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Ultrastructural Changes in Shoot Apical Meristem of Canola (*Brassica napus* cv. Symbol) Treated with Sodium Chloride

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Abstract: In the present research, structure and ultrastructure of shoot apical meristem of canola (*Brassica napus* cv. Symbol) under salinity conditions were investigated. The experiments were conducted in five groups (0, 3, 6, 9, 12 dS m⁻¹) under greenhouse conditions. Sampling of apical meristem and TEM tissue preparation procedure were carried out. Semithin and ultrathin sections were prepared and viewed in light and electron microscopy, respectively. The results included reduction of meristem size, disorders in meristem structure. Also formation of autophagic vacuoles was observed that is probably one of the plant responses to salt stress for more water storage in these vacuoles and decreasing of cell water requirements.

Key words: Salt stress, canola, shoot apical meristem, ultrastructure change, structure change, meristem size

INTRODUCTION

A heavy environmental concentration of salts unleashes various types of physical and chemical stress in plants, provoking complex responses that involve changes in plant morphology, physiology and metabolism (Cheesman, 1988; Sanders and Harper, 1999). Last investigations about meristems of growth inhibition and salt tolerance, often based on comparative studies between salt-tolerant and salt-sensitive cultivars, however, since most of the environmental stresses, influence tolerance level of various developmental processes, salt stress is considered a complex phenomena (Sanders and Harper, 1999).

A high concentration of NaCl greatly reduces of both the shoot and root (Staples and Tonniessen, 1984; Cheesman, 1988). Among the shoot cells, meristematic cells are especially interesting because mitotic activity and cell division indispensable for shoot growth and because meristematic cells are considered to be one of the salt-sensitive cells (Huang and Van Stevenmek, 1988). Canola is a genetically altered form of rapeseed with low erucic acid, a 22-carbon chain fatty acid that is used in a variety of polymer and lubricant products. Interest in canola is increasing steadily among consumers due to its lowest content of saturated fatty acids (<70 g kg⁻¹) among major oil seeds. Salt-tolerance of Brassicaceae family (including canola) is placed between Poaceae (*Poa* species) with the most tolerance and Fabaceae (*Viciafaba*) with the lowest tolerance. Ashraf *et al.* (2001) showed canola and tomato cv. Monkey maker continue to yielding under NaCl 200 mM. Stepphun and Volkman

(2001) compared the effect of different salinity levels on seed emergency, germination rate, plant height, biomass and yield in canola, pea, bean and wheat. The results showed that pea and bean no yield but canola and wheat yield 0.4 and 21% of yielding at non-salinity conditions. In studding of primary growth response of *Arabidopsis* to salt stress, the important of cell cycle regulation was indicated. Growth decline of stressed roots was due to reducing in cell production and smaller mature cells (West, 2004). Sacks and Silk (1997) showed that salt stress resulted in short-term changes in cell cycle duration, however, replaced with a long-term response that meristem size is reduced but cell cycle duration turn back to initial state.

The objective of this study was to determine the structural and ultrastructural changes of shoot apical meristem of canola (*Brassica napus* cv. Symbol).

MATERIALS AND METHODS

The experiment was conducted in Faculty of Science, Islamic Azad University of Mashhad, Iran (2004). The seeds of canola (cv. Symbol) were supplied by the Agricultural Research Center of Khorasan, Iran. The study was conducted in greenhouse condition. There were a control group which received no solution of NaCl plus four other groups which treated with salt solutions of 3, 6, 9 and 12 dS m⁻¹. Shoot apical meristems of control and treated plants at vegetative phase were collected and for TEM studies, specimens were fixed in 1% glutaraldehyde with 0.1 M cacodilate buffer, pH = 7.2, at 4 for 6 h; post-fixed in 1% osmium tetroxide at 4 for 6 h, then dehydrated in an

acetone series and embedded in spur resin. Semithin sections (0.5) were prepared using ultramicrotome and stained with toluidine-blue and viewed in light microscopy. After primary distinguishing, ultrathin sections (60-80 Å) were prepared and placed on 200 mesh grids. Tissue sections were stained with 5% uranyl acetate in methanol for 20 min and observed and photographed by a LEO 914 AB TEM.

RESULTS AND DISCUSSION

Shoot apical meristem of canola is small and cone-shaped, tunica consists of two cellular layers and corpus is recognizable. Initial Ring (IR) of the meristem is recognizable. Leaf primary base, Leaf primordium and leaf primary pattern is formed due to mitosis divisions of initial ring. Prepared semithin sections using ultramicrotome, after staining with toluidine blue and observing with light microscopy, showed structure of shoot apical meristem in details (Fig. 1, 2). In treated specimens, the shoot apical meristem was under disorder and the meristem size was declined (Fig. 3).

TEM studies on shoot apical meristem of canola and areas adjacent to the meristem showed that meristematic cells in control plants (Fig. 4) are small and every cell has thin cell wall, nucleus, bulky nucleolus and mitochondria are recognizable. Cytoplasm is dense and osmiophilic due to abundance of ground ribosomes. A part of cytoplasm is occupied with vacuole. In treated plants (12 dS m^{-1}) cell wall is thickened and suitable with cell demands for more relations, a considerable number of plasmodesmata have formed in the cell walls (Fig. 5). The important note is that a major part of cytoplasm is occupied with bulky vacuole and many of destructing organelles are observed in this autophagic vacuole. Traces of mitochondria, plastids, endoplasmic reticulum and cytoplasmic vesicles are viewed in this vacuole. In the medullary meristem cells, in addition of more vacuolizing of cytoplasm, differentiation of some organelles including convert of proplastids to chloroplasts and formation of thylakoids and grana is observed (Fig. 6). In areas adjacent to the shoot apical meristem, one of the differentiations that rapidly occurred, is formation of protophloem (Fig. 7). In these cells, remarkable extension of vacuolar system and destruction of a major of cellular contents can be observed. Moreover the intermediate wall including vast plasmodesmata which are differentiating to sieve pores. In the distance among them, the cell wall has thickened and formed the bars. A number of mitochondria in the cells are observed.

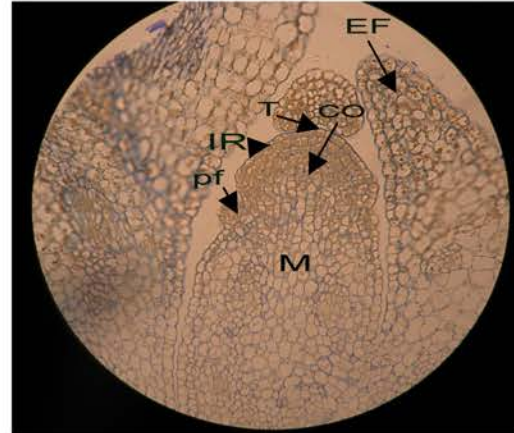


Fig. 1: Semithin section of shoot apical meristem of canola (control plant). Toluidine-blue staining (x 20). The cells of central part of shoot meristem are vacuolated and converted to medullary meristem (M), otherwise the cells of tunica and corpus have dense cytoplasm. T: Tunica, CO: Corpus, M: Medullary meristem, IR: Initial Ring, pf: Leaf primordium and EF: Leaf primary pattern

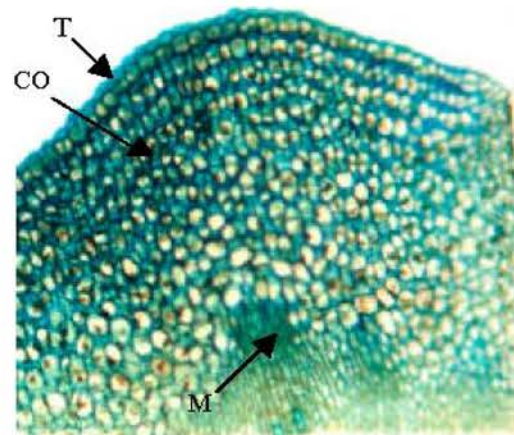


Fig. 2: Semithin section of shoot apical meristem of canola (control plant). Toluidine-blue staining (x 40). Two cellular layers of tunica are regular. Underlying the tunica, corpus cells with bulky vacuoles and less chromophilly are recognizable. T: Tunica, CO: Corpus and M: Medullary meristem

The results of present studies suggest that salt stress induced structural and ultrastructural changes in the shoot apical meristem of canola. Reduction of meristem size, disorders in the structure of shoot apical meristem, increasing of cell vacuolation and formation of autophagic vacuoles were all responses to salinity condition.

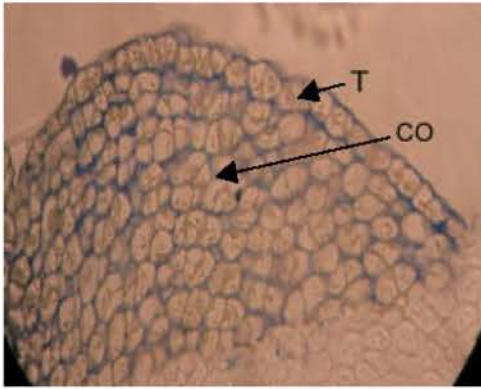


Fig. 3: Semithin section of shoot apical meristem of canola (12 dS m⁻¹ treatment). Toluidine-blue staining (x 40). Disorders in tunica organization are observable. T: Tunica and CO: Corpus

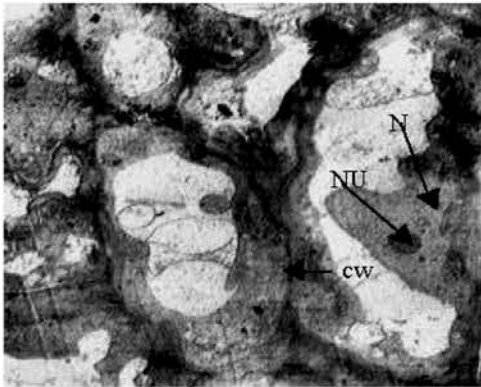


Fig. 4: TEM micrograph of shoot apical meristem of control plant (EC = 0). The meristematic cells contain recognizable nucleus and nucleolus, thin cell wall and dense cytoplasm (due to abundance of ribosomes) N-nucleus, NU-nucleolus, CW-cell wall

Similar to present results, previous investigations on the effects of salinity on apical meristem structure (Jbir *et al.*, 2003; Hanif and Davies, 1998) showed that the meristem size which is almost the same in the two wheat species (salt-tolerant and salt-sensitive), is decreased under salt stress especially in the salt-sensitive species. Sacks and Silk (1997) showed that water stress cause meristematic cells to be longer and reduced the rates of cell division that result in reduction of meristem size. One of the salt stress-induced changes in the ultrastructure of the shoot apical meristem was cell vacuolation. Jbir *et al.* (2003) reported that the cell vacuolation is increased by salt stress, especially in the salt-sensitive species. They showed that the salt stress-induced changes in the cells

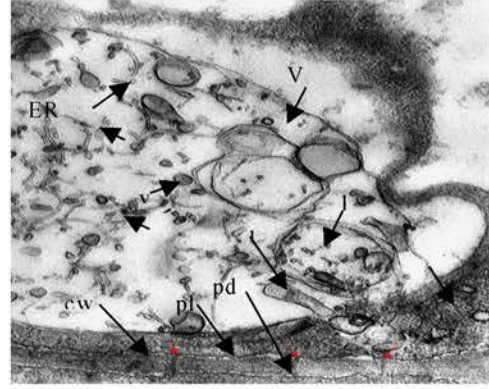


Fig. 5: TEM micrograph of shoot apical meristem of stressed plant (12 dS m⁻¹ treatment). One of the meristematic cells contain relatively thick wall with abundant plasmodesmata, plasmalemma, cytoplasm with ribosomes and endoplasmic reticulum. A bulky autophagic vacuole which surrounded most of organelles and destroyed them. cw-cell wall, pl-plasmalemma, pd-plasmodesmata, m-mitochondrion, p-plastid, vesicle, ER-endoplasmic reticulum.

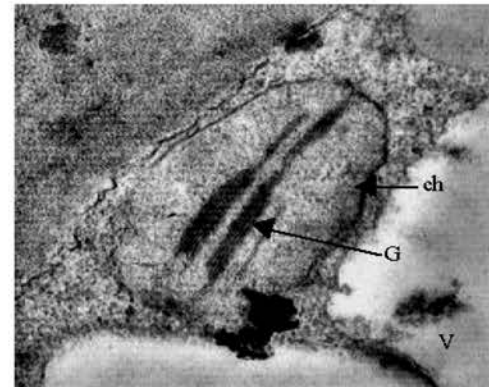


Fig. 6: TEM micrograph of shoot apical meristem. One of the medullary meristem cells: Extension of vacuolar system and formation of chloroplast with internal grana is observed. The cytoplasm is granular and contain abundance ribosomes. ch: chloroplast, G: grana, V: Vacuole

are characterized by further development of destructive processes expressed in changes of the ultrastructure of all cell organelles. Balnokin (1995) showed that the decrease in water content is accompanied by supply of Na⁺ in the cells. This can be explained by an increased permeability of the plasmalemma and other cell membranes due to a decrease in their barrier properties, which are realized through the function of ion pumping systems in the membranes.

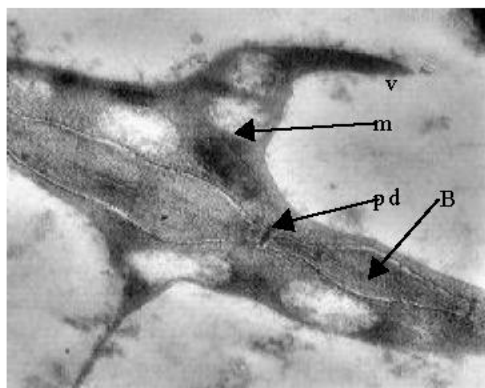


Fig. 7: TEM micrograph. One of the cells which is differentiating to protophloem in adjacent to meristematic zone of shoot apical. Plasmodesmata and mitochondria are recognizable. Thick cell wall with number of plasmodesmata that are differentiating to sieve pores. Thickened zones of cell wall (bars) are observed m: mitochondrion, V: vacuole, pd: plasmodesm, B: Bar

An excess of ions in the cytoplasm induced degradation of all organelles, except the nucleus, which is the most resistant to stress, but its structure is also affected (Werker *et al.*, 1983; Avetisova and Kadykov, 1985).

CONCLUSION

In conclusion, it can be said that salt stress induced a surge of changes in structure (reduction of size meristem and disorders in the meristem) and the ultrastructure of the shoot apical meristem (the formation of autophagic vacuoles that including damaged organelles in treated plants (thickening of cell wall for suitable with cell demands for more relations, forming a considerable numbers of plasmodesmata in the cell walls) in canola. These changes result in the reduction of water demands of meristematic cells.

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