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ROLE OF PROLINE, K/NA RATIO AND CHLOROPHYLL CONTENT IN SALT TOLERANCE OF WHEAT (*TRITICUM AESTIVUM* L.)

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Abstract

Studies to determine the role of proline, K/Na ratio and chlorophyll contents in salt tolerance of wheat genotypes were conducted in lysimeters using hydroponics technique. Seeds were allowed to germinate under normal condition (1.5 dS m^{-1}) and salinity treatment of 12 dS m^{-1} was imposed after one week of germination. Crop was irrigated at the interval of two weeks or whenever required with $1/4^{th}$ Hoagland nutrient solution of respective concentrations. Results clearly indicated that wheat genotypes with higher proline, K/Na ratio and chlorophyll contents had higher grain yield. On the basis of yield reduction, three genotypes viz. Lu-26s, Sarsabz and KTDH were found tolerant. These genotypes also maintained the higher concentration of proline, K/Na ratio and chlorophyll contents under saline conditions.

Introduction

High soil salinity is one of the important environmental factors that limit distribution and productivity of major crops (Ashraf *et al*., 2005; Chandan *et al*., 2006). Agricultural productivity in arid and semiarid regions of the world is very low due to accumulation of salts in soils (Ashraf *et al*., 2002; Munns, 2002). Saline medium causes many adverse effects on plant growth, which is due to low osmotic potential of soil solution (osmotic stress) specific ion effects (salt stress), nutritional imbalance or a combination of these factors (Marschner, 1995, Ashraf, 2004). All these factors cause adverse effects on plant growth and development at physiological and biochemical activities (Ashraf & Sarwar, 2002; Munns & James, 2003).

Wheat is the major cereal crop of Pakistan, which is grown all over the country. It is grown to meet the food demand of over growing population of Pakistan, but per hectare yield of wheat is far below than its yield potential, which may be due to different reason i.e., lack of proper water and nutrient managements, unavailability of fertile soils, salinity, water logging and drought. In Pakistan, salinity is a serious threat for wheat production. The most of underground water utilized for wheat cropping is brackish, however, some areas are irrigated with canal water but having lack of drainage system both the irrigation systems are increasing the soil salinity problem in the country due to which heavy losses in crop yields are reported (Khan *et al*., 2006). The wheat crop is a moderately salt tolerant (Khan *et al*., 2004) and for screening or developing salt tolerant wheat varieties, physiological and biochemical studies are necessary to identify the physiological and biochemical markers. By using these markers available wheat germplasm can be screened for salt tolerance or by incorporating them new high yielding salt tolerant wheat varieties can be developed. This is essential to fulfill the wheat grain yield demands of ever-growing population of Pakistan.

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Plants differ genetically in their response to salt stress (Ahmed *et al*., 2005). Different mechanisms of salt tolerance by plants have been suggested by different workers (Flowers & Hajibagheri, 2001; Gorham, 1994; Schachtman & Munns, 1992). Keeping in view the importance of wheat and salinity, present study has been planned to examine the role of proline, K/Na ratio and chlorophyll content in salt tolerance of wheat (*Triticum aestivum* L.).

Material and Methods

The experiment was conducted in lysimeters (cemented tanks), filled with river sand. The growing media was irrigated by 1/4th strength Hoagland solution, salinized by commercial NaCl salt to attain salinity level of 1.5 dS/m (control) and 12.0 dS/m. Six wheat genotypes viz., Lu-26s, Sarsabz, KTDH-22, V-7012, Khirman and Bakhtawar were sown in a randomized manner with three replicates. Growth observations were recorded at the time of maturity. Plant samples (flag leaves) were analyzed for soluble salts (Na, K and K/Na ratio), after extraction with 0.1 M. Acetic acid as described by Ansari & Flowers, (1986). Fresh leaves samples at flowering stage were analyzed for Chlorophyll (Lichtenthaler, 1987) and proline contents (Bates *et al*., 1973).

Results

Leaf chemical analysis of different wheat genotypes indicated that sodium (Na) contents increased under saline condition (Table 1). The genotypes Lu-26s Sarsabz and KTDH had comparatively less sodium contents than V-7012, Khirman and Bakhtawar. Results also indicated that Check wheat genotype (Lu-26s) had minimum Na contents than that of Sarsabz and KTDH-22 while potassium (K) contents reduced due to increase in salinity in all the wheat genotypes (Table 1). However, genotypes Lu-26s, Sarsabz and Khirman maintained higher potassium contents than others under saline conditions. The increase in sodium contents resulted a decrease in K/Na ratio in all the wheat genotypes (Table 1). However, salt tolerant check genotype (Lu-26s) showed minimum reduction in K/Na ratio which was closely followed by Sarsabz and KTDH-22.

Biochemical analysis of leaves of different wheat genotypes for proline accumulation and chlorophyll contents indicated that proline accumulation increased and chlorophyll contents decreased under saline condition (Table 2). Wheat genotypes Sarsabz, Lu-26s and KTDH showed higher accumulation of proline than others (Table 2). Similarly minimum reduction in chlorophyll content was noted in Sarsabz, Lu-26s and KTDH. Maximum chlorophyll contents were maintained by Sarsabz and KTDH followed by Lu-26s.

Grain yield of different wheat genotypes was significantly influenced by the salinity (Table 2). The genotype Lu-26s showed minimum reduction, when compared with control, whereas maximum reduction over control was recorded in Khirman. The genotypes Sarsabz and KTDH were successful in maintaining grain yield more than 60% under salinity stress (12 dS/m).

Table 1. Effects of salinity on sodium (Na), potassium (K) contents and K/Na ratio in different wheat genotypes.

S.No.	Variety	Control			Saline		
		Na (mg g^{-1} D.W.)	K (mg g^{-1} D.W.)	K/Na	Na (mg g^{-1} D.W.)	K (mg g^{-1} D.W.)	K/Na
1.	Lu-26s	0.18	2.19	12.20	1.36	1.43	1.05
2.	Sarsabz	0.18	2.41	13.17	1.57	1.56	0.83
3.	KTDH-22	0.16	2.67	16.74	1.72	1.44	0.69
4.	V-7012	0.20	2.20	12.03	2.74	1.20	0.43
5.	Khirman	0.16	2.15	12.49	2.50	1.30	0.56
6.	Bakhtawar	0.15	2.18	14.43	2.81	1.20	0.43
Mean		0.17	2.30	12.20	2.12	1.36	0.67
LSD (0.05)		0.0919	0.1404	1.084	0.0919	0.1404	1.084

Table 2. Effect of salinity on accumulation of proline, total chlorophyll and grain yield of different wheat genotypes.

S. No.	Variety	Control			Saline		
		Proline µmol g^{-1} F.W	Total chlorophyll mg g^{-1}	Yield kg ha^{-1}	Proline µmol g^{-1} F.W	Total chlorophyll mg g^{-1}	Yield kg ha^{-1}
1.	Lu-26s	5.02	2.96	3503	19.37	2.43	2728
2.	Sarsabz	4.93	3.08	3747	22.77	2.56	2458
3.	KTDH-22	5.17	3.07	3298	18.31	2.41	2017
4.	V-7012	5.07	2.75	2906	10.21	1.51	1458
5.	Khirman	5.15	2.79	3684	11.32	1.62	1358
6.	Bakhtawar	5.03	2.69	2345	10.60	1.62	1195
Mean		5.06	2.89	3247	15.43	2.03	1869
LSD (0.05)		0.993	0.2056	429.7	0.993	0.2056	429.7

Discussion

Sodium contents increased due to salinity in all wheat genotypes however genotypes Bakhtawar and Khirman maintained the highest leaf Na concentration which is closely followed by V-7012. Minimum Na content was recorded by Lu-26s followed by Sarsabz and KTDH (Table 1). The low sodium accumulation in Lu-26s, Sarsabz and KTDH-22 indicated that these varieties were more tolerant than those which translocated maximum Na in leaves. It is well established fact that Na is a toxic element whose higher concentration disturbs the different metabolic activities (Akram *et al*., 2007). The varieties which were successful in retaining the Na in the root were tolerant (Khan *et al*., 1990; Akram *et al.,* 2007). Based on these reports, it may be concluded that genotypes Lu-26s, Sarsabz and KTDH-22 maintained less amount of Na in their leaves and hence were tolerant to salinity.

In studies where salinity is developed with NaCl, a focus has been the transport systems that are involved in the utilization of Na as an osmotic solute (Yasar *et al*., 2006). Literature indicated that intracellular Na homeostasis and salt tolerance are modulated by calcium and high Na concentrations negatively affect K acquisition (Munns *et al*., 2002). Sodium competes with K for uptake through common transport system and does this effectively since the Na concentration in saline environments is usually considerably greater than that of K. It is also reported that sensitivity of some crops to salinity is due to the inability to keep Na and Cl out of transpiration streams (Gorham *et al*., 1990). Plants limiting the uptake of toxic ions or maintaining normal nutrient ion contents could show greater tolerance which was the case with present study. Uptake mechanism that discriminates similar ions such as Na and K could be useful selection criteria for salt tolerance in wheat and breeding for efficient nutrient uptake. A significant negative correlation was observed between grain yield and increase in sodium contents of the wheat genotypes grown under sodium chloride concentration.

In the present study all wheat genotypes showed decreasing trend in K content due to salinity stress. The decrease in K was due to the presence of excessive Na in the growth medium because high external Na content is known to have an antagonistic effect on K uptake in plant (Sarwar & Ashraf, 2003). It is also reported that salt tolerance is associated with K contents (Ashraf & Sarwar, 2002), because of its involvement in osmotic regulation and competition with Na (Ashraf *et al*., 2005). Regulation of K uptake and prevention of Na entry, efflux of Na from cell are the strategies commonly used by plants to maintain desirable K/Na ratio in the cytosole. In the present study, the tolerant genotypes are expressing the same trend for K/Na ratios. Genotypes Lu-26s, KTDH are comparatively higher in accumulating more K than sensitive ones. K/Na ratio is the criteria which is established by the scientist and the genetically approved for salt tolerance. So the varieties maintaining higher K/Na ratio are the salt tolerant and showing positive correlation between grain yield and K/Na ratio.

There are some reports where an increase in chlorophyll contents was observed in 6 genotypes of rice (Alamgir & Ali, 1999). However, the reduction in chlorophyll contents is to be expected under stress; being membranous bound, its stability is dependent on membrane stability, which under saline condition seldom remains intact (Ashraf *et al*., 2005). The decrease in chlorophyll contents under saline conditions is reported by Iqbal *et al*., (2006); Ashraf *et al*., (2005). Our results are in agreement with these workers where in all genotypes, chlorophyll contents are decreasing. The decrease is significant in sensitive genotypes in comparison to tolerant.

Accumulation of solutes especially proline, glycine-betaine and sugars is a common observation under stress condition (Ashraf *et al*., 1994; Naqvi *et al*., 1994, Qasim *et al*., 2003). It is reported by Ashraf *et al*., (1998) that proline is an important osmolyte to adjust the plant under drought/saline conditions. In the present study, the accumulation of proline was commonly observed in almost all genotypes however, the genotypes Sarsabz followed by Lu-26s and KTDH-22 had higher proline accumulation (Table 2). These genotypes are the best performing ones and had higher grain yield under salinity stress. Similar observations were recorded by other workers (Ashraf & Foolad, 2005) in different crops. There are however reasons to believe that proline accumulation may play a role in the salinity tolerance. Firstly it is an osmolyte accumulated under stress in almost all the plant species. Secondly a high proline concentration has been described in organs which naturally have low water contents such as seed and inflorescence. The results of present study showed that there is a positive relationship between proline accumulation and performance of wheat genotypes in terms of grain yield under salinity stress.

It was concluded that on the basis of less than 40% reduction in yield, three genotypes viz. Lu-26s, Sarsabz and KTDH-22 were better. It was also observed that the tolerant genotypes have higher proline accumulation, high K/Na ratio and less chlorophyll degradation as compared to sensitive ones.

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