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Research article

Photosynthesis and chloroplast genes are involved in water-use efficiency in common bean

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ABSTRACT

A recent proposal to mitigate the effects of climatic change and reduce water consumption in agriculture is to develop cultivars with high water-use efficiency. The aims of this study were to characterize this trait as a differential response mechanism to water-limitation in two bean cultivars contrasting in their water stress tolerance, to isolate and identify gene fragments related to this response in a model cultivar, as well as to evaluate transcription levels of genes previously identified. Keeping CO_2 assimilation through a high photosynthesis rate under limited conditions was the physiological response which allowed the cultivar model to maintain its growth and seed production with less water. Chloroplast genes stood out among identified genetic elements, which confirmed the importance of photosynthesis in such response. *ndhK*, *rpoC2*, *rps19*, *rrn16*, *ycf1* and *ycf2* genes were expressed only in response to limited water availability.

1. Introduction

One of the main components of climatic change is the alteration in the distribution of water by shorter and erratic rainy seasons, forcing many plant species to complete their life cycles under water stress conditions, restricting plant growth, development, survival and yield (Ahuja et al., 2010).

Water is a limited resource and agriculture consumes about 78% of it (de Fraiture and Wichelns, 2010), hence a recent proposal to mitigate the effects of climate change and to reduce agricultural water consumption is generating cultivars which use water efficiently (Boutraa, 2010), mainly in species of agronomic and alimentary interest such as common bean, which is mostly grown under rainfall, condition under which 60% of agricultural crops are produced (Molden et al., 2010). A crucial step in the colonization of terrestrial environments by plants was the evolution of genetic and physiological mechanisms which allowed them to control water loss while still fixing CO_2. This disjunctive and its implications in the plant water balance, its hydraulic and stomatal functionality are evident in the vascular plants evolution (Pittermann, 2010). The water-use efficiency (WUE) at physiological level is defined as the ratio between biomass and/or seed produced over water consumed (Xing et al., 2011). WUE in plants can be improved by increasing carbon assimilation while keeping the transpiration rate, or by reducing the transpiration rate while the carbon assimilation is kept (Yoo et al., 2009). Mittler and Blumwald (Mittler and Blumwald, 2010) mention that there is a genetic basis for WUE and is possible to perform breeding for this trait. Although WUE variation has been observed in plants, only recently its molecular characterization and dissection has started in the model specie *Arabidopsis thaliana*. So far, the engineering of major field crops for improved WUE with single genes has not yet been achieved (Karaba et al., 2007). Among the genes involved in the regulation of this phenomenon, ascorbate peroxidase (*apx2*), *erecta*, *hardy* and the transcription factor GT-2 LIKE (*gtl1*) stand out. The *A. thaliana* mutant *alx8* has a high housekeeping expression of the *apx2* gene,

Abbreviations: WUE, Water-use efficiency; PS, Pinto Saltillo; BM, Bayo Madero; TB, Total Biomass; *A*, Photosynthetic Rate; g_s, Stomatal Conductance; PRO, Yield of Seeds; WC, Water Consumed; ssDNA, Single Strand DNA; 20%, Limited Water Regimen; 60%, Optimal Water Regimen; dsDNA, Double Stranded DNA; pDNA, Plasmid DNA; cDNA, Complementary DNA; ROS, Reactive Oxygen Species.

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conferring water stress tolerance and a higher WUE (Wilson et al., 2009). The housekeeping (35S) overexpression of the *erecta* gene in an *A. thaliana* transgenic line increased its WUE by improving its photosynthetic rate, reducing its transpiration and stomatal density (Xing et al., 2011). The expression of *A. thaliana* gene *hardy* in rice improved its WUE by increasing the CO_2 assimilation through a high photosynthetic rate and a reduction in transpiration (Karaba et al., 2007). *A. thaliana* mutants with function loss of *gtl1* increased their water deficit tolerance and have a higher WUE by reducing the daily transpiration without a reduction in their biomass accumulation (Yoo et al., 2010). The aims of this study were to characterize the efficient use of water as a differential mechanism in response to water limitation in two bean cultivars contrasting in their water stress tolerance, isolate and identify fragments of genes associated to this response in a model cultivar and to evaluate transcription levels of genes previously identified.

2. Materials and methods

2.1. Plant material and treatments

Two bean cultivars contrasting in their water stress tolerance were selected, due to their differential ability to produce seeds under limited irrigation conditions. It has been reported (Rosales et al., 2012) that the Pinto Saltillo (PS) cultivar under drought reduced its pod biomass only by 17.0% with respect to irrigation, while for Bayo Madero (BM) cultivar the reduction was of 42.0%, considering the first one cultivar as tolerant and the second as susceptible. Both cultivars show prostrate growth habit type III, short photoperiod and belong to Durango race. In 2011 two experiments in Spring–Summer and Autumn–Winter cycles were established, at the Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP) Campo Experimental Bajío (CEBAJ), located at 20° 31′ north latitude and 100° 45′ west longitude to 1765 m above sea level. Experiments were established in a greenhouse using pots (16 cm height X 18 cm greatest diameter) with peat moss type substrate (Sunshine® mix No. 3) of which field capacity was determined as 1.89 g of retained water per gram of soil. Seeds were germinated in humidity chamber at 30 °C and five days later these were transplanted into pots containing the previously established treatments. In the spring-summer and autumn–winter cycles, four water regimens were established: 20%, 40%, 60% and 80% with respect to field capacity. To avoid water loss by evaporation and percolation, pots were covered with foil and drain holes were sealed with silicone, so that the only losses were by evapotranspiration. Water regimens were kept assuming that weight loss corresponded to consumed water and it was replenished through controlled daily irrigations. The only nutrients resource available was the previously contained in the substrate. For each water regimen, eight replicates were kept for 75 days, at that time plants reached full maturity and drastically reduced their water consumption. The average maximum and minimum temperatures in the spring-summer and autumn–winter cycles were 38 °C/14 °C and 31 °C/12 °C respectively, with average maximum and minimum relative humidities of 60%/36% and 86%/54%. The photoperiod was 14 and 12 light hours, according to the weather station located at CEBAJ.

2.2. Evaluated variables

In both agricultural cycles total biomass (TB) was evaluated, drying plants at 80 °C overnight. In the autumn–winter cycle the photosynthetic rate (A) and stomatal conductance (g_s) were evaluated with an infrared gas analyzer (LI-6400, LICOR) using the leaves which better represented the plants state. Radiation was set to 1000 mmol m^{-2} s^{-1} for 30 min, photosynthesis was subsequently calibrated to 1000 mmol m^{-2} s^{-1} with a CO_2 concentration of 400 mmol mol^{-1}, leaf temperature was kept at 25 °C and vapor pressure between 1 and 1.3 kPa. Measurements began between 12:00 and 13:00 h with a 70% average relative humidity. These variables were evaluated in three phenological stages: vegetative, reproductive and maturity, considering them when 50% of the plants had three composed leaves, when they were flowering and when pigmentation changed in mature pods, respectively. Seeds produced by plants (PRO) were weighted and water consumed (WC) was measured during the whole biological cycle. Integral WUE was evaluated considering the ratio between yield of seed and water consumed (PRO/WC), also the ratio between total biomass and water consumed (TB/WC). Instant WUE was measured as the ratio between photosynthesis and stomatal conductance (A/g_s). Results were subjected to analysis of variance (ANOVA) in a completely randomized design with eight replications and means separation tests of Tukey ($p < 0.05$) were performed, using SAS System for Windows 9.1. statistical software.

2.3. Housekeeping control genes evaluation

To identify the best reference housekeeping gene, expression profiles were generated by semiquantitative RT-PCR for actin, β-tubulin, cyclophilin, the elongation factor 1-β (fe1β), and ribosomals 26S and 28S genes. Primers pairs for each gene were designed using the Primer 3 Plus software based on their sequences housed in GenBank [GenBank accessions: KF033476.1, KF569615.1, X74403.1, KF033738.1, KF033619.1 and L36638.1]. Total RNA was extracted (Logemann et al., 1987) from leaf tissue of eight plants of both cultivars in the four water regimens in the vegetative and reproductive stages. Maturity stage was not considered, by the reduction in the physiological activity due to the aging of plants. RNA integrity was analyzed by electrophoresis, using 1.5% agarose denaturing gels with 12.3 M folmaldehyde and 10 X MOPS (Sambrook et al., 1989). Gels were visualized with ethidium bromide (0.5 μg mL^{-1}, EtBr) and UV light in a Chemidoc™ (BIO-RAD) photodocumenter. Concentration and purity of total RNA were determined by measuring the absorption of UV light using a Nanodrop 800™ (Thermo Fisher Scientific). Single strand DNA (ssDNA) was synthesized by reverse transcription with the enzyme SuperScript™ II (Invitrogen) from eight RNA's extracted of each treatment. Housekeeping genes were amplified by RT-PCR in a total volume of 20 μL containing 12 μL sterile water, 1 mM dNTP's, 1 X PCR buffer, 2 mM $MgCl_2$, 1 mM corresponding primers, 1 U taq polymerase and 300 ng of ssDNA. Reaction conditions were: 1 cycle at 95 °C for 5 min, 30 cycles at 95 °C for 1 min, corresponding melting temperature of each primer pair for 2 min, 72 °C for 2 min and a final cycle at 72 °C for 2 min. Products were analyzed by horizontal electrophoresis in 1.5% agarose with 1 X TBE buffer (1 mM pH 8 EDTA, 40 mM boric acid, 40 mM Tris), results were released and documented as described above. The densitometry analyses of images were performed using the TotalLab Quant TL120 1D v2009 software. Finally, variation among expression levels in quantitative terms was analyzed and compared.

2.4. Identification of water-use efficiency-related genes

The Pinto Saltillo cultivar was selected as study model. The instant and integral WUE increased under limited water and optimal conditions respectively, therefore based on the results of these evaluations, two contrasting water regimens were identified, one limiting (20%) and another optimal (60%) (Medrano et al., 2007). Ten plants were kept under these regimens until vegetative stage, considered as the most suitable to identify the genes of