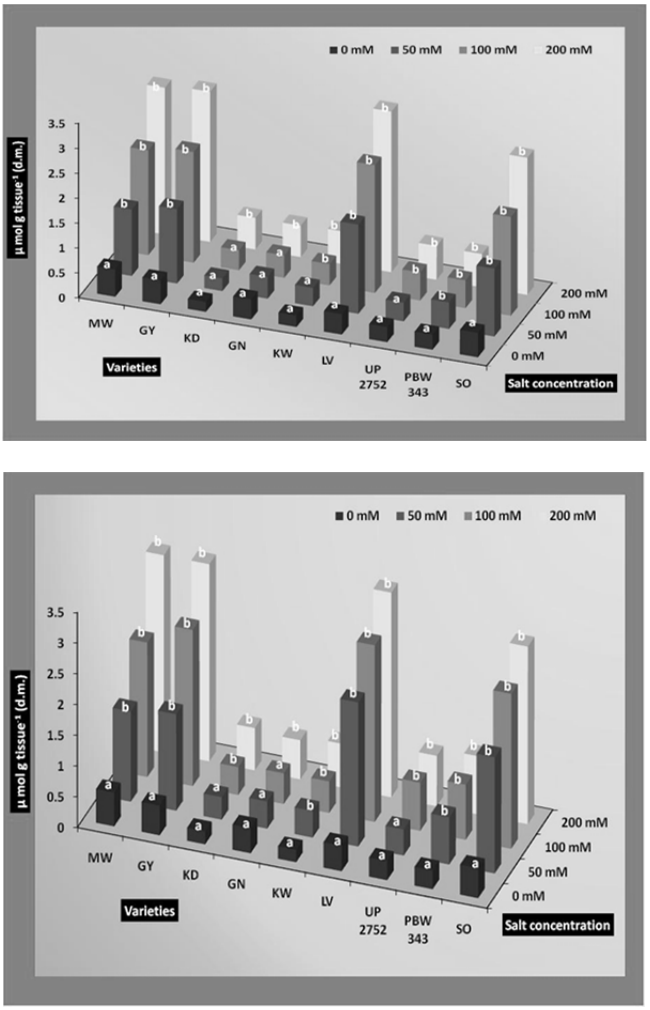


Fig. 3: Effect of salt (NaCl) stress on the lipid peroxidation (expressed as MDA content) in the leaf of nine wheat varieties for the 1st day (A) and 3rd day (B). Different letters indicate significant differences with respect to control (p≤0.01). 0mM, 50mM, 100mM, 200mM corresponds to the concentration of salt (NaCl)

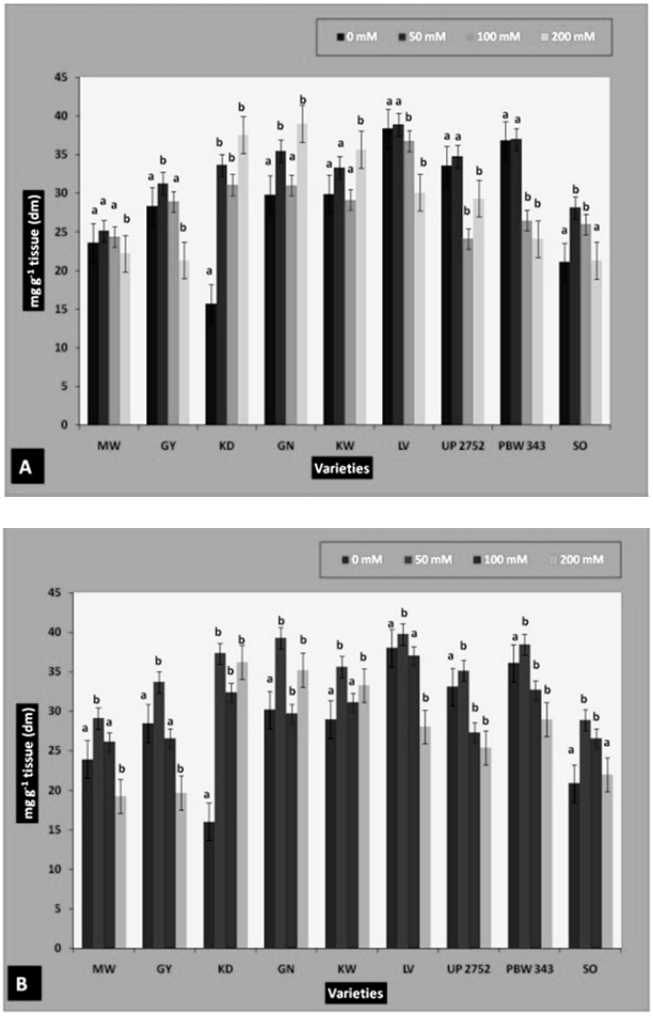
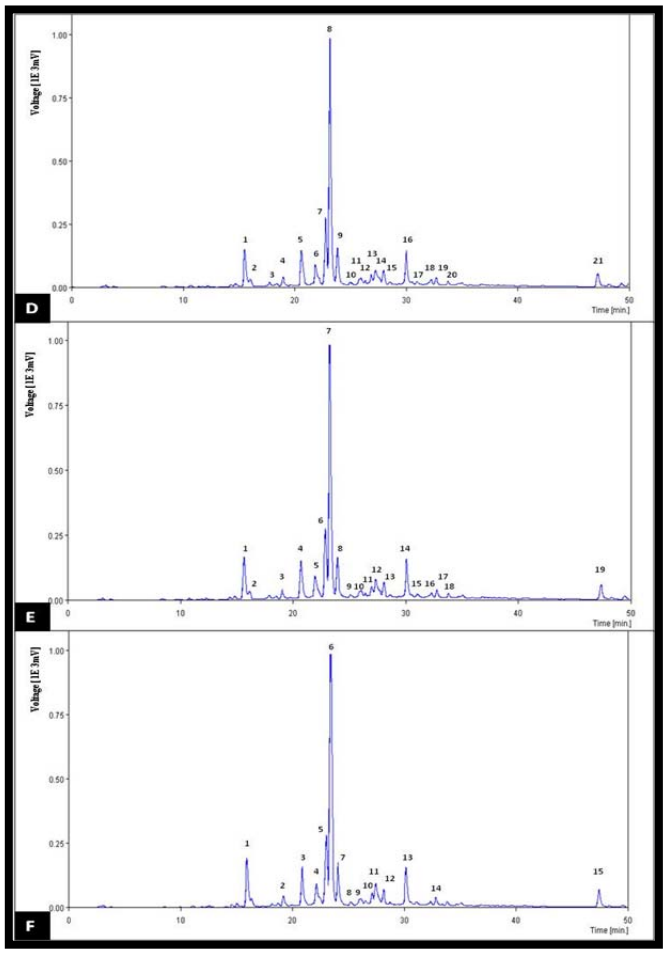


Fig. 4: Content of total phenol in the leaves of nine wheat varieties subjected to salt stress for 1 day (A) and 3 days (B). Dm, dry matter; Bars represent SE. Different letters indicate significant differences with respect to control (p≤0.01). 0mM, 50mM, 100mM, 200mM corresponds to the concentration of salt (NaCl)

Table 2: Content of H_2O_2 in the leaves following salt stress

Varieties	Day of sampling	Content of H_2O_2			
		Concentration of Salt			
		0mM	50mM	100mM	200mM
MW	1d	271.9	379.5	399.6	419.9
	3d	272.0	382.6	411.1	431.2
GY	1d	66.4	361.1	402.2	422.1
	3d	67.0	363.6	406.5	452.6
KD	1d	175.9	301.1	340.2	270.1
	3d	176.2	322.0	335.0	264.5
GN	1d	233.0	355.1	359.5	264.3
	3d	233.5	363.3	341.2	245.5
KW	1d	185.3	297.7	342.2	257.7
	3d	186.0	368.8	301.1	244.4

LV	1d	142.0	405.5	411.1	462.0
	3d	142.5	412.2	440.0	465.5
UP 2752	1d	159.1	295.5	340.1	312.2
	3d	160.2	321.0	336.4	289.9
PBW 343	1d	90.2	314.0	321.2	279.2
	3d	90.3	325.8	323.6	269.0
SO	1d	154.9	298.8	382.2	400.0
	3d	155.2	345.5	398.5	415.0



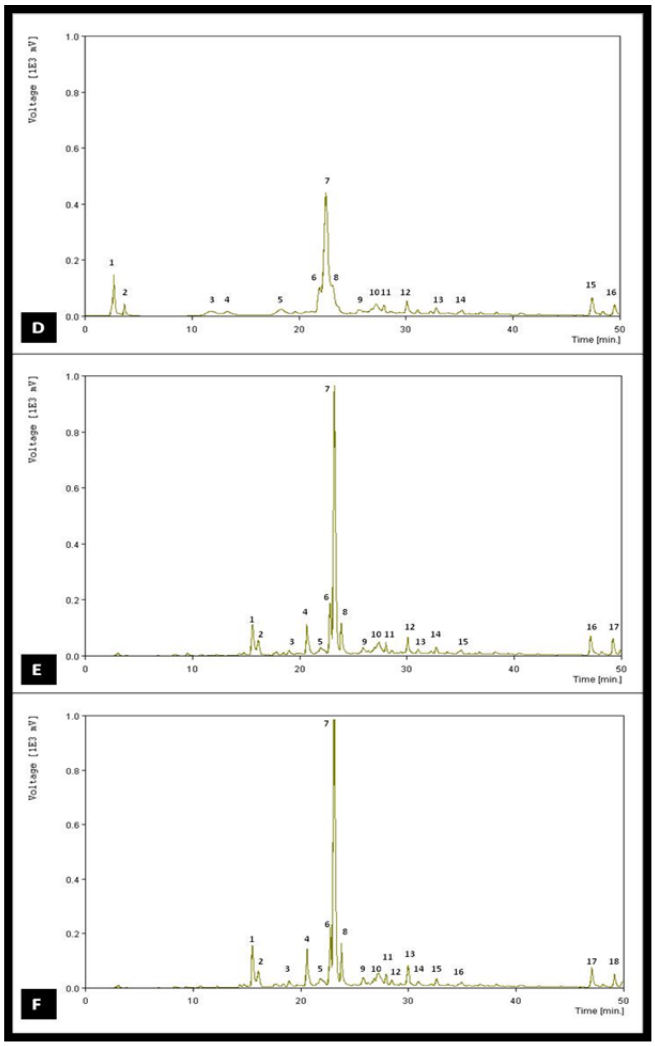


Fig. 5: HPLC profile of total phenols in the leaves during salt stress in one month old plant of GN & LV following NaCl treatment respectively; A–50mM, B–100mM C–200mM for the 3rd day

Table 3: Total antioxidant activity in wheat varieties during salt stress

Varieties	Day of sampling	DPPH (Total antioxidant activity) Concentration of Salt			
		0 mM	50 mM	100 mM	200 mM
MW	1d	8.43	9.20	7.45	4.66
	3d	8.42	9.50	6.11	3.36
GY	1d	4.56	6.56	5.59	4.21
	3d	4.61	7.78	8.20	5.22
KD	1d	8.81	29.35	36.65	55.32
	3d	8.82	39.55	42.21	50.01
GN	1d	4.83	28.88	37.87	54.95
	3d	4.84	35.36	43.65	48.64
KW	1d	8.92	19.98	29.80	36.60
	3d	8.91	21.20	33.30	38.90

LV	1d	5.21	8.50	6.99	4.60
	3d	5.19	8.21	4.87	2.98
UP 2752	1d	6.88	16.98	19.50	29.78
	3d	6.92	18.90	23.50	33.30
PBW 343	1d	5.76	15.68	21.20	28.80
	3d	5.76	19.60	22.74	32.70
SO	1d	7.12	11.80	8.78	5.30
	3d	6.99	13.35	9.10	3.65

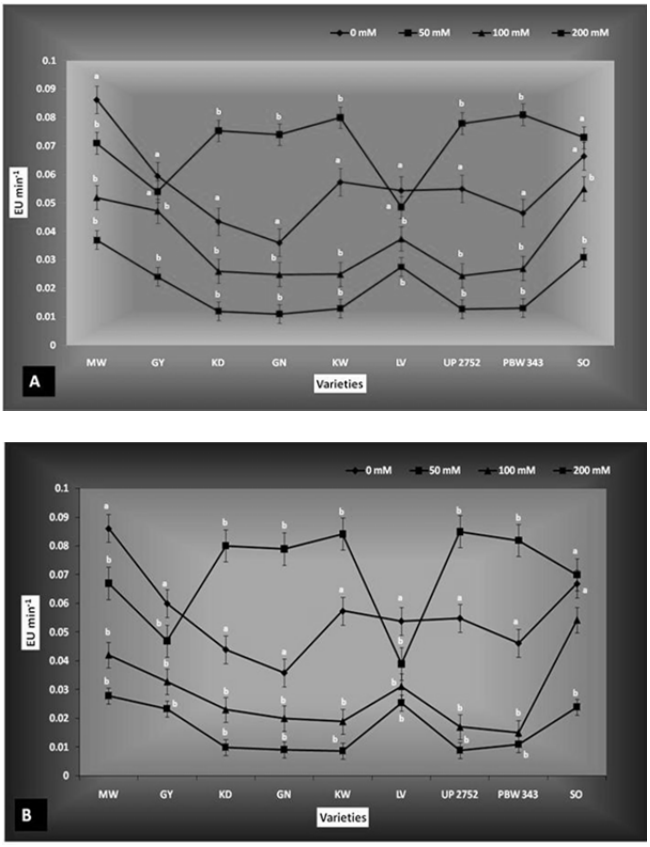


Fig. 6: SOD activities in nine varieties of wheat subjected to salt (NaCl) stress treatments for 1 day (A) and 3 days (B). Bars represent SE. Different letters indicate significant differences with respect to control ($p \leq 0.01$). 0mM, 50mM, 100mM, 200mM corresponds to the concentration of salt (NaCl)

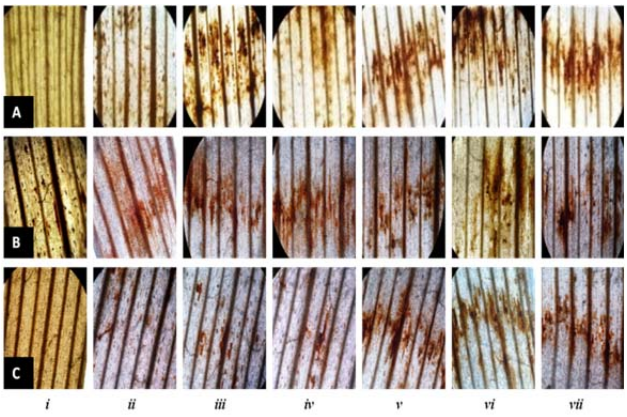


Fig. 7: *In situ* detection of H_2O_2 in mid–portions of leaves of wheat following salt stress: A–KW, B–LV & C–UP 2752; i–0 mM, ii & iii–50mM (1d & 3d), iv & v–100mM (1d & 3d), vi & vii–200mM (1d & 3d) respectively

4. CONCLUSION

The seedling stage of the plants, i.e., one month old plant was the best stage to study the effects of drought and salinity stress on the plant growth and development. During higher concentration of salt varieties such as LV, MW, SO and GY showed yellowing of the leaf and this yellowing and wilting increased with increase in the days of salt stress when compared to the other varieties. The RWC content and the MSI in the leaves decreased at higher concentration of salinity stress along with lower accumulation of non enzymatic antioxidants such as carotenoids and ascorbate along with phenol which was aggravated in LV, MW, SO and GY which also showed lower total antioxidative activity. HPLC analysis also showed the accumulation of few peaks of feulic acid, salicylic acid, chlorogenic and caffeic acid during salt stress. H_2O_2 accumulation in the leaves was higher in these varieties. The overall antioxidative activity expressed through the activity of CAT, POX, APOX, GR and SOD was lower in LV, MW, SO and GY as compared to the other varieties. The result of the present study shows that the KD, GN, KW, KD showed better tolerance to salt stress than the remaining varieties.

Table 4: Carotenoids and ascorbate in the leaves of nine wheat varieties subjected to salt stress

Varieties	Day of sampling	Carotenoid				Ascorbate			
		Concentration of Salt				Concentration of Salt			
		0mM	50mM	100mM	200mM	**0mM**	**50mM**	**100mM**	**200mM**
MW	**1d**	0.043	0.051	0.049	0.042	12.40	12.90	15.80	16.50
	3d	0.043	0.053	0.051	0.038	12.50	13.50	13.80	17.80
GY	**1d**	0.044	0.061	0.042	0.032	9.40	13.10	15.50	17.10
	3d	0.044	0.062	0.043	0.030	9.30	14.90	15.60	17.50
KD	**1d**	0.044	0.058	0.069	0.052	5.50	15.20	16.80	19.20
	3d	0.045	0.059	0.072	0.049	5.40	17.40	18.50	24.10
GN	**1d**	0.042	0.065	0.069	0.053	9.40	15.90	20.10	23.00
	3d	0.043	0.066	0.071	0.050	9.50	17.80	22.20	23.90

KW	**1d**	0.044	0.057	0.064	0.052	11.02	14.20	19.40	23.00
	3d	0.045	0.061	0.065	0.051	11.40	18.10	21.00	23.50
LV	**1d**	0.048	0.049	0.039	0.028	9.98	11.20	13.90	17.20
	3d	0.049	0.052	0.037	0.025	10.00	11.32	14.50	18.10
UP 2752	**1d**	0.050	0.058	0.060	0.041	12.11	13.50	16.60	21.20
	3d	0.050	0.059	0.061	0.043	12.00	14.50	17.90	23.00
PBW 343	**1d**	0.047	0.049	0.056	0.042	11.96	15.00	19.20	20.00
	3d	0.048	0.052	0.041	0.028	12.00	15.60	19.40	22.10
SO	**1d**	0.041	0.048	0.043	0.034	11.40	13.40	17.10	17.80
	3d	0.041	0.052	0.041	0.028	11.35	13.90	18.50	18.80

Table 5: Effect of salt stress on sodium (Na^+) and potassium (K^+) contents in the leaf of wheat varieties

Varieties	**Days of sampling**	**Content of Na^+**				**Content of K+**			
		Concentration of Salt				**Concentration of Salt**			
		0mM	**50mM**	**100mM**	**200mM**	**0mM**	**50mM**	**100mM**	**200mM**
MW	**1d**	6.21	7.11	10.13	14.24	19.87	16.31	12.32	6.63
	3d	6.19	8.97	15.65	19.98	20.01	14.51	11.21	5.65
GY	**1d**	7.11	6.97	11.51	17.64	21.11	16.55	13.66	7.22
	3d	7.10	8.97	16.52	18.99	20.08	13.96	11.35	5.99
KD	**1d**	4.51	6.89	11.11	13.21	22.45	19.25	15.35	14.55
	3d	4.49	7.32	11.99	15.22	21.02	17.55	14.85	11.35
GN	**1d**	4.88	6.12	9.94	10.99	23.01	21.03	17.41	15.64
	3d	4.89	6.25	10.01	12.02	22.98	18.91	15.42	13.63
KW	**1d**	5.01	7.01	12.21	13.99	25.14	18.96	16.55	13.64
	3d	5.09	7.22	12.52	14.32	25.03	16.52	13.25	11.05
LV	**1d**	7.01	7.85	18.84	21.01	18.97	14.52	8.64	4.36
	3d	6.99	7.96	19.98	22.10	18.94	11.01	6.67	2.99
UP 2752	**1d**	5.99	6.95	12.64	15.33	23.13	17.82	12.31	10.32
	3d	6.01	7.23	14.65	16.64	23.23	15.66	10.55	9.22
PBW 343	**1d**	6.47	7.12	12.32	15.97	19.64	16.98	12.66	10.8
	3d	6.45	7.56	13.99	17.52	19.61	14.22	11.52	9.99
SO	**1d**	5.89	7.02	14.33	18.85	19.64	15.55	12.02	8.85
	3d	5.91	7.22	15.22	18.91	19.81	13.25	11.51	8.23

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