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Anatomical Attributes of Different Wheat (*Triticum aestivum*) Accessions/Varieties to NaCl Salinity

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ABSTRACT

Anatomical responses of salt tolerant, moderate tolerant and sensitive wheat accessions/varieties were investigated at 10, 15 and 20 dS m^{-1} levels of NaCl salinity in a pot experiment in addition to control (2.5 dS m^{-1}). Salinity induced specific changes in the leaf, stem and root that entailed major effects on number and area of stomata, area of metaxylem and ground tissue of stem, and cortical and stele regions of root. The effect of salinity was more apparent at root level than other organs. Highly salt adapted anatomical changes were exhibited by tolerant Acc. 234/2 followed by moderate tolerant Acc. 243/1, while no adaptive response was noted in the sensitive Var. Fsd-83 in this regard.

Key Words: Anatomy; Leaf; Stem; Root; Salinity; Tolerance; Wheat (*Triticum aestivum*)

INTRODUCTION

Glycophytes as well as halophytes seem to depend on morphological, anatomical and physiological mechanisms, which enable them to survive in saline habitats (Maas & Nieman, 1978; Cheeseman, 1988; Lauchli & Epstein, 1990; Shannon, 1997; Isla *et al.,* 1998). Comparison of anatomical features of plants showing differential tolerance to salinity are important to understand the basis of salinity effects at cell, tissue and organ levels.

Imposition of salinity causes changes at cellular and tissue levels which resulted in the reduced overall size of various parts (Solomons *et al*., 1986). Waisel (1972) reported various structural changes in plant tissues under salt stress, viz., increase in succulence, changes in the number and size of stomata, thickening of cuticle, inhibition of tissue differentiation and changes in diameter and number of xylem vessels. Curtis and Lauchli (1987) reported a reduction in the expansion of leaf epidermis in Hibiscus cannalinus under salinity stress. Javed *et al*. (2001) reported that as an anatomical character, leaf in pearl millet was greatly affected than root. Enhanced development of sclerenchymatous tissue on the adaxial and abaxial sides of the leaf, maintenance of greater vascular bundle size and lesser reduction in interveinal distance was also noted in tolerant pearl millet genotypes in response to NaCl salinity. Increase in the width of the casparian strip in many halophytes (Poljakoff Mayber, 1975) and differentiation of root xylem parenchyma into transfer cells in *Zea mays* (Kramer *et al*., 1977) were also noted as anatomical features associated with salt tolerance.

The present study reports the comparative effects of different salinity levels on anatomical characters of leaf, stem and roots of some tolerant and sensitive accessions/varieties (Acc/Var) of wheat (*Triticum aestivum*).

MATERIALS AND METHODS

These wheat Acc/Var were screened out under NaCl salinity. Acc. 234/2 and 243/1 with salt tolerance limit of 17.95 dS m^{-1} and 16.91 dS m^{-1} and Var. Fsd 83 (11.07 dS m^{-1}) were declared as salt tolerant, medium tolerant and sensitive, respectively (Akram, 2001). Experiment was carried out in earthen pots of 25 cm in diameter. Pots were double layered with polyethylene bag to avoid the leakage and were filled with 10 kg sieve passed soil. Healthy grains of each wheat Acc/Var were sown at the rate of ten grains per pot. Thinning was done at first leaf stage to five plants per pot. Experiment was triplicated in completely randomized design.

Treatment application. Three salinity levels i.e. 10, 15 and 20 dS m^{-1} and control (2.5 dS m^{-1}) were developed at booting stage using sodium chloride (99% pure). The salinity levels were gradually developed @ 2 dS m^{-1} daily in order to avoid osmotic shock.

Anatomical measurements. Leaf, stem and root samples of all the three Acc/Var taken from same position were used for anatomical studies. Tissues were killed in formalin: acetic acid: alcohol: water (10:5:35:50) for 24 h and then store in 70% alcohol until use (Wahid *et al*. 1998). Free hand cut sections of stem and root were dehydrated in alcohol series and colour with safranine and light green stain while leaf epidermis was peeled off for studying stomatal frequency. Measurements of various cells and tissues were taken with ocular micrometer and exact values were computed with factor derived by comparing ocular and stage micrometer.

RESULTS AND DISCUSSION

Leaf characteristics. The wheat Acc/Var showed significant ($P<0.01$) differences for number of stomata per field, area of stomata and interveinal distance under increased level of salinization (Table I). The interaction of factors were also significant (P<0.01) for the parameters except for interveinal distance where no interaction was found. Salinity stress positively influenced the number of stomata, and negatively to the area of stomata and interveinal distance. However, different Acc/Var showed responses to applied salinity. Maximum increase in number of stomata and decrease in area of stomata and interveinal distance was exhibited by Fsd 83 while contrasting response was given by the 234/2 under the highest level.

Stem characteristics. Stem characteristics such as area of epidermal cell, largest metaxylem and cortex varied significantly (P<0.01) in different Acc/Vars of wheat with increase in root zone salinity. The interaction between Acc/Var and salinity was significant (P<0.01) for area of metaxylem and area of cortex while no interaction was found for area of epidermal cell (Table I). The tolerant Acc. 243/1 followed by medium tolerant 243/1 showed lesser reduction for these parameters but it was more pronounced in the sensitive var Fsd 83 (Table I).

Root characteristics. Wheat Acc/Vars indicated significant (P<0.01) differences under increased salinity for root anatomical features i.e. area of cortex, area of stele and area of pith. The interaction of both these factors for all the parameters studied were also significant (P<0.01). In case of area of metaxylem, the Acc/Vars did not differ significantly (P>0.01) but applied salinity significantly (P<0.01) affected the area metaxylem of root of both tolerant and sensitive Acc/Vars (Table I). The interaction for this parameter was also significant. The area of all the parameters decreased under increased salinity as expressed over control in all the three Acc/Vars. However, it was minimally affected in 234/2 followed by 243/1 and was greatly affected in Fsd 83 (Table I).

Salinity treated plants often show a considerable reduction in the water uptake which results in decline in water content of various parts including the leaves (Colmer *et al*., 1995; Curtis & Lauchli, 1987). Present results on anatomical studies of leaf and stem tissues revealed that there was a considerable reduction in the area of stomata, size of metaxylem and area of cortex under salinity stress. Moreover

Table I. Anatomical characters of three wheat Acc/Var growing under increased salinity levels

Acc/Var	Salt levels (dS m^{-1})	Leaf			Stem				Root		
		SF	AS	ID (μ)	AEC (μ^2)	ALM	GT (%)	AC (μ^2)	AM (μ^2)	AC (μ^2)	AST (μ^2)
234/2	Control	121.33	54.16	68.00	43.28	64.67	15.55	234.00	50.00	473.30	306.70
	10	132.33	51.02	60.67	40.14	62.33	14.82	223.30	46.67	460.00	301.00
	15	135.33	46.31	53.33	35.89	56.60	14.09	201.50	41.50	442.60	253.30
	20	142.67	43.17	50.00	31.02	51.48	12.36	180.10	34.20	396.30	214.60
243/1	Control	94.67	70.65	66.67	44.48	83.37	20.45	274.00	55.00	483.00	313.30
	10	115.33	65.94	55.00	39.06	66.50	18.85	253.33	45.00	453.30	280.00
	15	119.00	58.09	48.33	32.10	55.70	16.80	220.00	42.60	420.60	243.00
	20	129.33	52.38	41.00	26.04	47.53	11.97	180.67	30.10	382.70	207.30
Fsd 83	Control	114.67	56.52	77.00	44.85	89.24	25.66	330.00	63.30	498.30	376.70
	10	141.33	48.67	55.67	38.31	62.02	21.50	256.67	48.50	447.00	333.40
	15	152.33	47.60	40.33	29.78	48.37	15.07	233.33	35.00	401.60	264.00
	20	165.33	35.22	32.33	21.58	31.92	10.63	140.00	26.60	368.50	201.30
LSD (5%)		3.88	3.72	3.22	1.24	1.94	0.94	11.24	6.73	11.91	14.32

SOV	df	Leaf			Stem				Root		
		SF	AS	ID (μ)	AEC (μ^2)	ALM	GT (%)	AC (μ^2)	AM (μ^2)	AC (μ^2)	AST (μ^2)
Treatment (T)	3	79.25**	31.04**	78.04**	80.83**	21.52**	32.76**	330.36**	58.72**	114.92**	685.55**
Acc/Var (A)	2	81.67**	39.07**	48.85**	41.66**	37.60**	44.59**	126.09**	0.04^{NS}	6.50**	111.43**
T x A	6	23.39**	53.80**	1.40^{NS}	1.75^{NS}	14.94**	33.48**	32.95**	5.55**	7.27**	29.69**

SF=No. of stomata /filed; AS=Area of stomata; ID=Interveinal distance; AEC=Area of epi.Cell; ALM=Area of largest metaxylem; GT=% ground tissue at overall stem thickness; AC=Area of cortex; AM=Area of metaxylem; AST=Area of stele root; ** Significant at P<0.01 and NS= non significant.

the interveinal distance was also reduced considerably. These findings revealed that reduction in water and ion conducting tissues caused a reduction in transport of water. In addition, reduced area of xylem offered more resistance to the flow of water, which required more energy to transport any quantity of water from root to the leaves (Munns & Termaat, 1986). This ultimately resulted in more hampered growth performance of the sensitive Acc/Vars as compared to the tolerant Acc.

Among the many adverse effects of salinity structural changes in various tissue and cell (Solomons *et al*., 1986; Reinhardt & Rost, 1995; Gucci *et al*., 1998) in one. Data on leaf and stem revealed that irrespective of salinity tolerance potential of Acc/Var applied salinity substantially affected almost all the parameters under investigation. Among the dermal tissues, there was significant reduction in the epidermal cell area, area of stomata and increase in number of stomata per microscopic field. Among ground tissues a considerable reduction was noted in cortex area, and interveinal distance was substantially reduced. However, there was great genotypic difference for response to salinity stress. The tolerant Acc. 234/2 and medium responsive 243/1 showed almost comparable responses to applied salinity where as Fsd-83 was highly affected showing maximum decrease in the parameters studied.

There existed a wide variation with respect to stomatal frequency in different Acc/Var under increased salinity and it increased under saline conditions as compared to control. This increase was more pronounced in sensitive (Fsd-83) as compared to tolerant (234/2) Accs and was followed by 243/1. The increase in stomatal frequency was followed by decreased stomatal size. Wide variation in stomatal frequency under salinity offers a criterion of selection for salt tolerant material. An Acc/Var with lesser increase in stomatal number may be able to avoid excessive water loss due to transpiration without affecting photosynthetic capacity and hence causing lesser water stress. Thus the possibility exists for altering transpiration without altering photosynthesis by selecting Acc/Var with lesser stomatal frequency under salinity and getting better yield. These studies are in conformity with the finding of Curtis and Lauchli (1986).

In roots, the reduction in the cortical area, size of metaxylem, area of stele and pith indicated adverse effect of salinity. There was no significant change in the number of cortical cell layers but it was the size of the affected cells, which induced a reduction and form the basis of root constriction (Table I). An inhibitory effect of toxic ions on the root tissue mainly appeared due to their toxicity, which prevented the expansion and enlargement of different cells (Curtis & Lauchli, 1987; Javed *et al*., 2001). These changes are significant under salinity as they lead to the reduced uptake of water by root and its transport to the shoot but these changes were noted least in the tolerant acc (234/2) as compared to medium responsive and sensitive Acc/Var.

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