Article

Water stress tolerance in beans cv. Pinto Saltillo modified with the vacuolar pyrophosphatase-1 gene of *Arabidopsis thaliana*

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Abstract

The *Arabidopsis Vacuolar* Pyrophosphatase-1 (avp1) gene, which encodes the enzyme H+ pyrophosphatase, improves drought and salinity tolerance and increases biomass and yield when overexpressed in transgenic plants. The purpose of this study was to analyze the phenological, physiological and agronomic characteristics of beans cv. Pinto Saltillo (PS) transformed with the 35Sprom:avp1 gene under conditions of extreme water stress. The experimental design consisted of a random distribution of 10 homozygous plants for each of the 9 PS-avp1 lines, as well as unmodified control plants. Irrigation was suspended in the phenological stage of 50% flowering and water stress started from 100% to 9% volumetric water content (CVA), over 10 days. The results showed that the drought intensity index (IIS) was 0.78. Even though the stomatal conductance and perspiration showed a rate of decrease similar to the non-transformed plants, the photosynthetic rate of all PS-avp1 lines was higher than the control plants under conditions of water stress. The increase in total biomass in some PS-avp1 lines did not correlate with a higher yield. Seven of the nine lines evaluated showed a higher yield (35%-96%) compared to non-transformed PS plants under stress conditions, indicating greater efficiency in the translocation of photoassimilates to the target organs (seeds).

Keywords: drought tolerance, partition components, performance.

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Introduction

Beans (*Phaseolus vulgaris* L.) is an important basic crop which is consumed by approximately 400 million people in the tropics (CIAT, 2017). The origin and diversity of *P. vulgaris* before domestication, goes back to two main regions: the Mesoamerican and the Andean, where beans acquired several agronomic characteristics (Gepts, 1998). The races in the Mesoamerican group (Mesoamericana and Durango) show genetic sequences associated with water stress tolerance (Teran and Singh, 2002). There are two different types of water stress, intermittent and terminal.

Intermittent stress is caused by sporadic rainfall patterns that result in drought intervals that can occur at any time during the development process (Schneider *et al*., 1997). In contrast, terminal drought takes place when the plant goes through water stress during the reproductive stages and is the most decisive and decisive in terms of production and yield. The efficiency of some improvement methods for water stress tolerance, such as molecular marker assisted selection, has had limited results.

This has been due to genotypes with low level of polymorphism, presence of gene-marker recombination, variable resolution of QTL mapping and occurrence of genotype-environment interactions (Villordo-Pineda *et al*., 2015). All these are important factors required for the identification of genetic variability in the population, in order to identify the best genetic markers. The evidence indicates that the use of recombinant DNA has an opportunity to improve crops such as beans (Espinosa *et al*., 2013), allowing plants to tolerate water stress.

The recombinant avp1 gene of *Arabidopsis thaliana*, associated with water stress tolerance, codes for the H+-pyrophosphatase type I polypeptide of about 80 KDa (Maeshima and Yoshida, 1989; Sarafian *et al*., 1992). Overexpression of the avp1 gene has resulted in tolerance to water and saline stress in *A. thaliana* (Gaxiola *et al*., 2001), tomato (Park *et al*., 2005), lucerne (Bao *et al*., 2008), corn (Li *et al*., 2008), cotton (Pasapula *et al*., 2011), peanuts (Qin *et al*., 2013), lettuce and rice all of them with a similar phenotype such as higher shoot and root biomass, and higher yield.

The objective of this study was to evaluate the agronomic, physiological and phenological characteristics of bean cv. Pinto Saltillo lines transformed with the 35Sprom:avp1 gene under severe conditions of terminal drought in greenhouse conditions.

Materials and methods

The 35Sprom-avp1 expression cassette was previously designed by Gaxiola *et al*. (2001). The plasmid was introduced into *Agrobacterium tumefaciens* strain GV2260 via thermal shock (Höfgen and Willmitzer, 1988) and subsequently used to transform beans cv. Pinto Saltillo (Sánchez-Valdéz *et al*., 2004).

Bean transformation. Superficial sterilization, seed germination, hypocotyl dissection and environmental components were described by Espinosa-Huerta *et al*. (2013). The liquid induction medium (MI) consisted of B5 medium (Gamborg *et al*., 1968) [supplemented with myo-inositol

(100 mg L^{-1}), pyridoxine (1 mg L^{-1}), thiamine (10 mg L^{-1}), sucrose (2%), benzylaminopurine-HC1 (10 mg L^{-1}) (Sigma-Aldrich), at pH 5.8], was added to the culture of *A. tumefaciens*, strain GV2260 (OD600= 0.8) in a proportion 5:1 (v/v).

The hypocotyls were incubated for 10 min in this solution with gentle circular movements. The explants were grown in solid co-culture medium, which consisted of MI added with 200 mM acetociringone (3', 5' -Dimethoxy-4'-hydroxyacetophenone) (Sigma-Aldrich) and Agar (6 mg L^{-1}) (type A, for plant cell culture) (Sigma-Aldrich) and were incubated for 5 d at 25 °C, 16 h light (45-70 mmol m^{-2} s^{-1}) and 8 h dark.

After co-culture, the hypocotyls were transferred to Agrobacterium elimination medium which consisted of solid MI added with 300 mg L^{-1} of timentin (GlaxoSmithKline®) and remained in this medium for 5 d under the same culture conditions. Subsequently, the explants were transferred to selection medium with the same components of the elimination medium, added with 50 mg L^{-1} kanamycin (Sigma-Aldrich).

The differentiated buds that remained green in the selection medium were separated from the original explant (hypocotyl) and grown individually in Magenta® boxes containing elongation and rooting medium (MI without growth regulators), added with 50 mg L^{-1} of kanamycin and 300 L^{-1} mg of timentin to promote seedling growth and root formation. The culture conditions were identical to the previous stages.

In vitro regenerated seedlings were transferred to pots with Sunshine® Universal Mix substrate (Sun Gro Horticulture Canada Ltd.) and acclimatized to *in vivo* conditions. The plants grew in a greenhouse at 25-28 °C and a light intensity of 170 to 285 mmol m^{-2} s^{-1} until seed production.

Vegetal material

Nine homozygous lines bean cv. Pinto Saltillo (PS) generation T4 modified with a copy of the 35Sprom-avp1 expression cassette according to the segregation analysis (L1, L5, L7, L9, L10, L11, L12, L13 and L14) (PS-avp1) as well as the unmodified comparators of the same genetic background PS, were evaluated in bioassays of water stress in greenhouse conditions.

Molecular characterization of PS-avp1 bean lines

The DNA of each line was isolated according to the protocol of Murray and Thompson (1980) using 100-200 mg of foliar tissue per sample in order to identify via PCR the main elements of the inserted construction and confirm its state of genetically transformed plant.

Endpoint PCR amplification reactions consisted of DNA samples (50 ng), primers (0.2 mM), dNTP (0.25 mM), Taq polymerase (1U), magnesium chloride (2 mM) and Taq buffer solution (1X) (10 mM Tris-HCl, 50 mM KCl, 1.5 mM MgCl2, pH 8.3). The avp1 gene sequence primers consisted of sense avp1, 5'-GGC TCT GTT GAG GGA TTC AG-3' and avp1 antisense, 5'-GCA ATG ACA GCT GGG TTT CTT-3', amplification conditions were 10 min at 95 °C for one cycle, followed by 30 cycles of denaturation at 95 °C for 1 min, alignment at 55 °C for 30 s and extension at 72 °C for 1 min, plus a final extension at 72 °C for 10 min (Pasapula *et al*., 2011). The primers used did not amplify any endogenous unprocessed bean fragment.

Quantification of gene expression

The leaf tissue was collected from plants in 50% flowering stage, and for the total RNA extraction the commercial agent Trizol® (Reagent, Carisbad, CA, USA) was used, following the manufacturer's instructions. Total RNA was quantified by spectrophotometry (Nanodrop). The quantitative PCR expression analysis was performed with the ABI PRISM 7000 SDS real-time thermal cycler (Applied Biosystems). The oligonucleotides and probe were designed using Primer Express software version 2.0 (Applied Biosystems) and based on the sequence of the avp1 gene (NCBI, NM_001084073).

These consisted of avp1 5'-AGT GCA CTT AAG ATG GTT GAA G-3', AGP GCA 3'- ATG AGT CCA GGG ATG GTG TTG - 5, and AAC TGC CTG CGA ACT T probe labeled with fluorochrome FAM™. The endogenous control was the 18S ribosomal gene (4319413E, Applied Biosystems) labeled with the VIC™ fluorochrome. The cDNA synthesis was developed by reverse transcription (RT-PCR) using One-Step RT-PCR Master Mix of Applied Biosystems (Cat. No. 4309169). Each 25 µL reaction consisted of 12.5 µL of TaqMan® 2x Universal PCR Master Mix No AmpErase® UNG, 0.625 µL of 40X MultiScribe and mixture of RNAse inhibitor, 1.25 µL of the avp1 gene probe, 1.25 µL of the gene probe ribosomal 18S and 25 ng of total RNA.

The amplification conditions consisted of 30 min at 48 °C (reverse transcription), 10 min at 95 °C (denaturation) and 45 cycles (amplification): 95 °C for 15s and 60 °C for 1 min. The amplification results were analyzed using the ΔCt comparative method, which normalizes the avp1 readings with the 18S endogenous control readings such that the differences between expression levels will be solely due to overexpression of the gene in the tissue and not to variations in experimental RNA (ΔCt= Ct avp1-Ct 18S). Subsequently, each ΔCt of the replicas was compared with the highest value or calibrator [ΔΔCt = ΔCt (sample) - ΔCt (calibrator)]. Finally, the avp1 gene vs. relative expression was generated by squared value of the sample minus the calibrator (2-ΔΔCt) (Livak and Schmittgen, 2001).

Water stress treatment

Ten seeds of each PS-avp1 line and 20 seeds of unmodified PS beans were germinated in 2.5 L pots with 30% Sunshine® substrate and 70% vermiculite. The substrate was maintained at field capacity or 100% volumetric water content (CVA). The seedlings were fertilized twice during the growth process (flowering and seed filling initiation) with 3 g of urea. Water stress treatment began in the phenological state of 50% flowering, from 100% up to 9% CVA (10 days).

From 9% of CVA the plants were treated with daily recovery irrigation until the pod filling was completed. PS bean not modified under irrigation conditions or under water stress was used as a control. The experiment was established in the Bajío Experimental Field of the National Institute of Forestry, Agricultural and Livestock Research (INIFAP) during the spring of 2014, under confined conditions. The environmental conditions in the greenhouse consisted of a minimum temperature of 18 °C during the night and a maximum of 35 °C during the day. The natural luminosity occurred; through, glass roof and relative humidity was 70%.

Experimental design and statistical analysis

The experimental design of the phenological, physiological and agronomic evaluation was complete blocks, each PS-avp1 line and the PS control represented a block. The experimental unit consisted of a pot, which contained a plant and ten replicates were used per line or control material. The data collected were subject to an analysis of variance and the differences between means were compared using the Tukey test ($p \leq 0.05$). The correlation coefficients (r) between relevant variables were also calculated.

Physiological variables

The evaluation of the physiological variables was carried out using the portable system of photosynthesis CID-340 (CI-340 Handheld Photosynthesis System. Bio-Science), which was used to record the photosynthetic rate, stomatic conductance and perspiration rate. The readings of these variables were taken in young and succulent leaves of the 2nd layer of the plant, both in populations under stress and in irrigation. The readings were taken daily for 10 at the same time of day, in order to reduce errors derived from variations in solar incidence.

Phenological components

The variable days to flowering (DF) was recorded when 50% of the plants in the population exhibited at least one open flower, while the days at physiological maturity (DMF) were recorded when 75% to 90% of the pods lost their green pigmentation. The variables were recorded daily until the change in the phenological stage was identified. The variable days to seed filling (DLS) was calculated using the expression DLS= DMF-DF.

Yield

The crop yield (g) was represented as the weight of all the seeds harvested from the ten plants evaluated per line and by the PS control population.

Biomass components

When the plants of each PS-avp1 line and PS bean population reached physiological maturity they were sectioned into leaves, stems, roots, pods and seeds of all plants. The tissues, except the seeds, were completely dehydrated at 50 °C for 72 hours and both the fresh weight and the dry weight of each tissue were recorded. The biomass data by tissue (g) and total biomass (g) were collected by treatment (stress and non-stress) based on the blocks (10 plants).

Partition components

The partition variables refer to the selection indices that involve yield, biomass, days at physiological maturity and days to seed filling and determine the efficiency of the plants in the translocation of photosynthes to the target organs. These indices were calculated using the following expressions.

Biomass growth rate (TCB) refers to the average time of total biomass increase in relation to the days when the plant reached its physiological maturity.

$$TCB = \frac{\text{Total biomass}}{DMF}$$

The economic growth rate (TCE) is defined as the efficiency of crop yield in a given time.

$$TCE = \frac{\text{Yield}}{DMF}$$

The Seed Growth Rate (TCS) indicates the effect of the conditions between the state of flowering and the beginning of seed filling.

$$TCS = \frac{\text{Yield}}{DLS}$$

The harvest index (IC) refers to the efficiency of the metabolic energy used by the crop to synthesize the organic products necessary for its development.

$$IC = \frac{\text{Yield}}{\text{Total biomass}}$$

The relative demand force (FRD) is related to CO_2 emissions and indicates the amount of energy needed for the development of organs in the plant. Lines with a high FRD rate in any given light environment would be able to have a better response to CO_2 emissions.

$$FRD = \frac{TCS}{TCB}$$

The geometric mean performance (G) uses the performance of each PS-avp1 line under conditions of water stress and irrigation to calculate the effect and intensity of water stress on performance (Samper and Adams, 1985).

$$G = \left(Y_d \times Y_p\right)^{1/2}$$

Where: Y_d and Y_p refer to the performance or performance of each line under stress and irrigation, respectively.

The drought intensity index (IIS) is used to evaluate the performance of all lines with respect to both humidity conditions and determine the drought intensity value in the evaluated experiment (Fischer and Maurer, 1978).

$$IIS = \frac{1-X_d}{X_p}$$

Where: X_d and X_p are the average performance of all lines and controls under conditions of water stress and non-stress, respectively.

Results

Molecular characterization

Molecular characterization of PS-avp1 bean lines confirmed the presence of the avp1 gene according to the expected size of a gene fragment (630 bp) (Figure 1A). These lines were analyzed for their relative transcriptional expression levels with variable absolute values for each of the lines evaluated (Figure 1B). No quantitative expression of an endogenous gene homologous to the avp1 sequence was observed in unmodified plants.

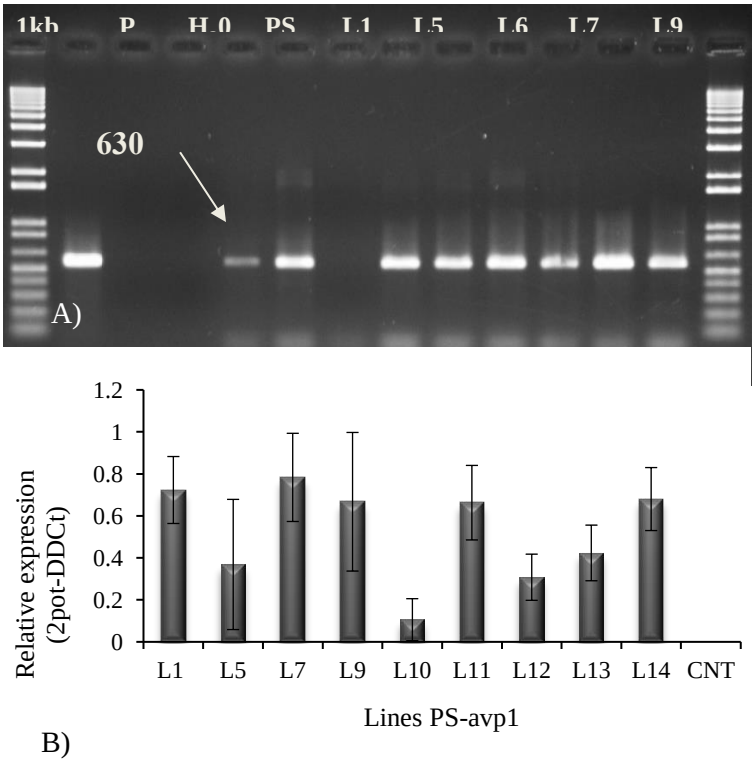

Figure 1. A) Avp1 gene amplification by PCR endpoint of homozygous bean cv. Pinto Saltillo lines. Lines PS-avp1= L1 to L14; P= plasmid with the PS-avp1 gene; H_2O, water without DNA; PS= bean cv. Pinto Saltillo not transformed; M= 1Kb Plus DNA molecular weight marker; and B). Relative transcriptional expression of avp1 in PS-avp1 lines and PS plants. Each value corresponds to the average of ten plants and three reaction replicates by quantitative PCR.

Drought intensity index

The drought intensity index (IIS) obtained under greenhouse conditions was 0.78, one of the most severe drought intensities reported up to now.

Physiological variables

The photosynthetic rate in most PS-avp1 lines was 1.4 to 3.1 times higher than PS plants, under water stress (Figure 2A). The increase in the photosynthetic rate correlates with the performance in most PS-avp1 lines, except for L5 which exhibited the photosynthetic rate lower than L12; however, both had similar performance under stress (13 g) (Table 1).

Table 1. Relationship of yield, biomass, phenological and partition components of bean cv. Pinto Saltillo plants modified with the avp1 gene under conditions of water stress.

Characteristic	L1	L5	L7	L9	L10	L11	L12	L13	L14	PS stress	PS irrigation
Yield (g)	10.1 ±0.4b*	13.4 ±0.2d	12.1 ±0.4c	14.4 ±0.3d	11.4 ±0.5c	16.5 ±0.4e	13.7 ±0.4d	8.4 ±0.2a	15.1 ±0.3d	8.4 ±0.2a	10.4 ±0.2b
Leaf	5.1 ±0.18b	8.4 ±0.18d	9 ±0.26e	3.9 ±0.24a	4.3 ±0.22a	7.9 ±0.18d	10.4 ±0.23e	12.7 ±0.16f	7.3 ±0.14d	10.3 ±0.4e	6.7 ±0.15c
Stem	9.7 ±0.19b	12.6 ±0.2c	19 ±0.23e	8.4 ±0.28a	8.2 ±0.21a	14.4 ±0.17d	17.1 ±0.21e	20.5 ±0.18e	14.6 ±0.14d	14.9 ±0.23d	8.9 ±0.19a
Pod	2.1 ±0.1b	2.7 ±0.06c	2.7 ±0.08c	3.4 ±0.13d	1.7 ±0.12a	3.4 ±0.10d	4.5 ±0.16e	0.8 ±0.07a	3.4 ±0.12d	3.8 ±0.2d	2.4 ±0.07b
Root	16.8 ±1.19c	12.7 ±0.71b	22.7 ±1.18d	9.82 ±1.32a	26.9 ±1.5e	15 ±0.68c	13.2 ±0.50b	34.8 ±0.97f	28.8 ±0.75e	20.2 ±0.8d	12.4 ±0.1b
Total	33.89	36.53	53.64	25.67	41.31	40.82	45.44	68.89	54.22	49.2	30.4
TCB	0.43 ±0.1a	0.46 ±0.1a	0.68 ±0.1b	0.33 ±0.07a	0.53 ±0.1b	0.52 ±0.1b	0.58 ±0.1b	0.88 ±0.1c	0.69 ±0.1b	0.63 ±0.1b	0.35 ±0.1a
TCE	0.13 ±0.09a	0.17 ±0.05a	0.16 ±0.02a	0.18 ±0.01b	0.14 ±0.09a	0.21 ±0.05b	0.17 ±0.08a	0.11 ±0.05a	0.1 ±0.03a	0.13 ±0.1a	0.09 ±0.01a
TCS	0.26 ±0.06a	0.34 ±0.1a	0.31 ±0.09a	0.38 ±0.1a	0.3 ±.08a	0.42 ±0.1b	0.34 ±0.1a	0.21 ±0.09	0.39 ±0.1a	0.27 ±0.09a	0.19 ±0.02a
IC	0.29 ±0.09c	0.37 ±0.1c	0.23 ±0.03c	0.56 ±0.09	0.27 ±0.06	0.4 ±0.09d	0.29 ±0.03c	0.12 ±0.04a	0.27 ±0.03c	0.21 ±0.09b	0.28 ±0.2c
FRD	0.6 ±0.2b	0.74 ±0.2b	0.45 ±0.2b	1.13 ±0.2c	0.55 ±0.2b	0.81 ±0.2b	0.58 ±0.2b	0.24 ±0.2a	0.56 ±0.2b	0.34 ±0.07a	0.64 ±0.08b
G	10.24 ±1.4ab	11.82 ±1.8b	11.22 ±2.3ab	12.27 ±2.7ab	10.86 ±1.7b	13.09 ±2.4b	11.7 ±1.5b	9.32 ±0.9a	12.53 ±2.5b	9.35 ±0.25a	10.39 ±0.1b

Yield (g 10 plants^{-1}); TCB= biomass growth rate (g m^{-2} d^{-1}); TCE= economic growth rate (g m^{-2} d^{-1}); TCS= seed growth rate (g m^{-2} d^{-1}); IC= harvest index; FRD= relative demand force; G= average geometric performance; *= means within the same row followed by equal letters, are not significantly different according to the means test of Tukey α= 0.05.

The perspiration rate and stomatal conductance of the PS-avp1 lines suffered similar reductions than the PS plants under stress conditions (Figure 2B and 2C). In the three physiological variables, the plants in irrigation showed higher values than all the plants under stress.

Phenological components

The values of the DF variables were similar in all PS-avp1 lines and unmodified PS plants (39 d), so no differences were found by transgenesis between the populations evaluated. However, the days required to achieve physiological maturity (DMF) were less in the PS-avp1 lines and PS plants

(78 days) under conditions of water stress compared to PS plants at risk (85 d). The effect of water stress on the number of days required for seed filling (DLS) indicates that PS-avp1 lines exhibited similar values (38 d) compared to PS under stress (39 d), but less than the days required under irrigation conditions (45 d) (Table 1).

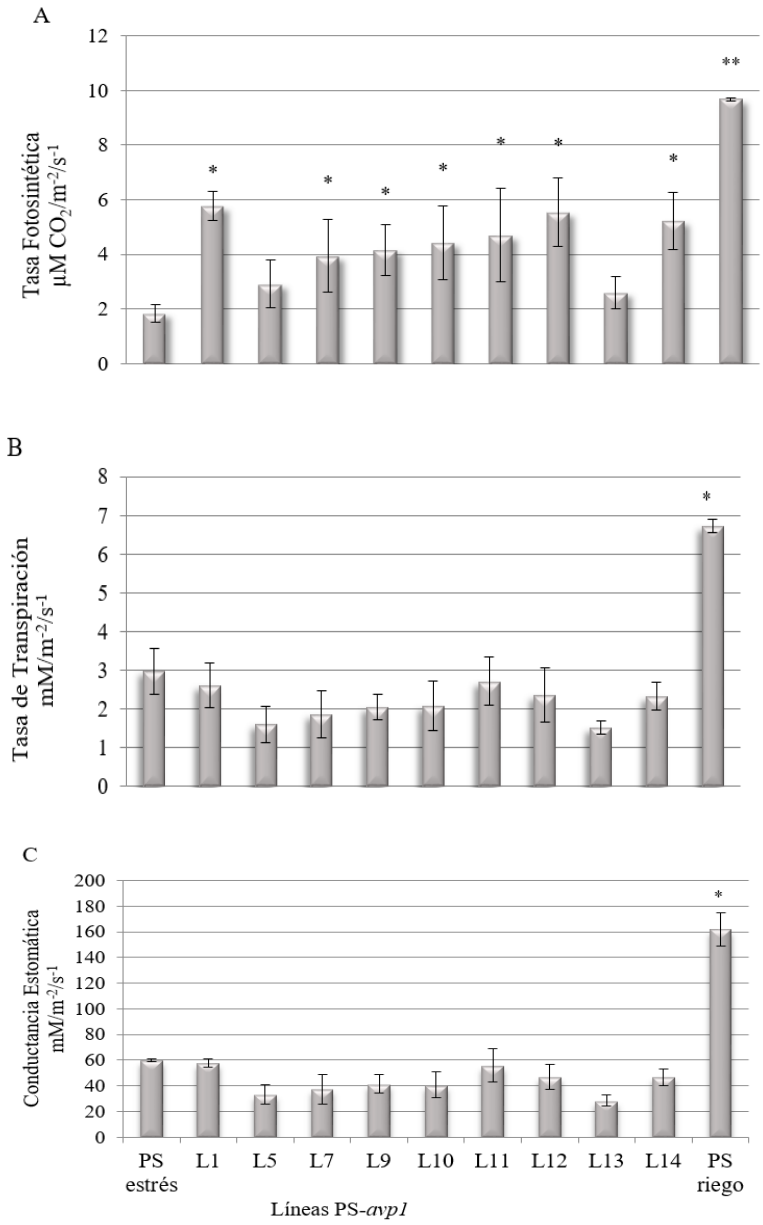

Figure 2. Performance of the physiological characteristics of PS-avp1 bean lines. A= photosynthetic rate; B= rate of perspiration; and C= stomatic conductivity of transformed bean lines with the avp1 gene (L1 to L14); PS stress= Pinto Saltillo bean not modified under water stress, PS under irrigation= Pinto Saltillo bean not modified under irrigation conditions. The values were taken at 20% of the volumetric water content in 50% flowering plants; **= statistically significant at 1%; *statistically significant at 5%.

In this experiment it was observed that even when the phenological variables in the PS-avp1 lines under water stress (L5, L7, L9, L10, L11, L12 and L14) had lower values compared to the PS plants in irrigation, these differences did not affect yield efficiency (Table 1).

Yield

Agronomically speaking, tolerance to water stress is perceived as the ability to generate yield, not just to maintain the vegetative tissue in the plant. Under this premise, the yield values of PS plants under irrigation (10.4 g 10 plants^{-1}) and water stress (8.4 g 10 plants^{-1}) were used to establish the comparison range for the performance of the PS-avp1 lines under stress. The yields of L1 (10.1 g 10 plants^{-1}) and L13 (8.4 g 10 plants^{-1}) were the smallest of the population of lines and were not statistically different from the unmodified PS plants (Table 1).

It should be noted that seven of the nine PS-avp1 lines exhibited not only better tolerance to water stress than control PS plants, but their yields under stress significantly exceeded PS yields under irrigation conditions. The yield values achieved by most PS-avp1 lines showed increases between 35% and 96% above the control plants under stress (Figure 3).

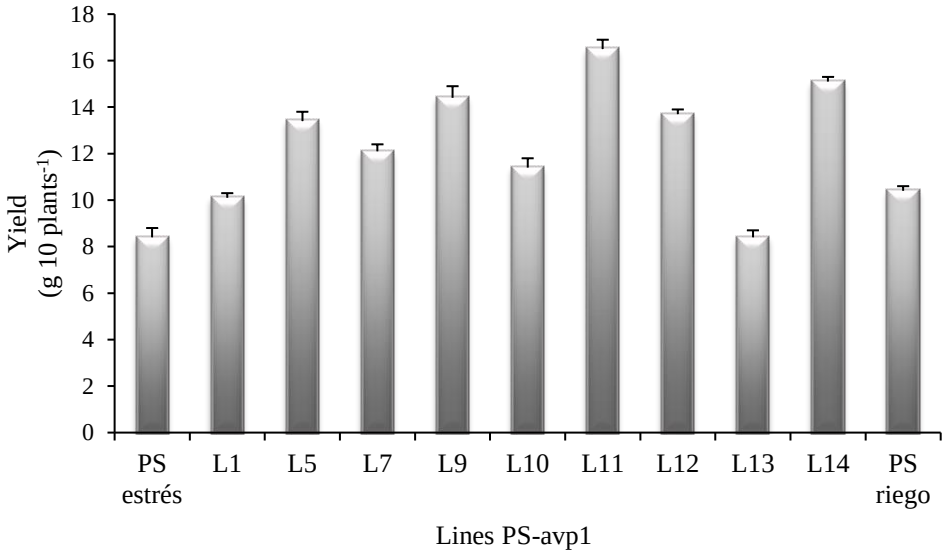

Figure 3. Yield values of PS-avp1 plants. PS stress: bean cv. Pinto Saltillo plants unmodified under water stress, irrigation PS= bean cv. Pinto Saltillo plants unmodified under irrigation, L1 to L14= PS-avp1 lines. Each column represents the yield of a total of 10 plants.

Biomass components

The biomass components showed variable values among the populations of PS-avp1 lines. Lines with higher yield showed no greater biomass for any of its components; for example, L5, L9, and L11 had a biomass within the range even below the control PS plants (Table 1).

On the other hand, plants of the L13 line, had a yield similar to PS under stress, and showed significant increases in leaf, stem and root biomass. Lines L7, L10, L13 and L14 had higher root biomass 21, 55, 118 and 70%, respectively (normalized with the biomass weight values of PS under water stress); however, this was not necessarily reflected in higher performance ($r= -0.29$; $\alpha\geq 0.05$). A negative correlation was observed between the total biomass and the harvest index (IC) (-0.783; $\alpha\leq 0.05$).

Similar results were obtained when total biomass and relative demand force (FRD) were compared (-0.81; $\alpha\leq 0.05$). Lines L1 (0.43), L5 (0.46) and L9 (0.33) showed a TCB statistically similar to PS plants at risk (0.35), while L7, L10, L11, L12 and L14 had values comparable to low PS plants water stress (0.63) ($\alpha\leq 0.05$). L13 (0.88) showed a higher value of TCB than the rest of the PS-avp1 lines and the PS population of control plants (Table 1).

Partition rates

The TCB showed a significant correlation with leaf biomass (r 0.76; $\alpha\leq 0.05$), stem biomass ($r= 0.85$; $\alpha\leq 0.05$), and root biomass ($r= 0.85$; $\alpha\leq 0.05$); however, it showed no correlation with any other partition indexes, such as pod biomass ($r= -0.25$; $\alpha\leq 0.05$) and geometric mean yield (G) ($r= -0.307$; $\alpha\leq 0.05$).

The TCE values for lines L9 and L11 were statistically different from the control plants. The rest of the PS-avp1 lines did not show significant statistical differences ($\alpha\leq 0.05$) (Table 1). In general, the absolute values of the PS-avp1 TCS index also had higher values for most of the lines except L1 and L13; however, no statistical differences were observed ($\alpha\leq 0.05$) (Table 1).

The harvest index (IC) indicated that line L13 showed lower values (0.12), still below the population of PS plants under stress (0.21 and 0.28 respectively) (Table 1). Lines L9 and L11 showed the highest values (0.56 and 0.4, respectively) which coincide with the performance values (Table 1). The rest of the populations of PS-avp1 lines (L1, L5, L7, L10, L12, and L14), had similar IC values to the PS plants in irrigation (0.28) (Table 1).

The FRD data indicated that line L13 (0.24) had values close to the values of PS plants under stress (0.34), on the other hand, plants of line L9 (1.13) showed statistical differences ($\alpha\leq 0.05$), not only with respect to the rest of the PS-avp1 lines but also with respect to the PS control plants under irrigation conditions and water stress.

Lines PS-avp1 L1, L5, L7, L10, L11, L12 and L14 had similar FRD values ($\alpha\leq 0.05$) to PS plants under irrigation (0.64) (Table 1). Finally, the genotypic yield (G) of PS-avp1 lines L5 (11.8), L10 (10.8), L11 (13), L12 (11.7) and L14 (12.5) was superior to the rest of the PS-avp1 lines and plants PS in irrigation (10.39). Line L13 (9.32), showed a low yield (G), similar to PS plants under stress (9.35); however, L1 (10.24), L7 (11.22) and L9 (12.27) had a high variation of G within populations which do not make them different ($\alpha\leq 0.05$) to PS plants (Table 1).

The genotypic yield (G) of the lines showed a high correlation with performance ($r= 0.99$; $\alpha\leq 0.05$), establishing a direct effect of water stress on productivity; however, no correlation was observed between G and total biomass. In contrast, the correlation coefficients of the TCE, TCS, IC and FRD partition rates with G were high to significantly high ($r> 0.6$; $\alpha\leq 0.05$).

Discussion

The level of water stress is the basis for explaining the phenotypic performance of the PS-avp1 lines. In this study, the drought intensity index (IIS) was similar to that reported by Ramirez-Vallejo and Kelly (1998), (IIS= 0.63 and 0.78), which defines an extremely high stress level to analyze agronomic characteristics of tolerance to this phenomenon.

Physiological characteristics

The physiological characteristics analyzed in this study showed an increase in the photosynthetic rate in plants transformed with the avp1 gene compared to the control PS plants under stress. This effect was previously reported by Khadilkar *et al*. (2016) who indicated an increase in the values of the photosynthetic rate of approximately 1.2 times higher in *Arabidopsis thaliana* avp1-1 plants.

This was explained as an improvement in the process of photosynthesis, consistent with the expectations of an increase in phloem transport due to the increase in sucrose and proton pump; through, of sympathizers inside the plasma membrane of the phloem companion cells to create a proton motive force.

On the other hand, the reduction in the values of perspiration and stomatal conductance in populations of PS-avp1 lines contrasts with that found by Qin *et al*. (2013), who stressed that the values of the three physiological characteristics showed an increase in modified plants peanuts of 35Spro:avp1 and subject to water stress (31 d) and saline (60 d). Similar increases in photosynthesis, perspiration and stomatal conductance were observed in 35Spro:avp1 cotton plants subject to saline stress (Pasapula *et al*., 2011).

Despite these differences, the values of the physiological variables of the PS-avp1 bean lines under water stress were within the range reported for Pinto Saltillo, under irrigation conditions (Chávez-Simental and Alvarez-Reyna, 2012), which indicates the efficiency and plasticity of the PS-avp1 lines for adaptation to limited water conditions.

Ramírez-Vallejo and Kelly (1998) reported differences in physiological variables at high IIS values (0.78), similar to the information reported here. Bean cv. Cacahuate 72 plants exposed to stress and non-stress treatments showed a reduction in their stomatal conductance. However, the plants showed no significant differences concerning water relations when compared to the plants under irrigation.

In this study the performance of the photosynthetic rate is independent of the stomatic conductance and the perspiration rate, this could probably be attributed to the osmotic adjustment achieved by the increase in the expression of H+ pyrophosphatase in the PS-avp1 lines along with an efficient translocation of photosynthes to seeds.

Phenological characteristics

The values of the phenological components of the PS-avp1 lines under conditions of water stress were lower than those reported by Acosta-Díaz *et al*. (2011), that is, the PS-avp1 lines were more efficient in conditions of water limitation. This acceleration to maturity is generally observed when there is a prolonged period of water stress during the reproductive stage and there are no favorable conditions for its recovery (Samper and Adams, 1985; Schneider *et al*., 1997; Ramírez-Vallejo and Kelly, 1998; Rosales-Serna *et al*., 2000).

Conclusions

Expression of recombinant H+ pyrophosphatase type I from *Arabidopsis thaliana* (avp1) provided protection from extreme water stress (ISS= 0.78) in PS-avp1 bean lines. The reduction in the rate of migration of CO_2 and loss of water vapor to the PS-avp1 lines during water stress was similar to those of the control plants under water stress; however, the PS-avp1 lines showed an increase in the growth rate during water stress by establishing a highly efficient translocation of photoassimilates to the seeds, suggesting that these seeds are the preferred target organ.

On the other hand, even though some lines showed an increase in root biomass, this characteristic did not provide an advantage in terms of water absorption and higher yields. All PS-avp1 lines with higher yield values than the PS plants in irrigation, maintained high values of partition indices associated with the accumulation of dry matter, particularly IC and FRD. Finally, these results show the initial evidence of a promising alternative for bean production in agro-ecological regions with severe water stress; to achieve this, it is maintained perspective environmental release of the most promising lines of PS-avp1 to analyze their performance under agronomic conditions.

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