Doctoral Thesis

Physiological Characterization of Salinity Tolerance in the Leafy Vegetable, Huckleberry (*Solanum scabrum* Mill.)

(Summary)

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The decrease in crop production as a result of salinization of agricultural lands is an important global concern. Most crops are glycophytes and since glycophytes are very sensitive to salinity, crop production will continue to drop if actions are not taken to develop salinity tolerant crops. Understanding the physiological adaptations of plants to salinity is an important prerequisite to engineering this salinity tolerance. *Solanum scabrum* Mill. (Solanaceae) (huckleberry) is a glycophyte and one of the most important leafy vegetables in many parts of Africa, especially in Cameroon where it is cultivated and exported. It is rich in Ca, Fe, vitamin A and protein and has been shown to have important medicinal attributes such as anti-inflammation and antioxidant activity. However, the growth of huckleberry has been shown to be hampered by drought stress, but its tolerance to salinity is unknown. Therefore, given the current global trend of increasing salinization of arable lands, it is important to understand the adaptation of the plant to salinity for better long-term management options and increased productivity. Thus this study was conducted with the aim of characterizing the physiological responses of huckleberry to salinity. This was achieved by 1. determining the growth and mineral uptake, 2. evaluation of the Na distribution pattern and the activity of some antioxidant enzymes, and 3. analyzing a high affinity potassium transporter (*HKT*) gene that excludes Na from shoots.

1. Comparative growth and mineral accumulation in huckleberry and eggplant

During salt stress, one of the factors affecting plant growth is nutrient deficiency. Thus, the mineral content of the plants grown under 50 and 150 mM NaCl was compared with that of eggplant. The results obtained revealed that the growth of huckleberry was better than that of eggplant and the accumulation of the major elements K and Ca in huckleberry was enhanced under salt stress. Especially, Ca was more accumulated in salinity condition than in control conditions. Na concentration was observed to be more elevated in the stem and root and much reduced in the leaf of huckleberry compared to eggplant. This yielded much lower Na/K and Na/Ca ratios in huckleberry compared to eggplant. These low ratios are important traits for plant adaptation to salinity and the reduced leaf Na accumulation is a characteristic of tolerant glycophytes and indicates the presence of a Na transport regulatory mechanism. Thus, it was concluded that huckleberry is more tolerant than eggplant owing to reduced transport of Na to the leaf, which helped the plants to maintain high levels of important minerals such as K, Mg and Ca.

2. Spatial Na accumulation and the activity of some antioxidant enzymes

Following the previous results showing reduced leaf Na accumulation in huckleberry, further studies were required to determine the pattern of Na uptake and distribution in the plant. To this end Na accumulation was measured in root, stem, petiole and leaf blade of plants grown under 150 mM salinity condition. The results revealed that the Na concentration in huckleberry increased from root to stem, decreased from stem to petiole and further decreased from petiole to leaf blade. The analysis of Na content revealed that of the total shoot Na content, only 50% was transported to leaf blade compared with 81% in eggplant, indicating that indeed there is the presence of a Na transport regulating mechanism possibly located in root, stem and petiole in huckleberry and seems to be lacking in eggplant. Thus, the tolerance of huckleberry could primarily lie in its ability to exclude Na from leaf blade.

Since under salinity one of the main causes of growth impairment is oxidative stress, induced my ROS, oxidative stress damage was evaluated by measuring malondialdehyde (MDA) content. The MDA content was found to be higher in leaf and root of eggplant than huckleberry indicating oxidative stress damage. Therefore, the detoxification of ROS is important if the plant has to survive salinity stress. In this light, antioxidant enzyme activity has been shown to be important in salinity stress tolerance through their scavenging properties of reactive oxygen species (ROS). Therefore, the activities of catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR), soluble peroxidases (sPOD) and cell wall peroxidases (cwPOD) were measured in the leaf and root of huckleberry and eggplant. The results showed that except for CAT, the activities of the enzymes were higher in the root than leaf of both plants. However, root activity was more enhanced in huckleberry than eggplant. More interestingly, the activity of cwPOD markedly increased in root and leaf of huckleberry, but decreased in eggplant for both tissues. Although this enzyme has been shown to be involved in lignin and suberin synthesis, which in the root intervenes in controlling Na transport to the shoot under salinity stress, it appears in the present study to be the main ROS scavenger in the leaf of huckleberry. However, in the root, aside ROS detoxification, it could also be involved in the reduction of Na delivery to the shoot through its function in lignin/suberin synthesis. On the contrast, the reduced enzyme activity in eggplant suggests the absence of such a barrier and consequently the free flow of Na to the shoot resulting in growth reduction.

3. Analysis of a high affinity potassium transporter (*HKT*) gene

The Na accumulation pattern in huckleberry suggest the existence of control points for Na exclusion in leaf blade. HKTs have been shown to control the transport of Na to leaf blade by extracting Na from the transpiration stream into xylem parenchyma cells, hence minimizing the amount of Na reaching the leaf. In light of this function, we measured the Na concentration in all four organs (leaf blade, petiole, stem and root) and analyzed the expression of the *Solanum scabrum HKT* (*SsHKT*) and *S. melongena* HKT (*SmHKT*) genes in these organs of plants grown in hydroponic culture under salinity stress.

The results revealed that in huckleberry, there was a progressive reduction in Na concentration from root to leaf blade as observed in soil culture and The Na accumulated was distributed as follows: root (52%), stem (29%), petiole (4%) and leaf blade (15%). The *SsHKT* expression pattern was proportional to that of Na concentration i.e. strongest in the root and progressively decreased to the leaf blade (lowest). Analysis of the SsHKT sequence revealed the presence of the SGGG selectivity filter pore-forming motif, which is selectively permeable to Na over K and is characteristic of the class1 HKT transporters. This characteristic selectivity filter enables the class 1 HKTs to regulate Na transport by retrieving Na from the transpiration stream and storing in neighboring xylem parenchyma cells. The highest induction of *SsHKT* expression observed in the root corresponded to the highest Na accumulation, indicating that *SsHKT* like other class 1 HKTs, would likely function in retrieving Na from the transpiration stream in all the plant parts, especially in the root and stem. In eggplant, the *SmHKT* was weakly expressed in the root and completely repressed in the other parts indicating insufficient capacity to control the transport of Na to the leaf blade resulting in excess accumulation of Na in the leaf blade (60% of total Na content)).

4. Conclusion

The results of the present study show that huckleberry is more tolerant to salinity than eggplant and that this tolerance is brought about by enhanced antioxidant activity, especially in the root, and reduced Na accumulation in leaf blade. Gene expression analysis revealed that the reduced accumulation of Na in leaf blade would be achieved by the induction of *SsHKT* expression in root and stem. This SsHKT function owes to the presence of the SGGG selectivity filter configuration, which renders it selectively permeable to Na. Thus, it would likely function in retrieving Na from the xylem of all the organs tested in response to salinity tress. Induction of *SsHKT* expression by 27.9-fold was observed in the root, corresponding to the region with highest Na accumulation and enhanced antioxidant activity. This suggests that, the rnechanisrn of salinity tolerance in huckleberry rnay involve a coordinated action of ROS detoxification and regulation of Na transport at the level of the root. In eggplant on the contrast, low antioxidant activity and weak expression of *SmHKT* could contribute to its susceptibility to salinity stress.