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Insha Zahoor
Department of Biotechnology, School of Biological Sciences, University of Kashmir, Srinagar, Jammu and Kashmir, 190006, India.

Mudasir A Mir
Centre for Plant Biotechnology, Division of Biotechnology, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Shalimar, 191121, India.

***Corresponding author**
Dr. AmrinaShafi,
Department of Biotechnology, School of Biological Sciences, University of Kashmir, Srinagar, Jammu and Kashmir, 190006, India.

Research Article **Biotechnology**

Primary and Secondary Cell Wall Changes in *Arabidopsis thaliana* under Salinity Stress

AmrinaShafi*[1], Insha Zahoor[2], Mudasir A Mir[3]

ABSTRACT

Salinity stress affects the growth, development and productivity of the crop world-wide. The first line of defense against any abiotic stress is the plant cell wall. Plant cell walls are composites of various carbohydrates, proteins and other compounds. Cell walls provide plants with strength and protection, and also represent the most abundant source of renewable biomass. The model plant Arabidopsis thaliana and the availability of its genome sequence have been invaluable for the identification and functional characterization of genes encoding enzymes involved in plant cell-wall biosynthesis. In the present study, *Arabidopsis thaliana* were used to study the effects of salinity stress on cell wall and morphology of plants. Bright, confocal and scanning electron microscopy was used to measure wall thicknesses of different cell types in freeze-fractured stem sections of *Arabidopsis thaliana*. Apart from cell wall studies, salinity also affected the yield and biomass of plants. It was concluded that the cell wall thickness was controlled by the salinity stress. These studies have improved our understanding of both the mechanisms of cell-wall biosynthesis under salinity stress, and have highlighted areas where further research is needed.

Keywords: Arabidopsis, Cell wall, Salinity stress, Anatomy, Confocal, Scanning, Brightfield microscopy

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INTRODUCTION

Salt stress imposes a major environmental threat to agriculture by limiting plant growth and reducing crop yield. The increased salinization of arable land is expected to have global effects, resulting in 30% land loss within the next 25 years (Wang *et al.*, 2003). Therefore, the efforts to increase salt tolerance of crop plants bear remarkable importance for sustainable agriculture. Abiotic stress comprises a major issue for contemporary agriculture in terms of low crop yield and increasing areas not suitable for planting (Reguera *et al.*, 2012). Among the abiotic stress factors high soil salinity and the lack of fresh water supplies are among the greatest obstacles for a high productive agriculture. Above 20% of the agricultural lands in the world are affected by high salinity and this

Percentage is expected to be further increased (Munns, 2005). Another concern is the global climate change that leads to more and more extreme fluctuation of the environmental conditions in agricultural areas (Soussana *et al.*, 2012). Probably, the high salt concentrations in a number of agricultural lands appear to be the major restriction for successful crop breeding, and therefore salt stress response and tolerance in plants tend to receive the greatest attention (Mudgal *et al.*, 2010; Shabala and Munns, 2012).

About 10% of the genes in Arabidopsis have been estimated to be involved in the various aspects of cell-wall metabolism, including polymer biosynthesis, transport, deposition, remodeling, turnover, and regulation of these processes (McCann and Carpita, 2008).The Arabidopsis genome sequence (Arabidopsis Genome Initiative, 2000) has provided an invaluable tool for efficient identification and systematic functional analysis of genes encoding cell-wall polysaccharide biosynthetic enzymes. Arabidopsis is an excellent model plant for cell wall studies as its cell walls resemble those found in many crop plants and trees. The aim of the study was to investigate the salt-acclimation process in the Arabidopsis and to address specific questions (i) what is the effect of salinity stress on plant cell wall (ii) whether salt assimilation affects the growth and yield parameters. The results concluded that there is change in cell wall morphology of plants under stress conditions which indicates the salinity stress has effect on cell wall morphology.

MATERIALS AND METHODS

Plant material and stress treatment

Arabidopsis thaliana seeds were surface sterilized, rinsed with sterile water and stratified at4 °C for two days on half-strength Murashige and Skoog (½ MS; 1962) medium supplemented with 1 % agar, 1 %sucrose. The seedlings were transplanted to the soil mixture of vermiculite: peat moss: perlite (1:1:1) in the greenhouse under a 16 h light and 8 h dark cycle at 20 ±1 °C and light intensity of 60–70 µmol PPFD m^{-2} $s^{-1}$and irrigated with ½ MS salts, weekly. For stress treatment, 21 days old seedlings were supplemented with desired concentration of NaCl (0, 50, 100 and 150 mM) dissolved ½ MS salts. Three biological replicates were collected from each sample at respective time points after salt stress treatment.

Microscopy

For confocal microscopic analysis, inflorescence stems of WT and transgenic lines were harvested and fixed in formalin, glacial acetic acid and ethyl alcohol (FAA, 1:1:18)at room temperature. Sections of 8–10 µm thickness were cut and stained with 1 % safran in and 4 % fast green. These sections were mounted and examined using Confocal Laser Scanning Microscope (Zeiss LSM510 metaGmbh, Germany).

For SEM analysis, segments from the apical 1 cm of stem cross-sections were fixed in a mixture of 2 % paraformaldehyde and 2.5 % glutaraldehyde in 0.1 mol/l cacody late buffer, pH 7.4 for 1 h and then with 1 % OsO4 in0.1 mol/l cacody late buffer, pH 7.4 for 30 min. After critical point drying, the samples were sputter-coated with gold, and the coated samples were viewed with a HitachiS-3400N field emission SEM using an accelerating voltage of 30 kV. For TEM study, stem slices were fixed in4 % (w/v) paraformaldehyde and 1 % (v/v) glutaraldehydein 0.1 mM phosphate buffer, pH 7.2, for 4 hat room temperature and then post fixed in 1.33 % OsO4 in cacody late buffer, pH 7.2, and stained with 1.5 % uranyl acetate. All samples were dehydrated in acetone series, followed by propylene oxide. Embedment was in Araldite, Epon, and dodecenyl succinic anhydride in proportions 1:1:3. Polymerization was carried out at 80 °C, and micrographs were taken with a Tecnai G2 TF20 electron microscope (FEI, Netherlands).

Cell wall Measurements

Measurements of cell wall thickness and shape were done with a HitachiS-3400N field emission SEM using an accelerating voltage of 30 kV.

Biomass and yield calculations

After completion of Arabidopsis lifecycle both under salt and stress conditions, the biomass calculations in terms of rosette diameter, number of leaves, root and shoot biomass was done. Also, yield measurements in terms of number of pods, seeds/pod and total number of seeds/plant were done.

Statistical analysis

All experiments were conducted with at least three independent repetitions in triplicates. All values are shown as the mean ± the standard deviation. The statistical analysis was performed using Statistical software (v.7). The statistical significance between the mean values was assessed by analysis of variance (ANOVA) applying Duncan's Multiple Range Test (DMRT). A probability level of $P \leq 0.05$ was considered significant.

RESULTS AND DISCUSSION

Effect of Salinity Stress on plants

Arabidopsis plants were given salinity stress (0, 50, 100 and 150 mM) and changes in the phenotype and morphological changes were recorded (Fig.1). Under control conditions, plants phenotype was normal, leaves were green and plants were healthy (Fig.1). At 50 mM conditions plants, some changes in the plant phenotype were observed but with the increase in stress i.e. 100 mM salt stress, wilting and chlorosis was observed which is an indication of stress acclimation in plants (Fig. 1). Plants exposure to low level salinity and

cold activates an array of processes leading to an improvement of plant stress tolerance. This has already been demonstrated for different herbaceous species such as soybean, rice, sorghum and Arabidopsis (Umezawa et al., 2000; Djanaguiraman et al., 2006; Shafi et al., 2014; 2015a; 2015b; 2017).

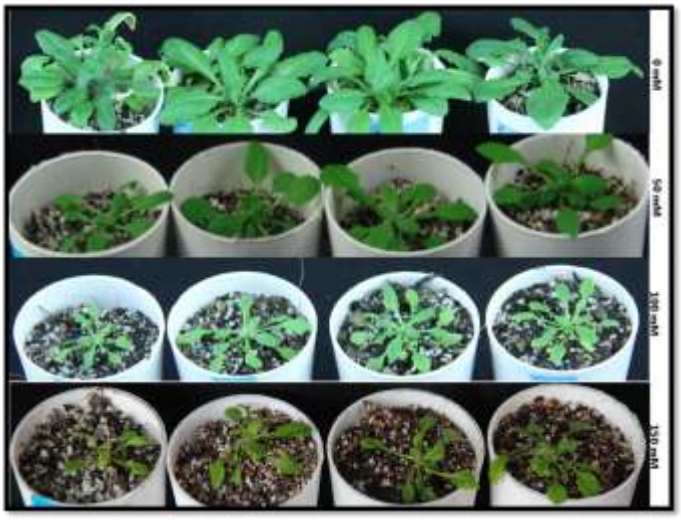

Fig.1. Phenotypic Changes in *Arabidopsis* under control (0 mM) and salinity stress (50, 100, 150mM).

Morphological and Developmental phenotypes under Salinity stress

The internal structure of the stems was characterized in *Arabidopsis* under control and stress conditions, which revealed that vascular bundles were well connected with inter fascicular fibers forming a continuous cylinder of lignified tissue around the pith. Under salt stress, thinner stems in plants had smaller vascular bundles and less developed fibers in the inter fascicular tissue under stress conditions (Fig. 2).This indicates that plant vascular system and the cell wall is experiencing the salt stress. Thus in the present study, anatomical investigation of vascular structures using confocal and electron microscopy clearly showed that disruption and distortion in the morphology of plants with salinity stress (Fig. 2).The wealth of information gained from the *Arabidopsis* genome sequence (*Arabidopsis* Genome Initiative 2000),coupled with the powerful tools available to *Arabidopsis* researchers (Seki et al., 2002; Rhee et al.,2003), has facilitated much progress within the cell-wall research community in identification of genes encoding enzymes involved in cell-wall biosynthesis.

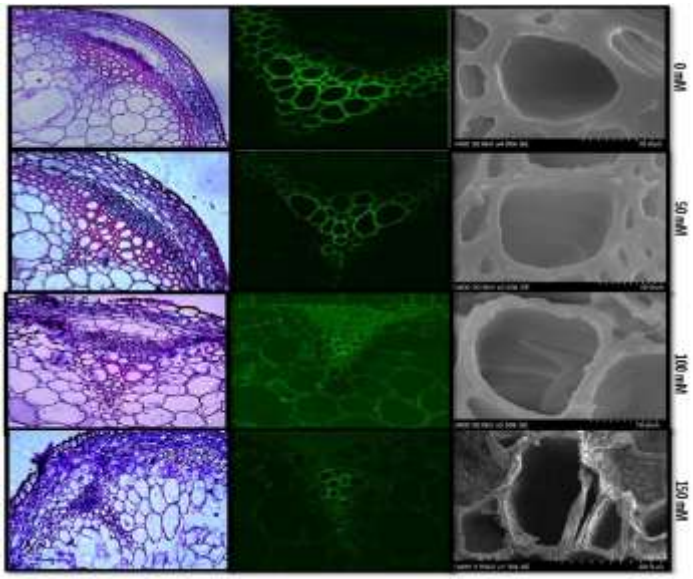

Fig.2. Stem sections of *Arabidopsis* stem observed with bright field, confocal and scanning microscope

Effect of Salinity stress on yield and Biomass

The *Arabidopsis* showed better performance under control conditions in terms of growth parameters such as, plant height, root length, rosette area and number of leaves (Table 1) as compared to stress conditions. Several yield parameters such as size of pod, number of pods and total seed yield were calculated (Table 1). It was observed that under stress conditions yield and biomass accumulation decreased, which indicates that plants are experiencing stress.

Table1. Biomass and yield attributes of *Arabidopsis* under control (0 mM) and salt stress (50, 100, 150 mM) conditions

S.No.	Attributes	0 mM	50 mM	100 mM	150 mM
1.	Plant Height (cm)	27.3	26.2	24	23.4
2.	Root Length (mm)	8	6	4	4
3.	Rosette Area (cm^2)	4	3	2	2
4.	No. of Leaves	14	10	6	5
5.	No. of Pods	43.6	39.2	37	37.4
6.	Pod size (cm)	1.5	1.3	1.2	0.94
7.	Total seeds	884	812	768	661

Changes in shape and size of Cell wall under Salinity stress

The detailed changes that occur in wall thickness during salinity stress were investigated in *Arabidopsis*. Using scanning electron microscopy (SEM), cell wall thickness was measured in stem prepared by rapid plunge-freezing in liquid nitrogen slush and then freeze-fracturing perpendicular to their long axes (Table 2). Thickening, thinning, and maintenance of wall thickness were observed within this growth conditions, and salinity stress. It was concluded that wall thicknesses are regulated by salinity stress, are dependent upon the carbon resources available to the cell(Table 2).Under salt stress, lignification finds it substantial role at anatomical and ultra-structural level, where it enables long distance water transport and sustain structural reinforcement of the vascular tissue (Sánchez-Aguayo et al.2004). Recently, it was shown that lignin serves as a major component of the casparian strip in *Arabidopsis thaliana* roots and also prevents ion diffusion in the root endodermis (Naseer et al. 2012).

Table 2.Wall thickness of vessels and fibers in stems of the WT and transgenic lines

S.No.	Sample	Interfascicularfibers (µm)	Vessels (µm)	Xylaryfibers (µm)
1.	0 mMNaCl	2.611 ± 0.09	0.808 ± 0.02	0.261 ± 0.02
2.	50 mMNaCl	2.839 ± 0.14	0.903 ± 0.03	0.18 ± 0.03
3.	100 mMNaCl	2.19 ± 0.18	0.75 ± 0.02	0.18 ± 0.02
4.	150 mMNaCl	1.548 ± 0.14	0.565 ± 0.05	0.135 ± 0.02

Wall thickness was measured from electron micrographs of fibers and vessels. Data are mean (µm) ±SE from cells. Means were compared using ANOVA.

CONCLUSION

Salt stress causes huge losses of agriculture productivity worldwide. Therefore, plant biologists aimed at overcoming severe environmental stresses needs to be quickly and fully implemented. Together with conventional plant physiology, genetics and biochemical approaches to studying plant responses to abiotic stresses have begun to bear fruit recently. *Arabidopsis* will continue to play an important role in the identification of genes and functional analysis of proteins involved in plant cell-wall biosynthesis. As more steps in wall biosynthesis are identified, there will probably be many more discoveries that challenge our current understanding of cell-wall biosynthesis and the functional roles of various cell-wall components. s

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