Molecular Mechanisms Involved in Salinity Tolerance in Barley



Salt tolerant (left) and salt sensitive (right) barley lines are grown on hydroponic culture under greenhouse conditions to show how salt treatment affects plants.



Visible effect of salt stress (#15dSm1-) on plant growth on hydroponic culture under greenhouse conditions. Tolerant line (left), thrives while sensitive line's (right)growth is hindered. This helps to explore the mechanisms of salinity tolerance

Thematic Area: Crop Productivity and Diversification

Purpose: Identify and isolate genes contributing significantly to salinity tolerance

Geographic Scope: MENA region

Timeline: 2013 - 2015

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Salinization poses a serious threat, particularly to countries with arid and semi-arid climates like those of the Middle East and North Africa (MENA) region. More than 1.5 million hectares of agricultural land is lost every year to salinity and it is estimated that as much as 20% of the world's arable land is saline. The end result? Crop yield decline, major constraints for agriculture production and increased global threat to food security. It is clear that we are at a crossroads where innovative methods are needed to render popular crops, such as barley, tolerant to high saline conditions.

Barley (*Hordeum vulgaressp. vulgare*), widely known as a "cereal grain" high in fiber and rich in key vitamins and minerals, is one of the most salt tolerant small grain crops. It has multiple uses as a food and can also be converted into malt or used for fodder. Developing barley varieties that are more tolerant to saline soil environments is critical for both developed and developing countries.

The International Center for Biosaline Agriculture (ICBA) initiated the "Molecular Mechanisms Involved in Salinity Tolerance in Barley" project to study the biochemical, genomic processes and the genetic mechanism involved in controlling several previously unstudied traits of barley that are likely to contribute significantly to salinity tolerance. Identifying single genes that play a key role in stress tolerance can improve breeding effectiveness while allowing for easy introduction and monitoring of the crop.

Activities and Outcomes

Pinpointing plant tolerance to salinity constraint involves both complex and integrated responses at the cell, metabolic and anatomic level. The project is aiming to identify novel mechanisms in barley that come into play under stress conditions such as high salinity, drought and heat. Not only is it investigating relevance to stress response, the project is also striving to provide information on genetic control, thus creating a set of tools that can be utilized in plant breeding programs.

In order to distinguish the mechanisms that are of general relevance for survival and yield under high salinity stress conditions, the project will:

> Monitor net sodium (Na+) uptake by roots and Na+ translocation and accumulation in leaves by measuring Na+/ K+ (potassium) ratio in roots, leaf sheaths and leaves





Field trial showing barley plants irrigated with salty water (15dSm⁻¹) at ICBA show salinity impact at field level which can be helpful for breeders in improving salinity tolerance in marginal environments.

- > Identify, isolate and clone candidate genes
- > Characterize the expression profile of the selected genes
- Screen selected genes for favorable alleles by development of SNPs markers
- > Generate transgenic barley lines tolerant to salinity stress

Ten barley lines (5 tolerant and 5 sensitive) were selected based on field trials for screening a worldwide core collection of barley. Thorough characterization of the stress response by measuring several physiological and molecular parameters of these 10 varieties followed.

When plants are cultivated in high salinity soils, ions are concentrated at the root zone, hindering water uptake and increasing transpiration (loss of water from leaves into the air). In turn, this results in ionic and electric imbalances, decreased growth, delayed

development and finally, plant death. An abundance of sodium ions is toxic for plants as they confuse them with potassium, an essential mineral for their growth and proper function. Young leaves and photosynthetic tissues are particularly sensitive to salt stress. Identifying and producing salt tolerant crops is therefore imperative to alleviate this problem.

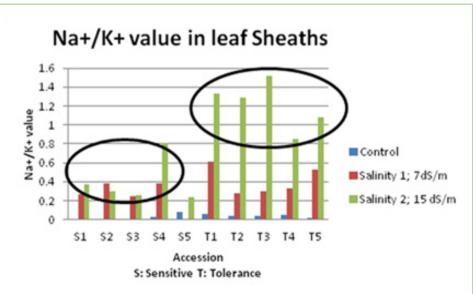
The project used a previously proven hypothesis that Na+ accumulation in leaf sheaths identified in durum wheat is an efficient way to protect cells from Na+ injury and plays a crucial role in plant tolerance to salinity. The same hypothesis was tested in barley by studying the expression pattern of one candidate gene

(HKT1;5) associated with Quantitative Trait Loci (QTL Nax2) controlling the unloading of Na+ to the xylem and in the control of root-to-shoot Na+ transfer.

High expression level by semi-quantitative RT-PCR of the HKT1;5 gene was observed in leaf sheaths of the tolerant barley varieties, while moderate expression was observed in sensitive varieties and the control non-stressed variety. In other words, what these studies suggest is that the HKT1;5 gene might be involved in the Na+/k+ transport through the plasma membrane in the leaf sheath with a more active role in the tolerant varieties. The HKT1;5 gene may in fact play a key part in regulating Na+ transport within the plant cells and is suggested as the most likely candidate gene associated with salt tolerance through ion exclusion or translocation.

Future Directions

Much work lies ahead as several previously unstudied traits that are to significantly contribute to salinity tolerance at agriculturally relevant salinities will be studied. Osmotic (minimum pressure which needs to be applied to a solution to prevent the inward flow of water) tolerance will be measured and related to yield maintenance under saline field conditions. The molecular genetic basis of various traits that are meant to contribute to salinity tolerance in mapping populations of barley will be examined. ICBA's approach will utilize recent advances in genetics and phenotyping directly in field trials. As a result, tools will be provided to increase crop yields under saline conditions. In parallel, the best transgenic barley varieties will be generated and validated for their tolerance to salinity stress. End results will enable breeders to improve salinity tolerance in available seeds and thus help successfully sustain rural populations in marginal environments.



Sodium and potassium accumulation in leaf sheaths of the tolerant and sensitive barley line indicates that tolerant lines retained and accumulated more sodium than the sensitive ones.