Indian J. Plant Physiol., Vol. 16, No. 2, (N.S.) pp. 141-146 (April-June, 2011)

ASSESSMENT OF VARIABILITY IN INTRINSIC MESOPHYLL AND CARBOXYLATION EFFICIENCIES IN WHEAT (*TRITICUM AESTIVUM* L.) FOR SALT TOLERANCE

RENU MUNJAL[1*], V. PHOGAT[2], S.K. SHARMA[2], SANJAY KUMAR[2], SATYAVAN[2] AND S.K. SETHI[1]

[1]Department of Genetics & Plant Breeding, [2]Department of Soil Science
CCS Haryana Agricultural University, Hisar-125004, India

Received on 25th June, 2010, Revised and Accepted on 23rd May, 2011

SUMMARY

Improving the salt tolerance of wheat requires access to genetic diversity and efficient techniques. Genetic progress in increasing yield potential is closely associated with increased photosynthetic activity. To assess the performance of 14 wheat varieties under different saline water irrigation treatments comprising canal water taken as contol (EC of 0.4 dSm^{-1}), EC of 5.0 and 7.5 dSm^{-1}, an experiment was carried out in micro-plots. The different genotypes performed differently in grain yield. The genotypes WH-1063, WH-1054 and WH-1080 were relatively salt tolerant as they had higher mean grain yield as well as higher yield at EC of 7.5 dSm^{-1}. On the contrary, genotypes WH-1053 and WH-1076 were relatively salt sensitive on the basis of grain yield obtained. The photosynthetic CO_2 fixation rate decreased by about 19 and 30% when subjected to salt stress of 5.0 and 7.5 dSm^{-1}, respectively, however, reductions in stomatal conductance (gs) and transpiration rate were much greater, amounting to 42 and 63% for gs, and 34 and 50% for transpiration rate under the intermediate (5.0 dSm^{-1}) and high salt stress (7.5 dSm^{-1}), respectively. The reduction in inter cellular CO_2 concentration (Ci) was much lower, amounting to only 8 and 12%, respectively, at stress levels of 5.0 and 7.5 dSm^{-1}. Salt-tolerant cultivars (WH 1045, WH1054, WH1062, WH 1063, WH 1077, WH 1078 and WH 1080) had high stomatal conductance ranging from 0·185 to 0·382 mmol m^{-2} s^{-1}. The intrinsic mesophyll efficiency showed a negative correlation with the yield (R^2 = 0·36), but a positive correlation of intrinsic carboxylation efficiency with yield (R^2 = 0·43) was observed. So it is assumed that intrinsic mesophyll and carboxylation efficiencies can be used as physiological parameters to screen wheat genotypes for salt tolerance.

Key words: **Photosynthetic rate, salinity, stomatal conductance, substomatal CO_2 concentration, wheat**

INTRODUCTION

Salinity is a widespread soil problem limiting productivity of crops worldwide. Plants vary, however, in their ability to cope with salinity as is evidenced by the wide diversity of plant habitats, ranging from nonsaline environments to the extreme salinities of sea. For crop plants, differences in salt flower exist not only among different genera and species, but even within a species (Ashraf and O'Leary 1996, Phogat *et al.* 2001, Phogat *et al.* 2008). Soil salinisation can reduce plant growth by perturbing biomass allocation, ion relations, water relations and other physiological processes or by a combination of such factors (Ashraf 1994, Munns and James 2003). Improving the salt tolerance of crop requires access to new genetic diversity and efficient techniques for identifying salt-tolerance. Physiological mechanisms that underlie traits for salt tolerance could

*Corresponding author, E-mail: munjalrenu66@gmail.com

be used to identify new genetic sources of salt tolerance. Genetic progress in increasing yield potential is closely associated with increased photosynthetic activity (Nagarajan 2004). Significant inhibition of photosynthesis results in considerable loss of potential productivity. Salinity is known to inhibit photosynthesis due to the direct affect of salts on stomatal conductance via a reduction in guard cell turgor and intercellular CO_2 (El-Hendawy 2005) Cheeseman (1988), Fedoroff (2006) and Ismail (2003) reported that the transpiration rate generally tends to decline with increasing rhizospheric salinity and this could be attributed to lower water potential in roots and the transport of abscisic acid (ABA) from root to shoot to induce stomatal closure. In wheat, James *et al.* (2002) observed that reduction in stomatal conductance, occurred under salt stress before an apparent decline in leaf water potential, and argued that chemical signals are likely to cause the decrease in stomatal conductance (gs). The early stomatal response to salinity also suggested the involvement of root–shoot communication in this initial acclimation stage of the tolerant lines which resulted in stomatal conductance getting decreased substantially before any noticeable change in leaf water potential. Stomatal conductance is a sensitive indicator of the salt stress because it is reduced immediately with the onset of salinity and is the initial and most profound cause of decline in CO_2 assimilation rate. Higher stomatal conductance under salt stress is related to higher CO_2 assimilation rate (James *et al.* 2008). The aim of this study was to determine whether there is genetic variation between intrinsic mesophyll and carboxylation efficiencies in genotypes that can be used as physiological parameters to screen wheat genotypes for salt tolerance.

MATERIAL AND METHODS

Fourteen wheat varieties (WH1045, WH1051, WH1052, WH1053, WH1054, WH1058 WH1061, WH1062, WH1063, WH1076, WH1077, WH1078, WH1080 and KRL 19) differing in their tolerance of salinity were evaluated during rabi 2007-08 under different saline water irrigation treatments comprising canal water (EC 0.4 dSm^{-1}), EC 5.0 and EC 7.5 dSm^{-1}, in micro-plots of 2 m x 2 m in size. Initially the soil was non-saline throughout the profile. Five irrigation schedule based on the recommendations for the non-saline irrigated soils were followed. The soil was allowed to stabilise before sowing the crop. Split plot design with three replicates was used. Recommended package and practices were followed in growing the crop. The crop was irrigated with saline water prepared by mixing highly saline ground water (EC 24-28 dSm^{-1}) with good quality water (EC 0.4 dSm^{-1}) in different ratios to get water of, EC 5.0 and EC 7 dSm^{-1}. Gas exchange parameters viz. A-assimilation rate (μmol m^{-2} s^{-1}), gs-stomatal conductance (mol $m^{-2}s^{-1}$), Ci-intercellular CO_2 concentration (ppm), T-transpiration rate (mmol $m^{-2}s^{-1}$) were measured 100 d after the imposition of salt stress in flag leaves using an open system LCA-4 ADC portable infrared gas analyser (Analytical Development Company, Hoddeson, England). These measurements were carried out from 10:00 to 14.00 hours with the following specifications/adjustments: leaf surface area 6.25 cm^2, ambient CO_2 concentration (C_{ref}) 371 μmol mol^{-1}, temperature of leaf chamber (Tch) varied from 25-28°C, leaf chamber volume gas flow rate (v) 296 mL min^{-1}, leaf chamber molar gas flow rate (U) 400 μmol s^{-1}, ambient pressure (P) 97.95 kPa, PAR (Q_{leaf}) at leaf surface maximum up to 770 μmol m^{-2} s^{-1}. A caution was also made that the measurements of control plants were immediately followed by that of the same cultivar under saline conditions. Intrinsic mesophyll efficiency (Ci/gs) and Intrinsic carboxylation efficiency (A/Ci) were calculated from the above observations. The data are mean values of three replications with four measurements per replication. The data was analysed statistically and the treatment means were compared using LSD technique at 5% probability for RBD (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

The grain yields of different genotypes of wheat showed a considerable variation and decreased with an increase in EC of the irrigation water (Table 1). Under salinity, mean grain yield of genotypes WH-1078, WH-1054, WH-1063 and WH-1045 was significantly higher than that of WH-1076, WH-1051 and WH-1053. However, genotype WH-1063, WH-1080, WH-1062, WH-1061, WH-1054 and WH-1077 gave higher yield at EC of 7.5 dSm^{-1}. Thus, different genotypes performed differently in grain yield recorded. Therefore, the genotypes WH-1063, WH-1054 and WH-1080 seem to

Table 1. Effect of salinity stress on grain yield (g m^{-2}) of different wheat genotypes

Genotypes	EC (dSm^{-1})			
	0.4	**5.0**	**7.5**	**Mean**
WH-1045	375	250 (33.3)	180 (52.0)	268.33
WH-1051	250	200 (20.0)	170 (32.0)	206.67
WH-1052	350	200 (42.9)	150 (57.1)	233.33
WH-1053	350	175 (50.0)	100 (71.4)	208.33
WH-1054	425	250 (41.2)	200 (52.9)	291.67
WH-1058	300	225 (25.0)	175 (41.7)	233.33
WH-1061	315	235 (25.4)	210 (33.3)	253.33
WH-1062	300	250 (16.7)	215 (28.3)	255.00
WH-1063	360	265 (26.4)	235 (34.7)	286.67
WH-1076	250	150 (40.0)	100 (60.0)	166.67
WH-1077	335	240 (28.4)	200 (40.3)	258.33
WH-1078	450	250 (44.4)	175 (61.1)	291.67
WH-1080	300	250 (16.7)	225 (25.0)	258.33
KRL-19	350	200 (42.9)	175 (50.0)	241.67
Mean	336.43	224.29	179.29	
CD (5%)	Genotype (G)	= 21.79	Salinity (S)	= 11.65
	G X S	= 43.59		

Values in parenthesis indicate percent reduction over control

be relatively salt tolerant as they had higher mean grain yield as well as higher yield at EC of 7.5 dSm^{-1}. On the contrary, genotypes WH-1053, WH-1078 and WH-1076 were relatively salt sensitive on the basis of grain yield obtained. This could be attributed to relatively higher photosynthetic capacity of tolerant genotypes at the vegetative stage which could have played a significant role in the grain yield variation of genotypes.

Variable genotypic responses with the salt-tolerant and sensitive lines showed a relatively greater decline in assimilation rate, stomatal conductance and transpiration rate (Table 2). Salinity also resulted in significant reduction in intracellular CO_2 concentration, but to a lesser extent than assimilation rate, stomatal conductance and transpiration rate. The reduction in Ci

Table 2. Effect of salinity stress on assimilation rate, stomatal conductance, internal CO_2 concentration and transpiration rate of different wheat genotypes

Genotypes	Assimilation rate ($\mu mol\ m^{-2}s^{-1}$)	Stomatal conductance ($mol\ m^{-2}s^{-1}$)	Transpiration rate ($mmol\ m^{-2}s^{-1}$)	Intercellular CO_2 concentration (ppm)
WH-1045				
0.4 dS/m	8.75	0.235	5.91	279.6
5.0 dS/m	6.98	0.185	5.15	176.5
7.5 dS/m	6.74	0.156	3.25	237.5
Mean	**7.49**	**0.192**	**4.77**	**231.2**
WH-1051				
0.4 dS/m	9.74	0.215	6.67	293.4
5.0 dS/m	8.25	0.188	6.02	265
7.5 dS/m	6.7	0.167	4.95	286.7
Mean	**8.23**	**0.190**	**5.88**	**281.7**
WH-1052				
0.4 dS/m	6.3	0.167	3.75	354.4
5.0 dS/m	5.68	0.095	2.62	169.2
7.5 dS/m	5.15	0.059	2.15	273.5
Mean	**5.71**	**0.107**	**2.84**	**265.7**
WH-1053				
0.4 dS/m	6.73	0.112	3.05	307.7
5.0 dS/m	4.91	0.08	2.9	237.2
7.5 dS/m	4.14	0.063	1.73	283.1
Mean	**5.26**	**0.085**	**2.56**	**276.0**
WH-1054				
0.4 dS/m	10.94	0.312	7.85	289.9
5.0 dS/m	10.23	0.265	6.81	143.2
7.5 dS/m	8.14	0.263	6.13	218.5
Mean	**9.77**	**0.280**	**6.93**	**217.2**
WH-1058				
0.4 dS/m	8.17	0.165	5.03	329.3
5.0 dS/m	7.65	0.105	3.7	225.0
7.5 dS/m	5.15	0.090	2.58	293.2
Mean	**6.99**	**0.120**	**3.77**	**282.5**
WH-1061				
0.4 dS/m	7.36	0.185	4.18	388.7
5.0 dS/m	6.29	0.112	3.87	172.0
7.5 dS/m	5.25	0.078	3.05	303.3
Mean	**6.3**	**0.125**	**3.7**	**288**
WH-1062				
0.4 dS/m	8.32	0.257	6.15	315.7
5.0 dS/m	7.93	0.181	5.26	243.7
7.5 dS/m	7.15	0.147	2.18	274.6
Mean	**7.8**	**0.195**	**4.53**	**278**
WH-1063				
0.4 dS/m	12.32	0.427	9.12	237.3
5.0 dS/m	11.46	0.365	7.85	135.3
7.5 dS/m	7.48	0.354	7.21	212.4
Mean	**10.42**	**0.382**	**8.06**	**195**

Genotypes	Assimilation rate (µmol $m^{-2}s^{-1}$)	Stomatal conductance (mol $m^{-2}s^{-1}$)	Transpiration rate (mmol $m^{-2}s^{-1}$)	Intercellular CO_2 concentration (ppm)
WH-1076				
0.4 dS/m	8.07	0.129	4.14	292.9
5.0 dS/m	6.25	0.085	3.3	172.9
7.5 dS/m	3.89	0.077	2.94	240.7
Mean	**6.07**	**0.097**	**3.46**	**235.5**
WH-1077				
0.4 dS/m	9.1	0.205	6.97	297.7
5.0 dS/m	7.75	0.143	4.81	265.3
7.5 dS/m	5.11	0.108	2.05	290.5
Mean	**7.32**	**0.152**	**4.61**	**284.5**
WH-1078				
0.4 dS/m	9.13	0.245	5.55	274.1
5.0 dS/m	8.96	0.182	5.27	178.3
7.5 dS/m	7.32	0.164	4.95	247.2
Mean	**8.47**	**0.197**	**5.35**	**233.2**
WH-1080				
0.4 dS/m	9.53	0.345	6.25	278.8
5.0 dS/m	8.67	0.290	5.45	165.2
7.5 dS/m	8.05	0.220	5.4	247.5
Mean	**8.75**	**0.285**	**5.7**	**230.5**
KRL-19				
0.4 dS/m	9.24	0.275	5.93	319.2
5.0 dS/m	7.8	0.173	5.17	293.2
7.5 dS/m	7.02	0.113	4.59	270.2
Mean	**8.02**	**0.187**	**5.23**	**294.2**
CD (5%)	G = 3.81 S = 1.52 GXS = 5.68	G = 0.18 S = 0.05 GXS = 0.39	G = 3.58 S = 1.23 GXS = 5.12	G = 115.5 S = 61.3 GXS = 203.5

was much lower, amounting to only 8 and 12%, respectively, at stress levels of 5 and 7.5 dSm^{-1}. The salt-tolerant genotypes (WH-1063, WH-1054 and WH-1080) had the higher mean stomatal conductance as compared to salt sensitive genotypes (WH-1053 and WH-1076). The tolerant line (WH-1063) had the maximum photosynthetic rate under salt stress followed by WH-1054 (Table 1). However, the higher photosynthesis of WH 1063 was also associated with higher stomatal conductance (gs) under salinity. The salt-tolerant cultivars seem to have better control over their stomata and maintained lower transpiration rates in the short term immediately after the imposition of salt stress (Table 2) probably to limit damaging effects of the influx of large quantities of salt and to allow further acclimation. James *et al.* (2002) observed a large decrease in stomatal conductance of two contrasting wheat genotypes under salinity, which was not associated with poor water relations, but presumably due to 'root signals' as the leaf turgor of both genotypes did not change under stress. Mechanisms underlying the longer term adaptive responses observed in the tolerant cultivar involve processes that resulted in better control of uptake and/or translocation of toxic salts to the shoot.

Intercellular CO_2 concentration (Ci) did not show a significant reduction under salt stress, and this is associated with higher gs. The lack of effects of salinity on Ci again suggested a direct effect of salinity on carbon assimilation, apart from its effect on gs. Salt accumulation in the mesophyll cells may inhibit carbon assimilation, resulting in an increase in internal CO_2 concentration, with eventual reduction in stomatal conductance (Salama *et al.* 1994, Maxwell and Johnson 2000, Ouerghi *et al.* 2000). The intrinsic carboxylation efficiency (A/Ci) showed a significant positive relationship with yield (r = 0.67). However, Ci/gs showed a significant inverse relationship (r = 0.63) with yield. The photosynthetic CO_2 fixation rate decreased by about 19 and 30% when subjected to salt stress of 5.0 and 7.5 dSm^{-1}, respectively, however, reductions in stomatal conductance (gs) and transpiration rate were much greater, amounting to 42 and 63% for gs, and 34 and 50% for transpiration rate (T) under the intermediate and high salt stress, respectively. The reduction in Ci was much lower, amounting to only 8 and 12%, respectively, at stress levels of 5 and 7.5 dS m^{-1}. The salt-tolerant cultivar (WH 1045, WH1054, WH1062, WH 1063, WH 1077 and WH 1078) had the highest stomatal conductance under both 5 and 7.5 dS m^{-1}, amounting to 0·382 and 0·185 mmol m^{-2} s^{-1}, compared with 0·120 and 0·085 mmol m^{-2} s^{-1}, respectively.

To investigate the correlation of yield with assimilation rate, transpiration rate, stomatal conductance and intercellular CO_2 concentration (Fig. 1) regression analysis was done. Positive value for correlation was found in all the four parameters but the value was high for intercellular CO_2 concentration (R^2 = 0·54) followed by stomatal conductance (R^2 = 0·39) assimilation rate (R^2 = 0·29) and transpiration rate (R^2 = 0·24). The intrinsic mesophyll efficiency (Fig. 1) correlated

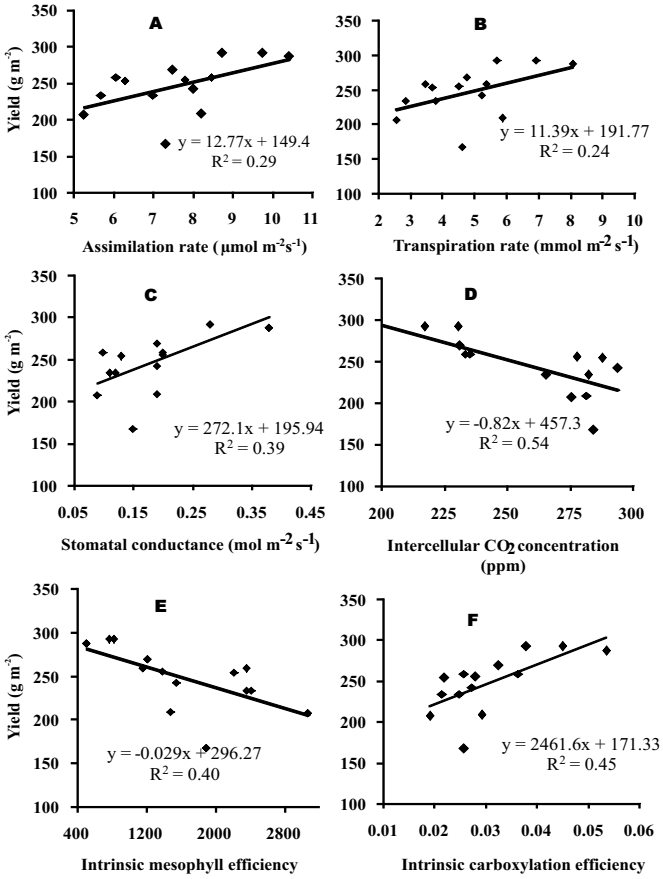

Fig. 1. Correlation of yield with assimilation rate (A), transpiration rate (B), Stomatal conductance (C), Intercellular CO_2 concentration (D), Intrinsic mesophyll efficiency (E) and Intrinsic carboxylation efficiency (F)

negatively with the yield (R^2 = 0·40), but a positive correlation of intrinsic carboxylation efficiency with yield (R^2 = 0·45) was observed.

Up regulation of the photosynthetic system appears to play a role in salt tolerance of wheat, with tolerant genotypes maintaining relatively higher photosynthetic function at reproductive stage leading to higher grain yield. This study showed that there was correlation between intrinsic mesophyll and carboxylation efficiencies in tolerant genotypes. So it is assumed that intrinsic mesophyll and carboxylation efficiencies can be used as physiological parameters to screen wheat genotypes for salt tolerance.

REFERENCES

Ashraf, M. (1994). Breeding for salinity tolerance in plants. *Crit. Rev. Plant Sci.* **13:** 17-42.

Ashraf, M. and O'Leary, J.W. (1996). Response of newly developed salt tolerant genotypes of spring wheat to salt stress, yield components and ion distribution. *J. Agron. & Crop Sci.* **176:** 91-101.

Cheeseman, J.M. (1988). Mechanisms of salinity tolerance in plants. *Plant Physiol.* **87:** 547-550.

El–Hendawy, S.E., Hu, Y. and Schmidhalter, U. (2005). Growth, ion content, gas exchange and water relations of wheat genotypes differing in salt tolerance. *Australian J. Agric. Res.* **56:** 123-134.

Fedoroff, N. (2006). Redox regulatory mechanisms in cellular stress responses. *Annals of Botany.* **98:** 289–300.

Gomez, K.A. and Gomez, A.A. (1984). Stastical Procedure for Agricultural Research (2nd ed.). John Willey and Sons. Inc. New York.

Ismail, A.M. (2003). Effect of Salinity on the physiological responses of selected lines/variety of wheat. *Acta Agronomica Hungarica.* **51:** 1-9.

James, R.A., von Caemmerer, S., Condon, A.G., Alexander, Z.B. and Munns, R. (2008). Genetic variation in tolerance to the osmotic stress component of salinity stress in durum wheat. *Functional Plant Biol.* **35:** 111-123.

James, R.A., Rivelli, A.R., Munns, R., von Caemmerer, S. (2002). Factors affecting CO_2 assimilation, leaf injury and growth in salt-stressed durum wheat. *Functional Plant Biol.* **29:** 1393–1403.

Maxwell, K. and Johnson, G.N. (2000). Chlorophyll fluorescence: a practical guide. *J. Expt. Bot.* **51:** 659–668.

Munns, R. and James, R.A. (2003). Screening methods for salinity tolerance: a case study with tetraploid wheat. *Plant and Soil.* **253:** 201-218.

Nagarajan, S. (2004). Sustaining the green revolution in India – A success story of wheat. APAARI Publication 2004/ 3, Bangkok, Thailand, p. 46.

Ouerghi, Z., Cornic, G., Roudani, M., Ayadi, A. and Brulfert, J. (2000). Effect of NaCl on photosynthesis of two wheat species (*Triticum durum* and *Triticum. aestivum*) differing in their sensitivity to salt stress. *J. Plant Physiol*. **156:** 335-340.

Phogat, V., Satyawan., Kumar, S., Sharma, S.K., Kapoor, A.K. and Kuhad, M.S. (2001). Performance of cotton (*Gossypium* species) and wheat (*Triticum aestivum*) genotypes under different salinity conditions. *Indian J. Agric. Sci.* **71:** 303-305.

Phogat, V., Satyawan, Kumar, S., Sharma, S.K. and Nandwal, A.S. (2008). Ion based selection of cotton (*Gossypium hirsutum* L.) genotypes for salinity tolerance. *J. Plant Biol.* **35:** 17-24.

Salama, S., Trivedi, S., Busheva, M., Arafa, A., Garab, G. and Erdei, L. (1994). Effects of NaCl salinity on growth, cation accumulation, chloroplast structure and function in wheat cultivars differing in salt tolerance. *J. Plant Physiol.* **144:** 241-247.