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EVALUATION OF SALT TOLERANCE IN WHEAT GENOTYPES ON GROWTH AND CARBON ISOTOPES DISCRIMINATION TECHNIQUE

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Abstract

Studies were conducted in green house to select suitable salt tolerant wheat genotypes on the basis of growth performance and carbon isotopes discrimination (CID) technique. Nine newly developed double haploids (DH) wheat genotypes were tested under gravel culture, along with salt tolerant (LU-26s) and high yielding (Sarsabz) checks. The crop was irrigated by non-saline (control) and saline (12dS/m) water and raised up to maturity, growth parameters (i.e. plant height, plant biomass, productive tillers, spike length, number of spiklets/spike, number of grains / spike, grain weight/ spike and grain yield/ 15 plants) were recorded after harvesting. Plant samples (straw) were collected and were analyzed for carbon isotopic ratio (C^{12}/ C^{13}) from IAEA laboratories Vienna Austria. The data showed that there was significant decrease in all the growth parameters due to salinity. On the basis of performance in different growth parameters it was found that wheat genotypes V3-DH, V9-DH, V10-DH, V13-DH, and LU-26s had good response at 12dSm^{-1}, thus can be categorized as better performing genotypes. Studies on carbon isotopes discrimination (CID) showed a decreasing trend under salinity. Mean CID values were 20.86 and 17.49‰ under two environments (non saline and saline, respectively), showing an overall 19% decrease under salinity. Generally the wheat genotypes having higher grain yield also had high carbon isotopes discrimination (CID). The relationship between grain yield and CID (Δ) was positive (R^2 = 0.695). The genotypes V10-DH, V13-DH with lower decrease in CID (i.e. 1.2 & 11.0%, respectively), also had high grain yield under salinity. Therefore the studies suggest that we can include CID technique as one of the selection criteria for salt tolerance.

Key words: Salinity, Wheat, Carbon isotopes discrimination, Growth performance.

Introduction

The major threats to world food security are increasing population, increasing food demand, shrinkage of cultivable land due to salinity and declining water availability. Abiotic stresses, including salinity and drought occur naturally (Dai 2011; Jacobson and Adams 1958) however both stresses are becoming more severe due to human activities such as deforestation, salt mining, poor irrigation of water and emission of greenhouse gases (Ghassemi *et al*., 1995; Marcum 2006; Anon., 2000). Szabolcs, 1989 reported that approximately over 1000 million hectares of land are affected by salinity worldwide. The ultimate results of these stresses lead to reduction in growth and biomass of plants (Ainsworth and Ort 2010). The limiting plant germination and early seedling growth is mostly due to water stress brought about by drought or salinity (Almansouri *et al*., 2001, Khan *et al*., 2007). As water and salt stresses occur frequently and can affect most habitats, plants have developed several strategies to cope with these challenges: either adaptation mechanisms, which allow them to survive the adverse conditions, or specific growth habits to avoid stress conditions. Stress-tolerant plants have evolved certain adaptive mechanisms to display different degrees of tolerance, which are largely determined by genetic plasticity. Plant species and the genotypes within a species vary in their growth response under stress.

Wheat is the most important food crop of Pakistan and ranks first among all the cereals. In Pakistan, it grows around 9.0 million hectares with annual production around 24.0 million tons (Anonymous, 2014). Wheat yield of the country is considerably affected by abiotic stresses such as high temperature, drought and salinity. It is obvious that present and future wheat food security face the problem of water scarcity or salinity. Screening of suitable salt tolerant crops has been attempted by many researchers on various crops at early seedling stage and maturity (Khan *et al*., 2006; Khan *et al*., 2007; Khan *et al*., 2009; Khan *et al*., 2014, Soleimani *et al*., 2011). Keeping in view the extent of reduction in crop yield due to salinity from 30-50%, a research study has been taken to screen salt tolerant wheat genotypes for saline areas of Sindh. The study comprised of Agronomical performance and use of CID (Carbon isotopes discrimination) as screening tool for selecting salt tolerant wheat genotypes. The study will provide a selective criterion for the selection of suitable wheat germplasm/ mutant in respect to stress condition and also provide the information to plant breeders in developing new high yielding genotypes for saline areas.

Material and Methods

Nine wheat genotypes (double haploid) were tested in green house using completely randomized block design (RCBD). Crops was irrigated by 1/4th strength nutrient solution. Two treatments i.e. non saline (1.4 dSm^{-1}) and saline (12.0 dSm^{-1}) were induced through irrigation salinized by commercial NaCl salt after two weeks of sowing. Irrigation was applied at the interval of two weeks or when ever required. Salinity (Electrical conductivity) of the nutrient solution was monitored regularly throughout the season by installing micro-tentionic. Growth and yield parameters (i.e. Plant biomass, plant height, productive tillers, spike length, number of spike lets/spike, number of grains/plants and 100 grains weight) were recorded at the time of crop maturity. Genotypes were categorized as tolerant and sensitive on the basis of less than 50% reduction in different variables. Carbon isotopes discrimination (CID) technique was also used to select suitable salt tolerant wheat genotypes for salt affected soils. In this regard leaf samples of selected wheat genotypes grown under saline and non saline conditions were analyzed for isotopic ratio of ^{12}C: ^{13}C from IAEA laboratories Seibersdorf Vienna Austria. The data was subjected to analyzed statistically (i.e., analysis of variance (ANOVA) and Duncan multiple range test (DMRT), using MStat-C computer package. Correlation studies between CID values and grain yield were also performed.

Results

The data for different growth parameters are presented in Table 1. Plant height recorded at the time of crop maturity, showed 33% relative reduction (Table 1). All the genotypes showed < 50% reduction in plant height. Under saline condition comparatively higher values for plant height were observed in LU-26s, V3-DH and DH-10, showing plant height 65, 64 and 62cm, respectively. However, the least reduction under salinity was observed in V16-DH (i.e. only 14%).

Effect of salinity with respect to plant biomass was significant. Mean reduction was 69.5%. Maximum biomass under salinity was observed in genotype V3-DH (4.0 gm) followed by V13-DH (3.4 gm) and V10-DH (3.2 gm). Almost all the genotypes had > 50% reduction in plant biomass except V16-DH, where the reduction was on the margin i.e. 50%.

The performance of wheat genotypes with respect to productive tillers was quite satisfactory, where the relative reduction in productive tillers was comparatively less (i.e. 28%). Maximum tillers under salinity (12dS/m) were observed in V16-DH. However the least reduction was observed in V3-DH (i.e. only 19%). The other genotypes which also had higher values of productive tillers were V1-DH, V18-DH and V13-DH., showing 25, 25, and 30% decrease under salinity.

The spike length of wheat genotypes was also reduced under salinity. The reduction in all the genotypes tested had < 50% reduction under salinity. Comparatively higher values for spike length were observed in V3-DH followed by LU-26s, and V10-DH. The check genotype (LU-26s) had successfully maintained the length of spike with only 11.6% relative reduction. It was observed that the genotypes V15-DH and V16-DH had least values of relative decrease but the spike length under salinity was recorded very low i.e. 6.cm.

Table 1. Growth performance of wheat genotypes under saline condition (green house studies).

Genotypes	Plant height (cm)			Plant biomass (g)			Productive tillers			Spike length (cm)		
	Con.	12 dS/m	R. dec (%)	Con.	12 dS/m	R. dec (%)	Con.	12 dS/m	R. dec (%)	Con.	12 dS/m	R. dec (%)
V1DH	98.6	58.7	40.47	12.3	2.8	77.3	2.7	2	25	9.52	7.42	22.08
V3DH	90.8	63.9	29.56	12.2	4.2	65.3	2.9	2.3	19.2	11.22	9.01	19.69
V9DH	85.9	58.1	32.34	9.5	3	67.8	3.1	2.1	32.1	8.44	7.35	12.94
V10DH	92.8	62.1	33.05	10	3.2	67.59	2.8	2	28	9.11	7.94	12.8
V13DH	89.9	56.7	36.96	13.7	3.4	75.2	3.3	2.3	30	9.35	7.38	21.09
V15DH	47.3	33.2	29.81	5.5	2.1	62.63	3.4	2.3	32.3	6.77	6.3	6.87
V16DH	47.3	40.6	14.32	6	3	50.19	4	2.4	38.9	6.95	6.41	7.79
V18DH	89.4	55.2	38.26	7.5	2.8	63.11	2.7	2	25	7.59	7.06	7.00
V20DH	95.3	54.9	42.42	10.8	2.4	77.87	2.8	2	28	9.17	6.69	26.97
Sarsabz	81.8	55	32.74	10.5	2.1	80.06	3.7	2.1	42.4	9.01	7.01	22.18
LU-26s	94	65	30.85	12.3	2.8	77.3	3.8	2.1	44.1	10	8.84	11.57
Mean	**83.01**	**54.85**	**32.80**	**10.03**	**2.89**	**69.49**	**3.20**	**2.15**	**31.36**	**8.83**	**7.40**	**15.54**
LSD (0.05)	**8.31**			**2.82**			**0.61**			**1.24**		
Genotypes	**Number of spikelets/ spike**			**Number of grains/ spike**			**Grain wt/ spike (g)**			**Grain yield/ 15 plants (g)**		
	Con.	**12 dS/m**	**R. dec (%)**	**Con.**	**12 dS/m**	**R. dec (%)**	**Con.**	**12 dS/m**	**R. dec (%)**	**Con.**	**12 dS/m**	**R. dec (%)**
V1DH	15.5	10.9	29.83	46.66	31.28	32.96	1.96	0.60	69.41	42.7	9.1	78.81
V3DH	16.6	10.3	37.93	51.19	24.41	52.32	1.41	0.59	58.29	30.8	7.3	76.17
V9DH	12.5	10.4	16.58	29.36	24.91	15.17	1.31	0.56	57.18	46	6.2	86.43
V10DH	14.2	11.7	17.86	38.61	25.39	34.24	1.76	0.93	47.20	41.2	22.5	45.40
V13DH	14.9	10.1	32.18	40.46	23.91	40.92	1.79	0.99	44.70	44.6	24.2	44.50
V15DH	9.0	7.4	18.48	16.18	12.48	22.84	0.79	0.51	35.28	33.3	6.3	81.22
V16DH	8.4	8.2	2.4	16.53	15.8	4.43	0.82	0.65	19.90	29.2	7.9	72.86
V18DH	11.4	11.1	2.61	27	18.83	30.25	1.13	0.48	57.77	32.4	8.0	75.44
V20DH	13.9	9.3	32.98	40.46	19.11	52.77	1.63	0.46	71.63	38.4	18.2	52.6
Sarsabz	13	9.1	30.44	40.47	16.69	58.77	1.35	0.74	46.90	50.4	25.5	49.40
LU-26s	13.2	10.6	19.69	29.76	22.59	24.08	1.32	0.77	41.99	53.9	28.7	46.8
Mean	**12.96**	**9.92**	**21.91**	**34.24**	**21.40**	**33.52**	**1.39**	**0.66**	**50.02**	**40.26**	**14.90**	**64.61**
LSD (0.05)	**2.30**			**8.82**			**1.22**			**10.05**		

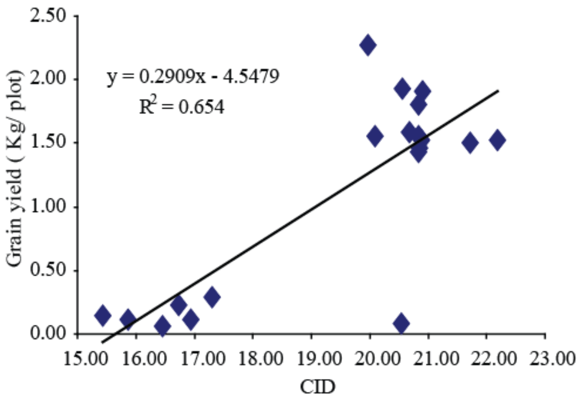

Fig. 1. Relationship between CID and grain yield / plot under non-saline and saline conditions.

The reduction in number of spikelets/spike was slightly high. The genotypes having higher values for number of spiklets/ spike are V10-DH, V18-DH and V1-DH, however relative reduction was comparatively low in genotypes V16-DH and V18-DH.

Like in other growth parameters, high reduction in number of grains was observed. The mean values for relative reduction were 34%. It was also observed that though the relative reduction in V1-DH was bit higher but the numbers of grains were quite high than all the other better performing genotypes. The other genotypes which also had higher numbers of grains were V10-DH, V9-DH, V3-DH and V13DH respectively.

The results with respect to grain weight/spike also showed reduction. The genotypes which showed < 50% reduction in grain weight were V10-DH, V13-DH, V15-DH, V16-DH, Sarsabz and LU-26s, showing 47, 45, 35, 20, 47 and 42% decrease, respectively.

The relative decrease in grain yield/15 plants was also significant. The genotypes comparatively having higher grain yield were V10-DH,V13-DH,V20-DH,Sarsabz and LU-26s, showing grain yield > than 18 grams/ 15 plants.

On the basis of different growth parameters it was concluded that wheat genotypes V10-DH, V13-DH, and LU-26s had better response at 12dSm^{-1} thus can be categorized as better performing genotypes.

Carbon isotopes discrimination (CID) studies: There was a significant positive correlation (R^2 = 0.695) between CID and grain yield (Fig. 1.). Generally the genotypes having higher values for CID also had better performance in term of grain yield. The data with respect to individual wheat genotypes grown under high salinity patches showed decrease in CID (Δ‰) values. There was an overall 15.5% decrease in CID (Δ‰) values under salinity. The CID values under normal conditions were ranged 19.9 to 21.7 and 16.5 to 20.6 under saline condition. The mean values under two environments (non saline and saline) were 20.9 and 17.6 (Δ‰).

Under saline condition the genotypes V10-DH, V13-DH and V20-DH were categorized as high carbon isotopes discriminating genotypes, with less reduction in discriminating ability. Whereas, the genotype V16-DH and V18-DH can be categorized as low carbon isotopes discriminating genotypes, showing maximum decrease under salinity i.e. 25.94 and 23.62%, respectively. On the other hand the genotypes V1-DH, V3-DH, V9-DH, V15-DH, Sarsabz and LU-26s may be categorized as medium carbon isotopes discriminating genotypes (Fig. 2).

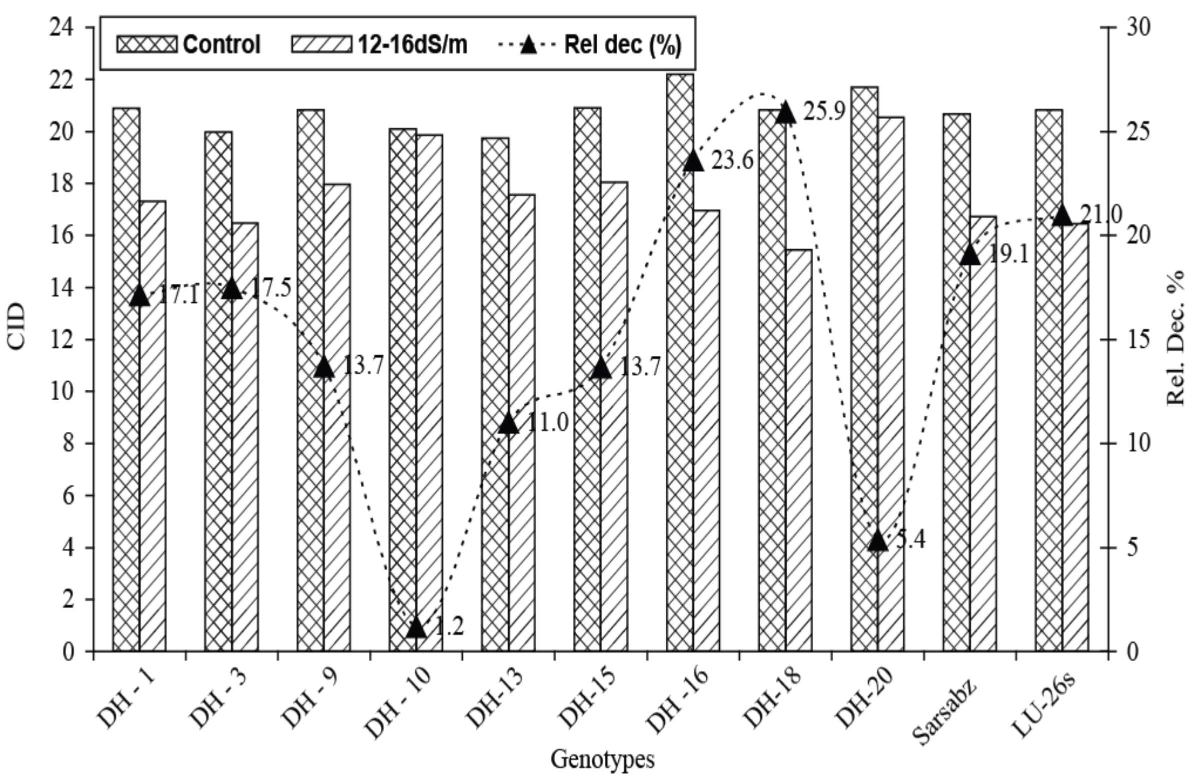

Fig. 2. Carbon Isotopes discrimination (CID) studies in wheat genotypes grown under non-saline and saline field conditions.

Discussions

Presence of salts in the growing medium reduced the availability of water to plants, which may results in overall reduction in plant growth (Cramer *et al.,* 1990). This is the osmotic or water deficit effect of salinity, where yield reduction may range from a slight loss to complete crop failure depending upon severity of the salinity problem (Chang & Sipio, 1991). In the present investigation all the wheat genotypes showed reduction in yield and yield contributing components. However it varied among the genotypes. Turki *et al.* (2012) have the opinion that the variation in response to salt in varieties and accessions closely relates to genetic diversity among these species. The data with respect to plant biomass was found maximum in V3-DH, V10-DH and V13-DH. On the other hand maximum grain yield under salinity was observed in salt tolerant check (i.e. LU-26s) followed by Sarsabz, V13-DH and V10-DH. Better performance of LU-26s was also reported by Ahmad *et al*. (2005) and was found the most salt tolerant among the tested genotypes. They reported that the highest grain yield of LU-26s was due to its low Na^+ uptake, high K^+/Na^+ ratio, higher dry weight of shoots and spikes and better grain development. It was quite interesting that in spite of less relative reduction in most of the parameters in V-16-DH, grain yield was much less as compared to LU-26s and V13DH. Lower values of biomass and grain yield of V16-DH might be due to its less vegetative growth under two growing environments (i.e. normal and saline) or short stature of this genotype. According to Kamkar *et al*. (2004) the salinity induced source limitation reduces yield primarily by a severe reduction in grain number and then by reduction in grain yield. This was found true in case of V16-DH, which had minimum no of grains under salinity. Lower values of no of tillers, spike length and no of grains/ spike also reflected on lower grain yield in case of genotype V20-DH. It has also been reported (Mass *et al*., 1983; Mass & Poss, 1989) that the effect of salinity on tiller number and spikelet number, which both initiate during early growth stages, has a greater influence on final grain yield than on yield components in the later stages. The numbers of spiklets were also higher in LU-26s and V13-DH as compared to V16-DH. Salah *et al.* (2005), have the opinion that an increase in number of spikelet per spike will improve the salt tolerance of wheat genotypes in breeding programs. In view of many earlier studies grain yield is the net outcome of the synthesis of assimilates by leaves during photosynthesis and translocation of these assimilates to the developing seed where they are utilized to synthesize other organic compounds. (Pettigrew & Meredith, 1994; Eagli, 1999). The pronounced effect of salinity is the reduction in photosynthesis during vegetative growth (Dadkhah & Griffiths. 2004, Kafi *et al.,* 2007). During the fixation of carbon by photosynthesis, the naturally occurring stable isotope ^{13}C is discriminated against, because of fractionation of carbon stable isotope (^{12}C and ^{13}C) mainly by Rubisco (Farquhar, *et al*., 1989). Plants therefore, contain a lower ratio of ^{13}C to ^{12}C than the air that supplies to them (Farquhar and Richard, 1984). Under salinity the discrimination ability of plants is reduced in wheat as reported earlier (Ansari *et al.*, 1998). The data with respect to ratio of ^{13}C to ^{12}C, showed a decreasing trend under salinity. There was an overall decrease in carbon isotopes ratio under salinity as compared to non saline environment. Generally higher values of carbon isotopes discrimination (CID) were recorded in wheat genotypes having high biomass. There was an overall positive relationship between biomass and CID (Δ) ($R^2 = 0.695$). The genotypes having more biomass i.e. V10-DH, V13-DH under salinity comparatively had lower decrease in CID (Δ) (i.e. 1.2 & 11.0%, respectively) while in case of V16-DH, which had low grain yield also had higher decrease in CID (Δ) in (i.e. 23.6% relative reduction). Thus the results support our findings. However, the trend in case of LU-26s did not support agronomical parameters completely, where instead of high grain yield, the relative reduction in CID values were bit high (i.e. 21%).. Therefore the studies suggest that we can include CID technique as one of the selection criteria for salt tolerance but cannot rely completely on this trait. Phenotypic studies for selection of salt tolerance in wheat are necessary for correlation. Studies are in agreement with the findings of Hokmabadi *et al.* (2005). They concluded that carbon isotopes discrimination in pistachio may be a useful indicator of cumulative salinity history of plant but is not a suitable indicator for pre screening of pistachio rootstocks for salinity resistance.

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