Ultrastructural changes in potato (Solanum tuberosum) under NaCl mediated salinity stress in vitro

B. Al-Safadi (*), M. Nakar
Department of Molecular Biology and Biotechnology, Atomic Energy Commission of Syria, PO Box 6091, Damascus, Syria.

Key words: flavanone glycoside, NaCl, Solanum tuberosum, trichomes, ultrastructural changes.

Abstract: Histological analysis was employed to investigate the way potato plants (Solanum tuberosum cv. Draga and Spunta) face salinity stress. Different concentrations of NaCl (50, 100, 150, 200 and 250 mM) were used on potato plantlets growing in vitro to simulate salinity stress condition. Potato plants treated with 50 and 100 mM concentrations of NaCl went into the osmotic stage, and responded with changes: the flavone naringin was created and accumulated in the cells of the aerial parts, and a different type of trichome was observed, in addition to the original types, in potato plants treated with concentration 100 mM. This new type of trichome appeared similar to type B trichomes therefore they were called “type B-like trichomes”. While no substance was exudated from these trichomes in cv. Draga, the trichomes, in cv. Spunta, green droplets were noted on the glandular vesicle. Furthermore, the non glandular trichomes had some swollen stem cells, and branched ones were also observed. Thanks to these new trichomes, the plants had increased leaf pubescence.

1. Introduction

Plants frequently face adverse environmental conditions, such as drought, salinity, chilling, freezing, and high temperatures which may delay growth and development, reduce productivity and, in severe instances, cause plant death (Krasensky and Jonak, 2012). Salt shock is an extreme form of salt stress resulting from the sudden exposure of plants to high levels of salinity (Shavrukov, 2013). Cellular responses to stress include adjustments in the membrane system, modifications in the cell wall architecture, and changes in cell cycle and cell division. These responsive mechanisms help plants cope with their surrounding environment and tolerate these stresses (Sairam and Tyagi, 2004; De Oliveira et al., 2013).

Plant defense mechanisms against biotic and abiotic stresses can be either constitutive (continuous) or inducible under stress conditions (Freeman and Beattie, 2008). In the case of low level salinity stress, for example, the cells undergo osmotic phase due to the none lethal salinity. As a result, they accumulate organic non-toxic solutes such as sugars, proline, mannitol, sorbitol, and amino acids in the vacuoles of the cytoplasm, even if cells do not produce such compounds in normal conditions (Läuchli and Grattan, 2007; De Oliveira et al., 2013). These solutes are called osmolytes, and the reason cells amass them is to achieve osmotic balance and to protect enzyme activity. Furthermore, they can play a role as scavenger of oxygen-free radicals produced by salinity (Shannon, 1997; Sairam and Tyagi, 2004; Zhu, 2007; Etehadnia, 2009; De Oliveira et al., 2013).

Other compounds can also play a role in plant tolerance to stresses. The flavonoid compounds, for example, perform as free radical scavengers and antioxidants against oxidative damage during exposure to various biotic and abiotic stresses such as heavy metals, drought, salinity, excess solar radiation etc. (Tattini et al., 2000; Ali and Abbas, 2003; Brown, 2005; Zhu, 2007; Chutipaijit et al., 2009; Samantal et al., 2011).

Trichomes comprise another type of plant defense response and they play mechanical and chemical roles in controlling water loss by transpiration, increasing tolerance to extreme temperatures, protecting plants against attacks by herbivores, and
reducing excessive radiation (Gonzales et al., 2008; Kang et al., 2010; Adebooye et al., 2012). Solanum species are well-known for employing their trichomes (especially the glandular types) against insects, for example, the resistance of wild potato cultivars against Colorado potato beetle, potato tuber moths, the parasitoid Copidosoma koehleri (Blanchard) (Pelletier et al., 2013), green peach aphid (Myzus persicae Sulzer) (Vallejo et al., 1994), and potato leafhopper (Medeiros and Tingey, 2006). The role of potato trichomes in abiotic stress tolerance has not been reported. However, Tattini et al. (2002) found an integrated role of glandular trichomes and flavonoid glycosides in the mechanisms of acclimation of Phillyrea latifolia to excess solar radiation.

Tuber-bearing Solanum species have two major types of trichomes: glandular and non-glandular. Glandular trichomes are recognized as type A or type B and have been extensively studied (Pelletier et al., 2013). Type A has a short stalk and a four-lobed glandular head which contains phenolic compounds, while type B is characterized by its long stalk and small glandular vesicle which continuously exude acyl sugar secretions. Wild potato Solanum berthaultii bears all types of trichomes, some, or none (Pelletier et al., 2013), but some commercial Solanum tuberosum cultivars, for example Elba and Allegeny, have non-glandular and type A glandular trichomes only (Medeiros and Tingey, 2006).

The aim of the present study was to use histological analysis to investigate how potato (Solanum tuberosum) responds to salinity shock.

2. Materials and Methods

Plant material

Tubers from the potato cultivars Draga and Spunta were obtained from General Organization for Seed Multiplication (Aleppo, Syria). Draga variety, a cross SVP 50-2017 x MPI 19268 (HZPC, Netherlands) is an early variety, consistent performer, with medium yield, medium to large uniform tubers, round shape, cream-coloured skinned with creamy-white flesh and medium depth eyes (EUROGROW, 2011).

Spunta variety, a cross Béa x USDA 96-56 (Higgins Agriculture Ltd) is a medium-early ripening variety, with very high yield, long, slightly kidney-shaped, bulky tubers and shallow eyes (Ahdb, 2011).

Explants (nodal segments containing one or two buds) were prepared as previously described (Al-Safadi and Arabi, 2003, 2007).

Salinity stress

In order to simulate salinity stress in vitro, the explants were grown in tubes containing MS medium (Murashige and Skoog, 1962) with different concentrations of NaCl (50, 100, 150, 200, 250 mM) and grown as described by Al-Safadi and Arabi (2007).

Histo-anatomical study

Six plants from each salinity treatment were examined. The whole potato plant (shoot, mature leaves, root) and peels of orange fruit were prepared as follows: The samples were fixed in Carnoy’s solution (3 Ethyl alcohol: 1 acetic acid) for 2 h, then transferred to 70% alcohol and stored at 4°C until analyses. Cross-sections were prepared manually by using a blade razor. Some sections were stained with Safranin O for 5 min (Al-khatib et al., 1995; Tiţă et al., 2010); for sample examination, a bright field microscope (Nikon Eclipse 80i) was used and photos were captured by a digital camera (Nikon DS-Ri1).

Identification of the crystals

Solubility. Five different plants from each salinity treatment were used. Two leaves per plant were taken and immersed in hot water (90°C) for 10 min, and grapefruit juice was diluted with water (50%) and heated to (90°C) for 10 min. The crystals were then studied as cross-sections under Nikon Eclipse 80i microscope to observe whether any dissolution occurred.

Reacting to ferric chloride. Since physiological parameters showed no significant differences (data not shown) between plants treated with 50 mM and those treated with 100 mM NaCl, and for simplicity purposes, only leaves and cross-sections of plants treated with 100 mM of NaCl, plus the control, were soaked in a solution of 1% Ferric Chloride and heated at 90°C for 3-4 min, then left at room temperature for 10 min, to observe the change in colors.

Crystallization. To obtain and compare the sugar crystals under a Nikon Eclipse 80i microscope, Carnoy’s solution was added for 2 h to the samples for comparison: potato leaves and cross-sections of control and those treated with 100 mM NaCl, the pericarp of orange fruit and grapefruit juice.

3. Results

Our first observation when we studied the tissues of plants treated with 150, 200 and 250 mM of NaCl
was that most of the cells in studied plant parts (roots, shoots, leaves) suffered plasmolysis. This means that the cells were already dysfunctional and the plants were no longer in the tolerance stage but had shifted to the toxic stage, when ions accumulate up to fatal levels in the cytoplasm and cells cannot overcome the damage from accumulating solutes (Lauchli and Grattan, 2007; De Oliveira et al., 2013). On the contrary, at concentrations of 50 and 100 mM, plasmolysis was limited, therefore we focused on these two treatments to detect changes in the cytoplasm. Consequently, when referring to treated plants we intend those treated with concentrations of 50 and 100 mM only.

Studying cross-sections of the stems and leaves of the treated plants from both cultivars (Draga and Spunta) revealed some interesting and curious structures which did not exist in the tissues of control plants. Scattered cells, with rosette-shaped crystals inside, were observed in the cortex layer of the stems and in the spongy mesophyll layer of the leaves (Fig. 1 a, b).

The crystals resembled the shape of flavanone glycosides found in citrus (hesperidin and naringin) when crystallized. Hesperidin is a sweet tasting glycoside usually found in the mesocarp layer (the white pith, Albedo) of the pericarp (the peel) of orange fruit Citrus auratium (Hendrickson and Kesterson, 1956; Al-khatib et al., 1995), while naringin is a principal flavonoid in grapefruit and gives it its bitter taste and can be found in the juice (Kesterson and Hendrickson, 1953). Both glucosides are very similar in appearance to crystals under the microscope, as the crystal needles agglomerate in a rosette pattern and have very close chemical structures (Kesterson and Hendrickson, 1953; Hendrickson and Kesterson, 1956).

To confirm our analysis, we took cross-sections of the pericarp of orange fruit and grapefruit juice treated with Carnoy’s solution. Comparing the results to those in the treated potato, we found the two shapes to be similar (Fig. 1 c, d). These crystals were not seen in tissues not fixed with Carnoy’s solution. This is considered to be further evidence that these were crystals of a flavanone glycoside dissolved in the cytoplasm and only could be seen under microscope when crystallized by adding alcohol, acetic acid or cooling (Kesterson and Hendrickson, 1953; Hendrickson and Kesterson, 1956; Al-khatib et al., 1995).

Moreover, in the control plants, these crystals were not found in the tissues before or after treating with Carnoy’s solution or cooling.

The crystals, in addition to their rosette shape, had specific Naringin’s crystal features: i) brownish tan color while Hesperidin crystals are colorless or pale yellow as seen in the mesocarp layer of the peel of orange fruit (Fig. 1 a-d) (Kesterson and Hendrickson, 1953; Hendrickson and Kesterson, 1956); ii) when interacted with ferric chloride, the color of the leaves of treated plants changed from pale grey to light caramel. On the contrary, when the leaves of the control plants were immersed in ferric acid no difference in color could be seen (Fig. 2 a, b). The color of the crystals in the cross sections of the leaves changed from brownish caramel to a very dark red wine color (Fig. 3 a, b). The interaction of ferric chloride with Naringin, especially at a sufficient level of concentration, caused the change in color which gets darker with the increase in concentration, becoming black at very high concentrations (Kesterson and Hendrickson, 1953; Sinclair, 1972; Radhakrishnan et al., 2013); iii) when the leaves were immersed in hot water for 10 min, the crystals in the cross sections and grapefruit juice started diffusing (Fig. 3 c-e); it is known that Naringin’s crystals are much more soluble in hot than cold water, unlike Hesperidin which does not dissolve in hot water (Kesterson and Hendrickson, 1953; Hendrickson et al., 1954; Hendrickson and Kesterson, 1956). Therefore, we assume that the crystals were
Naringin.

The control plants and those treated with the 50 mM concentration had only straight non-glandular and Type A trichomes on the aerial parts. However, surprisingly, type B-like trichomes were observed in addition to the other type of trichomes in plants treated with the 100 mM concentration. While they had type B trichomes, there was no evidence that they secreted any substance in ‘Draga’ plants, although green droplets were noticeable on glandular vesicles in ‘Spunta’ plants (Fig. 4).

In control plants and those treated with 50 mM NaCl, non-glandular trichomes were straight, linear with conical shape, and the size of the stem cells were gradually reduced from the base cell to the top. However, in the 100 mM NaCl treatment, the trichomes were very long and branched with swollen stem cells on very large base cells (Fig. 5).
4. Discussion and Conclusions

In this study we found that potato plants treated with low concentrations of salinity (50 and 100 mM) went into the osmotic stage. Consequently, cells in the aerial parts created a new substance, which was identified as ‘Naringin’ (the rosette-shaped crystals we found in the cortex) (Fig. 2 a, b). Naringin is a flavanone-7-O-glycoside between the flavanone naringenin and the disaccharide neohesperidose. It is mainly found in grapefruit to which it gives its typical bitter flavor (Belajova and Suhaj, 2004).

Flavonoids, such as catechin, epi-catechin, eriodictyol, kaempferol, and naringenin in different amounts, are very common in the cultivated potato Solanum tuberosum (Brown, 2005). It has been reported for rice (Chutipaijit et al., 2009), barley (Ali and Abbas, 2003) and many other plants (Samantal et al., 2011) that flavonoids accumulate in the tissues of stressed-plants. Furthermore, Gupta and Huang (2014) reported that anthocyanin (also a flavonoid) is accumulated in plants exposed to salt stress. Flavonoids become more soluble in the form of flavonoid glycoside and thus more effective (Chutipaijit et al., 2009; Samantal et al., 2011).

Therefore, we assume that the flavonone naringenin in treated potato became available in larger quantity than normal, as a result of the salinity stress. In potato, naringenin is a byproduct of the pathway of anthocyanin and flavonoles biosynthesis (Gramene, 2016). It is unclear, from our work, how naringin was synthesized and accumulated in the tissues of potato plants treated with NaCl. However, it is likely that under stress conditions, potato plants produced naringrin through the naringenin glycoside pathway (Caspi et al., 2016), thus making it possible to visualize naringrin under the microscope as crystals.

Our study also revealed that potato plants treated with 100 mM concentration resulted in an additional type of trichomes, to the original types (non-glandular and type A) (Fig. 4 c-f). These new trichomes were similar in shape (long stalk with glandular vesicle) to those in the wild type potato Solanum berthaultii (Vallejo et al., 1994), hence, we called them “type B-like trichomes”. Here, we suggest several hypotheses to explain why potato plants formed these trichomes.

With regard to the genetics of type B trichome-density inheritance, we found that most of the studies in the literature were carried out on diploid potato. However, some researchers (Gibson, 1979; Mehlenbacher et al., 1983; Mehlenbacher et al., 1984; Vallejo et al., 1994; Jansky et al., 1999) have convergent conclusions, for example: a) the absence of type B trichomes is controlled by a few genes, at least one of them being recessive due to structural genomic differentiation; b) most of the variation among individuals of offspring of back-crosses studied were not due to heritable genetic differences; c) the inheritance of type B trichome density could be influenced by non nuclear genetic factors such as maternal cytoplasmic DNA, as suggested by Vallejo et al. (1994), and Jansky et al. (1999) who presumed that the cytoplasm in the somatic tetraploid hybrids they studied could repress the gene expression; d) in contrast, the droplet size of type B trichomes was highly heritable.

Therefore, we wonder if the genes responsible for the presence of type B trichomes were silenced in one way or another in cultivated potatoes, due to structural genomic differentiation during the hybridization, segregation and recombination processes. Especially in our case, ‘Draga’ and ‘Spunta’ are tetraploids with 2n = 4x = 48 chromosomes (Caprutoi et al., 2000, 2003). Additionally, the expression of these genes could be affected by changes in the cytoplasm (Vallejo et al., 1994; Jansky et al. 1999; Hanson and Bentolila, 2004). Therefore, we hypothesize (although more genetic studies are called for on this subject) that at least one of the genes that control the presence of type B trichomes was silenced or changed epigenetically, which affected its expression and, subsequently, the other genes could not be expressed during the evolution of the potato cultivars.

In the current study we did not investigate the genes involved in formation or activation of trichomes under salinity stress. However, other studies have looked into the WRKY transcription factors (TFs) family in plants which comprises numerous members that regulate genes involved in seed germination, seed dormancy, trichomes development, lignin biosynthesis, and both biotic and abiotic stress responses (Pnueli et al., 2002; Guillaumie et al., 2010; Wang et al., 2010). Some of the WRKY genes have been reported to be activated in response to various abiotic stresses including high salinity (Jianchao et al., 2015).

Furthermore, it has been documented that salinity could induce modifications in the genome, like other abiotic stresses, by making changes in the histone modification pattern, thus activating some genes and/or silencing others (Kapazoglou and
chomes with swollen stem cells (Fig. 5 a, b, c). The same results were reported by Kang et al. (2010) when they studied trichome distortion caused by hairless mutation of tomato (Solanum lycopersicum). Salinity may thereby change the genome epigenetically (changes in the chromatin) and genetically (changes in DNA). It is also possible that salinity affected the resultant proteins of those genes; it has been reported that salinity can make changes in the protein patterns (Barakat, 2003; Kim et al., 2012).

On the other hand, without regard to genetic reasons, Gonzales et al. (2008) referred to some reports which presumed that plants under salt stress could use the glandular trichomes as facultative salt glands and to eliminate excessive salt (Gonzales et al., 2008). In our case, it is possible that a conversion from non-glandular to glandular trichomes under salt stress occurred. However, as we indicated in the results section of this work, we did not observe any substance exudates from the type B-like trichomes in ‘Draga’, while in ‘Spunta’, these trichomes exuded some kind of green droplets. If the droplets had the same chemical makeup as the exudate from the glandular vesicle of type B trichomes (sucrose esters viscous droplets) (Pelletier et al., 2013), it would mean that they may form an irregular local layer on the surface of the leaves; it is known that these trichomes secrete droplets continuously (Pelletier et al., 2013; Wollenweber et al., 2005). This layer would play a role of additional covering to coat and reduce the absorbance of radiation and water loss.

Furthermore, we detected some non glandular trichomes with swollen stem cells (Fig. 5 a, b, c). The same results were reported by Kang et al. (2010) when they studied trichome distortion caused by hairless mutation of tomato (Solanum lycopersicum). They found that type I trichomes (equivalent to type B trichomes in potato) on the hl mutant were crooked and had highly swollen stem cells (Kang et al., 2012). In contrast, Gomes et al. (2011), after treating Salvinia auriculata Aubl. with different doses of NaCl salt, found that the trichomes became more slender as the dose was increased.

We also noticed branched trichomes (Fig. 5 d) which are probably caused by the extensions in the swell of stem cells, giving the appearance of branching. Kang et al. (2010) reported the same observation in tomato affected by hairless mutation (Kang et al., 2012). Also, irregularity in trichome shape was reported in some plants which were exposed to salt stress (Adebooye et al., 2012). Nevertheless, the branched trichomes are more effective in protecting the plant from losing additional amounts of water by forming a shield against sunlight, thus maintaining a good quantity of water to adjust the turgor pressure in cells. Somehow, the branched trichomes and type B-like trichomes contributed to increasing leaf pubescence, which is a familiar phenomenon among some plants subjected to drought or salinity stress (Gonzales et al., 2008; Makbul et al., 2011; Adebooye et al., 2012).

In our study, potato gained new traits: naringin (the flavanone glycoside), branched non-glandular trichomes and type B-like trichomes. The new trichomes allow these plants to have increased leaf pubescence and perhaps different chemicals on the leaf surface, making them more resistant to insects.

We believe more research must be carried out on how salinity and drought could be exploited to produce more resistant plants. After all, as some research has pointed out, salinity does not always cause negative effects in plants (Shannon and Grieve, 1999).

In conclusion, potato plants (Solanum tuberosum L.) have mechanisms to help them tolerate salinity stress at the cellular, biochemical, and physiological levels. In the present study, some changes were observed in potato plants growing in vitro, including the creation of the “flavanone glycoside” naringin in the cells of the aerial parts (playing the role of antioxidant) and the formation of a new type of trichome. Further research is needed to study the role of these cellular changes in potato plants reacting to salinity stress.

Acknowledgements

The authors would like to thank the Director General of AECS and the Head of Molecular Biology and Biotechnology Department for their support. The assistance of N. Tafesh and I. Karajoli in conducting the experiments is much appreciated.

References


ALI R.M., ABBAS H.M., 2003 - Response of salt stressed


FREEMAN B.C., BEATTIE G.A., 2008 - An overview of plant defenses against pathogens and herbivores. - The Plant Health Instructor, APS.


