DREB genes and deeper rooting phenotype in common bean (*Phaseolus vulgaris*) for drought tolerance

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INTRODUCTION

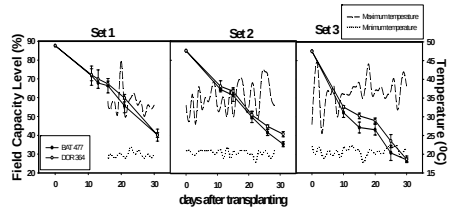
Common bean (*Phaseolus vulgaris* L.) is a staple food crop for many developing countries, mainly in Latin America and Africa. However, most regions of cultivation are also areas where extreme drought conditions strike in specific seasons or even throughout most of the year, affecting growth and seed productivity. Drought tolerance is found in different bean races of wide genetic diversity. This suggests that genetic diversity in drought mechanisms and genes could exist. One important mechanism is rooting depth as manifested in line BAT 477 of Middle American race. This line has expressed tolerance in many sites in international trials. Our research focus is now to identify genes for the deeper rooting phenotype that could be tagged by molecular techniques to facilitate their selection in breeding programs. One family of genes of great interest are the *DREB* genes, which are master switches of downstream genes whose coordinated expressions lead to abiotic stress tolerance, including drought, in the plant. In this study, we tested the hypothesis that *DREB* gene(s) are associated with the deeper rooting phenotype.

MATERIALS AND METHODS

Plant materials and stress treatments

Drought shock treatment, three-weeks old plants of two bean genotypes, BAT477(drought tolerant) and DOR364 (drought sensitive) grown in pots in the greenhouse were placed on a table in the greenhouse after washing soil from the roots and collected at 0, 2, 3, 6, 12 and 24 hours. For each time point roots and leaves of four plants were collected for gene expression analysis.

Terminal drought experiments, Three greenhouse experiments were conducted using a mix of andisol from Darien of Colombia with sand (2:1 w/w). Two genotypes described above were grown under terminal drought (starting with 85% field capacity-FC level and drying out) in transparent plastic cylinders (80 cm long and 7.5 cm diameter) covered with black and white paper. The experiments consist of split-plot design with 6 time points (5, 10, 15, 20, 25 and 30 days after treatment-date) as main plots, genotypes as sub-plots and three replications. Control plants were grown under 80% FC during the whole experiment and they were harvested at different time points as indicated in "RESULTS". Twenty cm of root tissues from root tip was collected for gene expression analysis.



Effect of maximum temperature in the greenhouse on the field capacity level of soil during three sets of experiments of terminal drought on two common bean genotypes.

Gene isolation and characterization

*DREB1*homologs from bean were isolated from bean genomic DNA by PCR based cloning techniques using conserved AP2 domain sequences of *DREB* genes. A local *P. vulgaris* ESTs database compbio.dfci.harvard.edu/ was used to identify ESTs for *DREB2*, ESTs sequences were use to design PCR primers to perform a chromosome walking using DNA walking Speedup™ (Seegene)

Gene expression analysis

cDNA were obtained from total RNA. It was extracted from leaves and roots using TRIzol® reagent (Invitrogen). Gene expression analysis was done by Real Time PCR with gene specific primers and as internal control r18S primers in an OPTICON II (MJ research) using the Brilliant® SYBR® Green QPCR Master Mix (Stratagene).

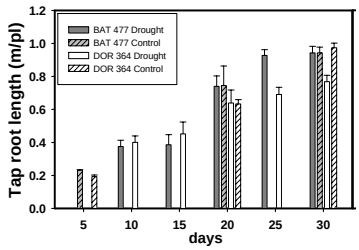
RESULTS

Physiological and morphological analysis of drought tolerant bean

Under the terminal drought conditions, BAT 477 developed a deeper rooting system in the tap (main) root than DOR 364 and the larger difference in tap root length was found at 25 days. Before 30 days of the treatment, BAT 477 reached the bottom of the cylinder, searching for and depleting available water. When the tap root length in drought stressed plants was compared with that of control plants, the root growth was maintained or even promoted in the different sets of the experiment in BAT477 while the tap root growth of DOR364 was inhibited.

Experimental set up for terminal drought

Root morphology under terminal drought for 30 days



Tap root length of two common bean genotypes under terminal drought conditions

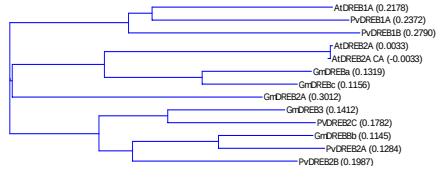
DREB gene expression analysis under drought shock and terminal drought conditions

Drought shock treatment: No significant induction of the gene expression was found for *PvDREB1A, PvDREB1B* and *PvDREB2C* although all the genes showed constitutive expressions in leaves and roots of BAT477 and DOR364. On the other hand, we found that *PvDREB2A* and *PvDREB2B* were induced by drought in both genotypes in leaves and roots (data not shown). The increase of expression of *PvDREB2A* in roots was found especially between 6 and 24 hours of the treatment. For *PvDREB2B* the transcript level was especially induced between 6 and 12 hours in both varieties.

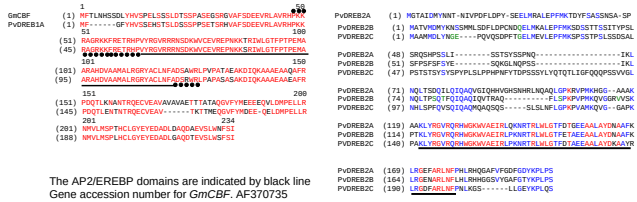
Terminal Drought: As same as in drought shock stress, *PvDREB1* genes did not show increased transcript level in both genotypes. Interestingly, *PvDREB2A* and *PvDREB2B* genes are induced by the terminal drought only in drought sensitive line, DOR364, but not in BAT 477 which has deeper rooting phenotype under drought conditions.

Isolation of *DREB* genes in common bean

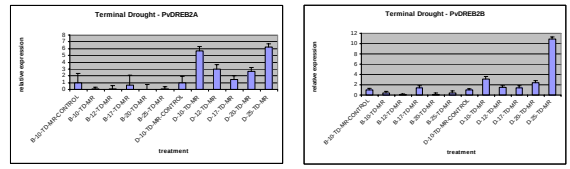
Two *DREB1* homologs named *PvDREB1A* and *PvDREB1B*, and three *DREB2* homologs, *PvDREB2A*, *PvDREB2B* and *PvDREB2C* were identified in this study.



Multiple alignment of DREB1 and DREB2 genes from P. vulgaris Arabidopsis (At) and soybean (Gm) orthologous.



The AP2/EREBP domains are indicated by black line
Gene accession number for *GmCBF*. AF370735



PvDREB2A and 2B gene expression under terminal drought B: BAT477 D:DOR364
Number: duration of treatment (days) TD: Terminal Drought MR: Main root

CONCLUSIONS AND PERSPECTIVES

- Drought tolerant genotype, BAT477 showed deeper rooting phenotype in terminal drought conditions.
- Physiological data suggested that BAT477 has high water-use-efficiency during drought stress, which is achieved by through reduced transpiration through stomatal closure while maintaining photosynthetic efficiency (data not shown).
- Two types of *DREB* genes were isolated from *P. vulgaris* . One, the *PvDREB1*-type genes, are cold-inducible (data not shown) and other are drought-responsive *PvDREB2*-type genes.
- *PvDREB2A* and *PvDREB2B* genes may not contribute to deeper rooting phenotype, which is one of genetic traits for drought tolerance in bean.

ACKNOWLEDGEMENT

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