Development and Evaluation of Transgenic Chickpea for Tolerance to Drought and Low Temperature Stress by Using P5CSF Gene and Drought Responsive Regulatory Elements

Water deficit is a major constraint to chickpea crop productivity where the genetic engineering approaches can provide a novel and effective strategy to enhance its tolerance to drought. Genetic engineering of chickpea for enhanced tolerance to water stress has been carried out by using the osmoregulatory P5CSF129A gene and the DREB1A transcription factor which acts as a major "switch" that triggers a cascade of genes in response to abiotic stress. In the first phase of this project, an efficient and reproducible transformation protocol was developed to facilitate the production of transgenic chickpea plants. During this period, 48 transgenic events of chickpea var. C 235 were produced by using the 35S:P5CSF129A through Agrobacterium tumefaciens –mediated genetic transformation. These events were characterized at the molecular and biochemical level where most were positive for gene integration and followed the Mendelian segregation ratios in T1 and T2 generations.

During the beginning of phase 2 (2004-2007), 18 events carrying rd29A:DREB1A that were developed in a separate study were introduced into this project and advanced to T4 generation. During this period, a dry down procedure was also optimized to screen the transgenic chickpeas for traits contributing towards potential drought tolerance. A procedure with a more realistic physiological response to progressive soil drying was adopted so as to include a proper control of soil moisture depletion. This also ensured that the test plants were exposed to stress levels and kinetics of water-deficits similar to those likely to occur under field conditions. The transgenic events, containing either the CaMV 35S:P5CSF129A gene or the rd29A:DREB1A transcription factor having low copies of the respective transgenes (1-2 copies) were phenotypically evaluated in dry-down experiments to study various physiological parameters including plant responses to soil drying as measured by the fraction of transpirable soil water (FTSW), stomatal conductance and transpiration efficiency (TE). The events exhibiting a diversity of stress response patterns, especially with respect to the Normalized Transpiration Rate Response (NTR) -FTSW relationship were selected from that ranking. Seven events, each carrying the P5CSF129A or DREB1A genes were evaluated for the kinetics of transpiration response to progressive soil drying. All the selected transgenic events differed from the wild type parent in their normalized transpiration rate response (NTR) to FTSW, showing a decline in transpiration at lower FTSW values (drier soil). Several events had superior transpiration efficiency (TE), photosynthetic activity, stomatal conductance and total transpiration under water limited conditions in comparison to the untransformed control variety (C 235). All the selected transgenic events had a transpiration decline upon soil drying in drier soil than for untransformed parent. The total biomass produced during the dry down cycle (Δ biomass) showed differences amongst the transgenic events.

A series of experiments were then performed with the selected transgenic events carrying the DREB1A or P5CSF129A genes. The response of transpiration to progressive soil drying and TE was measured using our standard protocol. The assessment of 11 transgenic events carrying the 35S:P5CSF129A gene...
under dry-down conditions in two different experiments showed that they differed in their transpiration responses to soil drying. The TE of only two transgenic events carrying the 35S:P5CSF129A gene showed a modest increase in TE compared to the wild-type (WT) parent. These results indicated that, although, the overexpression of osmolyte proline in these transgenic chickpeas resulted in a sort of osmotic adjustment by maintaining the cell turgor and physiological processes of these plants, thereby, resulting in the postponement of dehydration as the water deficits developed under the dry down conditions. However, there was no significant advantage in the TE values, which is one of the potential component traits of drought tolerance. On the other hand, several transgenic events carrying the DREB1A transcription factors driven by a stress-responsive promoter rd29A showed significant increase in transpiration efficiency compared to the WT. Hence, the results obtained in this study indicated that a single gene approach involving engineering of an osmoregulatory pathway is unlikely to solve the drought issue by itself, and it seems a good idea to focus on the ongoing efforts using transcription factors driven by a stress-responsive promoters that are likely to switch on a battery of genes in response to stress. These hypotheses will be further tested in studies involving lysimetric and contained field evaluations of the selected transgenic events.