INTERNATIONAL JOURNAL OF PLANT, ANIMAL AND ENVIRONMENTAL SCIENCES

IJPAES

Volume-4, Issue-1, Jan-Mar-2014

ISSN 2231-4490

Coden : IJPAES www.ijpaes.com

Received: 15th July-2013 *Revised: 29th July-2013* *Accepted: 01st August-2013*

Research article

TEPARY BEAN GERMPLASM, A RESOURCE FOR DROUGHT TOLERANCE

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ABSTRACT: Tepary bean germplasm was studied for drought stress tolerance based on quantum photosynthetic yield in the leaf duringearly vegetative growth stage in greenhouse and high tunnel. The purpose of the study is to evaluate the cultivar's potential to withstand water stress and to identify the potential cultivars for use in breeding tepary bean for drought tolerance. The experiment was conducted twice in a replicated block design imposing irrigated, control and water stress, treatments both in greenhouse potted plants and high tunnel. The entire germplasm was characterized into low, mediumand highly tolerant lines based on yield (Fv / Fm). Other traits like relative injury (RI %), relative water content (RWC), relative greenness, biomass and transpiration efficiency (TE) were also scored to view the cultivar diversity for tolerance to drought.The results obtained to select the cultivars as tolerant (4, 7, 8, 10,15,18, 29, 31),and susceptible (1,2,3,6,20, 23, 25,27,30)for drought stress tochoose for crossing program and to develop mapping population for genetic studies on drought tolerance in tepary bean were presented. The expected impact is the development of drought tolerant crop resources for arid agriculture in USA.

Key Words: *Phaseolus*, Florescence, food legume
Abbreviations: Fv=Variable florescence; Fm=maximum florescence

INTRODUCTION

Non-availability of water (drought stress)to the crop, during its early and active vegetative growth stages has negative impact on yield potential due to irregularities in osmotic potential [15, 16] and quantum yields [23]. Drought severely affected 24 - 41.07 % area during summer of 2012 in United States of America (USA) with severe impact on economic yields of traditional crops like corn and soybean (www.drought.gov). The extent of yield loss is dependent on timing, duration and intensity of water stress [1,4]. Food legumes are well known for environmental sustainability and drought tolerance in arid agriculture [28]. Tepary bean (*Phaseolusacutifolius* A Gray) is highly drought tolerantnative food legume under cultivation since 19th century [10]. It is self-pollinated crop [34] and a potential alternative for cultivation in Virginia with very short duration of 2.5-3 months [12] in summer after wheat crop. Chlorophyll fluorescence was efficiently used as a tool to determine the damage in photosystems under water stress in legumes [26] and other cereal crops [6]. The genotype-environment interactions were better assessed by measuring relative greenness of leaf [17], chlorophyll florescence (Maxwell and Johnson, 2000) under water stress which impact the crop's efficiency of photosynthesis [28], nitrogen [23] and water [31] use. The transpiration efficiency (TE), relative water content (RWC) of leaf [7] and electrolyte leakage were indicators ofthe damage to the cell membrane. Drought tolerant cultivars gave highest yield compared to drought sensitive cultivars under drought stress in chickpea [18], mungbean [24] and pigeonpea [15] due to high water use efficiency with stable quantum yield potential [13]. Research was in progress to identify drought tolerant tepary plant introductions (PI 200902, PI 312637, PI 440788, and PI 440789), develop breeding lines for drought and heat tolerance, seed size and growth habit in Puerto Rico [27] and successfully transfer tepary'sheat and drought tolerant genes to common bean respectively in New York [29] and California [33] through inter specific hybridization.

Teparybean yields were significantly higher in Virginia during late May (2239 kg/ha) compared to June (1899 kg/ha) and July (1310kg/ha) plantings (Bhardwaj et. al., 2002). There were 180 tepary bean selections maintained at Virginia State University (VSU) including germplasm collections from native seeds and United States Department of Agriculture (USDA) repository showing variability in seed color, shape and size. These collections were not evaluated to date for their potential for adaptability to water and heat stress tolerance in Virginia. Other new food legumes, chickpea, fababean, lupin, mungbean and pigeonpea, evaluated for their production feasibility since 1991 at VSU in Virginia and identified adaptable high yielding cultivars in these crops [5] which were also not studied for their drought tolerance. The purpose of the present investigation was to 1) characterize the Native American tepary bean germplasm based on variations in chlorophyll florescence under drought stress 2) Analyze other parameters such as relative water content, transpiration efficiency and relative injury for each cultivar during water stress period 3) compare the drought tolerance capabilities of tepary bean with other legumes to identify the highly drought tolerant cultivars for tepary bean breeding.

MATERIALS AND METHODS

Greenhouse plant material and growing conditions

The seed material used in greenhouse pot experiments for chlorophyll florescence evaluations werefrom 31 Native Americancollections grown in Randolph farm during 2010 and 2012 at VSU. The seed material was screened for purity based on uniform color and selected sixteen seeds for each cultivar and planted in pots filled with regular potting mix with all the nutrients required for normal plant growth under 10-12hr photoperiod, 60 - 85% relative humidity with 28^0C / 18^0C day and night temperatures on an average. The experiments were conducted twice in replicated block design with two sets of31 genotypes along with a control, drought tolerant soybean cultivar (S6-PI 471938,TC Carter) and other legumes crops (chickpea, mungbean, mothbean and pigeonpea). The experiments for TE and Biomass were conducted twice in replicated design seperately.

The moisture parameters like water deficit, volumetric water content were measured thoroughly to avoid maximum possible experimental errors during execution of the water stress treatment and fix the watering schedule as briefly described below.

Water deficit (D)

In millimeters was calculated for three replicated pots during early vegetative stageof tepary bean plants to decide the optimal conditions for water stress for the water stress treatment of the experiment. The calculations were done separately at constant atmospheric conditions of greenhouse at moisture availability ranges of 50, 75 and 100 percent.D = R * (FWC-VWC) where R is the rooting depth, FWC is the field water capacity and VWC is the volumetric water content. We observed plants reaching wilting two days early in 50% moisture availability compared to 75 and 100%.The volumetric soil moisture content in each pot and treatment was measured using soil moisture probe (Moisture meter HH_2, version 4, Delta T devices Ltd., Cambridge, UK). The available moisture content of each pot was measured as difference between the volumetric water content of saturated soil and moisture content at the wilting point (in case of drought stress) and at normal conditions (control) of crop growth. Further, we calculated the amount of water used by stressed plants compared to control by measuring the volumetric water content on every day for the first few weeks to know the daily uptake of water and weekly from each line from the beginning of the stress period till the end of the stress. The water use efficiency of water stressed ($0.01m^3/m^3$; 2.3%) plants was high compared to control ($0.068\ m^3/m^3$; 14%) plants in a 24 hour period on an average, was indicated by the mean values of volumetric water content of all the lines from both control and stress treatments during experimental period (data was not presented). The mean content of water present during active vegetative stage of tepary bean when suffering from water stress (2.77%) was less compared to control (4.52%).

Watering Schedule

The potted seeds in both the sets were watereddaily till one week to have uniform germination, followed by gap filling. Watering done at weekly intervals up to field capacity (0.450 - 0.50 m^3/m^3; 431-730mV) until fifth week with recommended fertilizer, insecticide and fungicide dose as necessary in treatment and control pots. Watering was completely stopped on the sixth week in one set (treatment) and the other set (control) was continued at weekly schedule.

Sample Collection

We selected the size (3 cm) of the leaf for water stress at USDA-ARS using the standardized light adapted test. We re-analyzedto locatethe selected leaf size (3cm) and identified third leaf from tip of the vine to use for characterization of the entire germplasm. The first fully expanded young leaf (third leaf) from the tip was selected as test sample for chlorophyll florescence as well as for other parameters tested. Leaf discs of 100mm size were collected twice in triplicates from control and treatments exactly 45 days after planting (DAP) for five days. Day one was treated as before water stress, day 2 and 3 were the drought treatments (47 and 48 day) and day 4 and 5 were to study the stress recovery in comparisons with irrigated control for the similar days. The discs were collected in 24 well plates (CELLTREAT scientific products, LLC, Shirley, MA01464) in triplicates in liquid water and transported to research lab at Agricultural Research Station (ARS) at VSU.

Fluorescence for drought tolerance

Chlorophyll pigments were excited (fluorescence) by a 660nm solid state light source with filters blocking radiation longer than 690nm and with an average intensity of modulated light adjustment from 0 to 1 μE. Saturation of the photosystem being measured is provided by a filtered 35 W halogen lamp (350□690nm) and was adjusted to default duration of 0.8 seconds. The variable fluorescence (Fv) is the difference between maximum (Fms) and steady state fluorescence (Fs). The ratio of variable fluorescence to the maximum fluorescence (Fv/Fms) under steady state conditions decides the quantum efficiency of photosystem II. This ratio indicates the efficiency of energy capture and electron transport from PSII which determines the carbon dioxide (CO_2) assimilation efficiency under drought stress situation. It is the best estimated method for efficiency of CO_2 assimilation due to its fluorescence measurements of chlorophyll in the outer leaf surface [6].

Measurement of chlorophyll fluorescence was performed using modulated chlorophyll fluorometer (Model: OS 1-FL, www.optisci.com) using dark as well as light adapted tests. The leaf discs were read using light adapted test (test type 2) for yield measurements of quantum efficiency under photosynthetic conditions immediately after sample collection and after exposing to heat by incubating the leaf discs at 42^0C in a hot air oven for 20 min. The incubated samples were allowed to cool for 20 min and were used for fluorescence measurements to know amount of energy lost due to heat. The variations in chlorophyll fluorescence readings obtained before and after incubations were used to estimate the water and heat stress tolerance due to non-photochemical quenching and impairment in the PS II efficiency in each individual cultivar to identify the potential cultivars with water stress tolerance. The dark adapted test (test type 1) measurements done after placing the discs inside dark cabinet for 30 min to understand the efficiency of energy capture and carbon assimilation.

Relative greenness of leaves

The Minolta SPAD-502 (Soil Plant Analysis Development) meter, a handheld light meter was used to measure relative greenness of leaves as a quick estimate of chlorophyll content in both water stressed and control plants of all the genotypes in replicates before and at the end of the stress period (Table 5) from the same leaf (third fully expanded leaf, 3 cm size) used for chlorophyll florescence analysis above.

Relative Water Content (RWC) of leaf discs

The relative water content of the leaves was measured by measuring fresh weight (FW), weight of fully turgid leaf disc (TW) and dry weight (DW) of leaf discs (Mohamed et. al., 2002) before and at the end of the stress period for cultivars with high, medium and less tolerance to drought. The leaf discs were floated in deionized water for 16 hours to determine their turgid weights. The discs were dried to constant weight at 80^0C for 48 h. The RWC was computed as percentage= 100* (FW-DW) / (TW-DW).

Thermo-stability of membrane

Ten leaf discs from selected drought responsive and control plants in triplicates were scored for relative injury (RI) percentage (Srinivasan et.al., 1996). The leaf discs were collected from the fully expanded third leaf from both water stress and control plants of the selected cultivars and were washed three times with distilled water.The clean leaf discs were incubated between 40-50^0C temperatures at an interval of two degree in pre-heated (2mL) water for 15 min and made up the volume to 10mL to incubate for 16 h at room temperature and read the conductance. The samples were then autoclaved at 120^0C for 15 min and read the conductance of the solution. The identified temperature for critical injury (46 ^{0}C) was used to characterize the germplasm for drought tolerance. The RI % was calculated using the formula,

RI (%) = (1-(1-(T1/T2)/1-(C1/C2)))*100.

Biomass and Transpiration efficiency (TE)

The plant biomass accumulated at the beginning and end of water stress were measured for both control and treatment plots and the percent change in biomass was calculated. The shoot and total transpiration efficiency was calculated using the difference in the saturation point to the volumetric water content at the end of sixth week to study the variation among the selected lines for TE [35]. We didn't use the plastic bags to cover the pots as there was no significance difference observed due to use of polybags in measuring TE. The dry mass of the shoot (above ground) and root was calculated after drying the samples to constant weight at 80^0C. The shoot and total TE was calculated by dividing the shoot and total (with root) dry mass with the amount of water used by the plant during the stress period. The average biomass and TE values for the selected cultivars from high, medium and low tolerance to drought stress were presented (Table 5).

Comparisons with other legumes

During two experimental periods, the selected high yielding cultivars in five legumes pigeonpea, chickpea, soybean, mothbean and mungbean were studied for chlorophyll florescence along with tepary bean 31 native lines in triplicates and were analyzed statistically for chlorophyll fluorescence values under water stress to compare with those of tepary bean (Table 3).

Statistical Analysis

The ANOVA procedure with PROC GLM was performed using SAS 9.3 softwarefor randomized block designto determine the statistical significance between and within treatments (drought X cultivar) using mean replicated data from all the cultivars for all the treatments. The missing sample data was calculated using SAS – LS means for LSD. PROC FORMAT for scoring/ranking the cultivars based on treatment means. Clustering was done using UPGMA-Pearson's correlation co-efficient analysis based on all the data for the traits studied for drought tolerance.During the experimental period, we experienced several constraints to maintain the stable conditions due to our limited greenhouse facilities and due to rainfall in field experiment which lead to few missing and uncorrelated values in certain cultivars, which were eliminated from analysis. To create a dendrogram (Fig. 1) from the set of these variables chosen for current diversity study, similarity coefficients were transformed into distances to make a cluster using the Un-Weighted Pair Group Method with Arithmetic mean (UPGMA) algorithm. The Pearson Coefficient (r) was chosen to calculate the distance values (d) using the formula d = (1-r)*100.

RESULTS AND DISCUSSION

Chlorophyll Fluorescence

Means of two experiments were compared for treatments and their interactions to identify cultivar performance for PSII efficiency of energy absorption and non-photochemical quenching (Table 2).Significant difference (0.001) between experiments was observed with average Fv/Fm values ranging from 0.68 to 0.77 in tepary bean. The photo-synthetically active radiation (PAR) was high in tolerant cultivars (0.6) compared to susceptible cultivars (0.15) to water stress. Non-significant difference between control and treatment plots for all the cultivars before drought stress and one day after resumption of watering. All the cultivars recovered within 24 hours after resuming watering and were with fluorescence value for Fv / Fm of 0.80 and are on par with the preliminary results observed using three cultivars at USDA-ARS, Lubbock in 2011(Unpublished).Non-significant differences ($0.97>p>0.10$%) were observed among 31 cultivars for water stress treatment and their interactions within 24 hours period of stress.

Significant differences for heat and drought stress tolerance were observed among the cultivars and between treatment and control on second day of stress based on which the genotypes were classified into three major categories (Table 3). The genotypes were grouped as highly tolerant to water stress if the Fv / Fm is more than or equal to 0.8 and moderately tolerant if Fv / Fm is more than or = 0.7 and less tolerant or susceptible to both drought and heat if Fv / Fm is less than 0.7). We used Fv / Fm reading of 0.80 to classify the cultivars as it isthe standard value in most crop plants underwater stress [11]. The results suggested that chlorophyll florescence measurements with high values of Fm indicate high amounts of chl a/b [19] and high amounts of nitrogen [14] while reduced Fv / Fm ratio was due to iron deficiency associated with low chlorophyll content [22] and water stress during vegetative growth stage [2]. Significant differences in fluorescence during first experiment indicated more than 50 % of the population with quantum yield above the mean value of 0.73 (water stress) and 0.6 (Heat). The control soybean produced 0.8 under heat and water stress conditions during all the experiments and treatments.

Table 1: Analysis of Variance (ANOVA) for chlorophyll fluorescence (Fv/Fm) for control (irrigated) and water stress (drought) treatments for tepary bean.

Item	Degrees of Freedom	EXPT I	EXPT II	Year 1(2012) Combined
Mean Psii value for drought		0.68	0.77	0.73
Mean Psii value for Irrigation		0.66	0.73	0.73
Replication *p* value	2	<0.0001	0.07	<0.0001
Cultivar (C)*p* value	31	0.52	0.10	0.13
Water Treatment (W) *p* value	1	0.66	0.39	0.71
Incubation (I) *p* value	1	<0.0001	<0.0001	<0.0001
Day (D)) *p* value	2	<0.0001	<0.0001	<0.0001
Interaction (C*W) *p* value	31	0.32	0.97	0.97
Interaction (C*I) *p* value	31	0.67	0.55	0.69
Interaction (C*D)) *p* value	62	0.65	0.02	0.35
Interaction (W*I) *p* value	1	<0.0001	<0.0001	<0.0001
Interaction (W*D) *p* value	2	0.32	0.05	0.88
Interaction (I*D)) *p* value	2	<0.0001	<0.0001	<0.0001

Mean quantum yield (Fv/Fm) values are averages of 32 samples (31teparybean genotypes + one soybean control) from three replications. The value in parenthesis indicates loss of energy captured due to heat (incubation at 42 degrees C). W: Water Stress (Irrigation treatment); I: Incubation; D: Days

Table 2: Mean quantum yields (Fv/Fm) of tepary bean during two experimental periods.

Item	Experimental period	Before Stress	During Drought / Water stress	24hr After resuming water
EXPT I _ Drought	Aug-Sep2012	0.75 (0.79)	0.73 (0.78)	0.76 (0.78)
Heat		0.73 (0.76)	0.63 (0.60)	0.63 (0.58)
CV% (R^2)	6.6 (0.44)			
EXPT II _ Drought	Sep-Oct2012	0.78 (0.78)	0.79 (0.76)	0.77 (0.78)
Heat		0.74 (0.75)	0.75 (0.76)	0.71 (0.74)
CV%	5.4 (0.43)			
Drought	Mean data from two experiments	0.76 (0.78)	0.74 (0.77)	0.73 (0.77)
Heat		0.76 (0.73)	0.68 (0.66)	0.64 (0.65)

The values indicate the mean quantum yields of water stress and control in parenthesis for 31 tepary bean cultivars from three replications and two experiments. The control soybean yielded 0.8 in all the experiments and treatments.

There was non-significant variation (p=0.36) among the genotypes during second experiment in 2012 and most of them were with Fv / Fm of 0.8. The reason could be avoidance of shade and providing uniform experimental soil and climatic conditions of greenhouse environment which were unavoidable while conducting first experiment. The reduction in values of chlorophyll florescence during first experiment during 2012 in tepary bean was attributed to several stress factors that were influencing the crop growth period in the greenhouse besides drought stress including but not limited to insect (mite, whitefly), fungal and viral infestation which reduced the leaf area and shade which influenced the leaf thickness. These were supported by the previous observations in soybean and chickpea, stating that that environmental stress during crop growing period will impact the growth due to reduction in chlorophyll content and associated quantum energy absorption, decreasing the amount of assimilates to grain [11]. Significant variation ($p<0.0001$) observed for photochemical efficiency of leaf disc under heat stress and the values were always reduced when compared to water stress indicating that there is decrease in the quantum yield due to impairment of the PSII system due to heat treatment for 20 min. These observations are in line with the previous observations for variations in several crops [8] and legumes like chickpea [28], fababean and soybean [14, 19] due to decreased supply of water due to abiotic stress [8]. During 2013, the experiment was repeated in the greenhouse under uniform conditions of drought and observed significant differences among the genotypes during two experimental periods and observed non-significant variation between the two year experiments (data not presented). The florescence experiment was also repeated using the samples from high tunnel to compare the tolerance variations among the cultivars (data not presented). The results were on par with the previous results achieved in 2012 except that the values were lower than the greenhouse experiment. In high tunnel, we observed the yield (Fv/Fm) values ranging from 0.20 to 0.57 under light adapted stable photosynthetic conditions. The cultivars7, 23, 28 and 31 least tolerant (Fv/Fm< 3); 15, 18 29, and 30 are highly tolerant (Fv/Fm> 4) and 21 and 22 were moderately tolerant to heat stress. The ANOVA (Table1) for the two experiments conducted in 2012 and DUNCAN groups were presented (Table 3). The RWC, relative greenness, RI %, biomass and transpiration efficiency of selected lines from high, medium and low drought tolerance category were presented (Table 5).

Table 3: DUNCAN grouping of 31 tepary bean cultivars based on quantum yields for drought tolerance in comparisons with soybean control during two experimental periods.

DUNCAN GROUPING	Tepary bean Cultivar IDs	Quantum yield (Fv/Fm)
Group A (High)	Soybean S6;Teparybean cultivar IDs: VSU 4, 7,8,9,12, 15, 17, 19, 21, 22, 24, 25, 26, and 29	0.80 (0.79-0.77)
Group B (Medium)	Teparybean cultivar IDs: VSU 2,3, 5, 10, 11,13,14, 18, 20, 28, 30 and 31	0.70 (0.73 - 0.74)
Group C (Low)	Teparybean cultivar IDs: VSU 1,4,6,23,25,27	< 0.69
P value		<0.0001
CV%		5.12
R^2		0.52

The data presented is the mean of three replication and three experiments for Psii photochemical efficiency (Fv/Fm).

Table 4: Comparative analysis of drought tolerance based on photochemical efficiency (Fv/Fm) in tepary bean with other legumes.

Treatments/Crops	Pigeonpea	Soybean	Tepary bean	Chickpea	Mungbean	Mothbean
Drought Stress treated	0.80a	0.79ab	0.80b	0.81b	0.80b	0.77ab
Irrigated Control	0.71b	0.73b	0.77a	0.80b	0.80b	0.75b

In each row, means with different letter (a/b) were significantly different (p=0.07%). The means were averages of three replications and cultivars,2, 11, 4, 6 and 31 respectively in chickpea, mungbean, mothbean, pigeonpea, soybean and tepary bean.

Relative greenness of leaves

Significant differences (p=001) for relative greenness of the leaf among the cultivars and treatments as well as before and at the end of stress and non-significant differences (p=0.001) between the two experiments was observed. The mean SPAD reading to indicate the relative greenness of the leaf in treatment (36.08) was high compared to control (35.00) during the stress period supporting the cultivar's water stress response and it was a quick estimate of chlorophyll and Nitrogen content (Kao and Forseth, 2006,Loh et. al., 2002). The SPAD reading was high in cultivar 2 due to infestation with virus.

Relative water content of leaf discs

Significant differences for RWC were revealed among treatments (p=0.003) and cultivars (p=0.0001) and their interaction (p=0.0001) with a mean RWC of 22.59 % in control and 19.07 % in treatment. The RWC was high in TB # 16 (86.13 %) followed by TB # 10 (53.45 %) and low in TB 25 (7.19 %) and TB22 (7.09 %). The drought tolerant line TB # 30 (35.43 %) was with high RWC compared to susceptible line, TB #23 (11.12 %) as supported by Castonguay and Markhart (1991) and was due to high leaf water-use efficiency and high photosynthetic electron transport efficiency under drought (Fenta et. al., 2012) with significant interactions between cultivar and drought.Some of the lines which were drought tolerant were observed with low RWC and the susceptible line, with high RWC, which could be attributed to unexpected changes, occurred in greenhouse environmental conditions during experiment 1.

Thermo-stability

The electrical conductivity (EC) was measured in terms of µsams/cm for each leaf disc sample and was expressed as relative injury percentage (RI %). All the cultivars were with highest EC values after incubation compared before incubation in both control and treatment samples. Significant differences (p=0.003) for cell membranethermo-stability were observed among the cultivars, treatments and their interaction (p=0.009). The cultivar thermo-stability values (RI%) were ranging from 3.76 (TB # 10) to 33.07 (TB #25). The susceptible lines were with highest EC values (high RI%) and tolerant lines with low EC (low RI %) values (Table 5). The mean RI % of the cultivars in treatment, water stress, was lower (11.7) than those in control (12.5) plants indicating that the thermo-stability of the membrane increased with water stress in tepary bean. The drought tolerant lines TB #30 (5.58 %), TB# 18 (10.10 %) TB #15 (11.20 %) with less RI % compared to susceptible lines, TB# 23 (14.17 %), TB# 20 (31.10 %) and TB# 25 (33.07 %).The electrolyte leakage and fluorescence ratio were negatively correlated in tepary bean under water stress and was on par with previous results in legumes [32].

Table 5: Biomass and transpiration efficiency of the drought responsive tepary bean cultivars

Tepary bean Cultivar ID	Dry mass (g/plant)	Total TE (g/kg)	Shoot TE (g/kg)	RWC of leaf disc (%)	RI %	SPAD reading for leaf disc
1	0.8±0.8	2.6±2.3	2.3±2.1	17±1.4	7.3±3.4	32.09±2.7
2	3±0.4	32.7±4.7	31±5.5	18.5±2.8	4.8±2.1	42.25±2.6
3	8.6±3.8	36.1±15.8	34.8±15.0	15.2±4.8	7.8±1.4	39.17±3.9
4	4.8±4.9	15±14.9	14.1±14.2	16.1±6.6	8.3±11.2	28.49±3.1
7	3.8±3.9	25.2±23.4	23±21.9	13.8±7	3.2±2.7	36.22±4.1
15	7.2±0.0	41.1±0.0	39.7±0.0	24.3±2.1	8.9±5	38.55±1.4
18	-	-	-	-	-	-
23	4.4±1.9	20.1±8.1	19.8±8.0	8.9±6.2	7.8±6.1	32.2±4.9
24	1.9±2.0	7±7.5	6.8±7.5	8.9±12.7	10±5.6	38.9±4.7
29	4.5±3.6	26.4±17.8	25.2±16.8	24.4±13.5	33.1±13.6	38.3±2.3
30	2.9±1.8	12.4±7.3	11.9±6.9	35.4±21	5.6±2.7	39.46±3.1
31	1.5±1.5	6.7±6.8	6.2±6.4	17.3±11.4	5.2±8.3	37.46±3.1

The values are means of two experiments and three replications in each experiment. Cultivar 18 was died in all the replications during TE experiment

Biomass and Transpiration efficiency

Non-significant differences (p=0.28) for biomass and significant differences (p<0.001) for transpiration efficiency were observed among the cultivars and between control and treatment (Table 5). The total biomass accumulated at the end of stress period was 4.1 g on an average in treatments (cultivars under stress) and 5.3 g in control. The biomass accumulated was high in 9, 18 and 11 (10-12g) and low in 19 and 22 (1-2g). Plant biomass accumulated during stress period was less in treatment (2.7 g) compared to control (3.2 g) on an average. The root biomass (0.2g) was less compared to shoot biomass (3.8 g), unlike soybean, with a very low root to shoot ratio in teparybean, but the length of the root was increased with water stress in cultivars. Dehydration-avoidance responses of tepary bean lines vary during drought stress due to deep penetrating root system and sensitive stomata of leaf [20].

Significant differences among the cultivars for total and shoot transpiration efficiency revealed that cultivar 9 and 8 with high values (373-1237 g/m^3) and 99 and 22 with low values (8-9g/m^3). The TE and SHTE (shoot transpiration efficiency) values were high in treatment (25.54 g/m^3;24.46 g/m^3) compared to control (90.77 g/m^3; 88.76 g/m^3) on an average. The cultivars 9 and 8 were with high values for TE and SHTE as the biomass accumulated was high compared to the water used due to their shady location in replication one in experiment one that influenced the mean values. The biomass, total and shoot TE for the selected nine drought responsive cultivars were presented (Table 5).The results were supported by previous observations on drymatter reduction in mungbeanunder water stress during six weeks after planting [30] and cereal crop sorghum [35]. Multiple parameters were measured during the current water stress studies as screening cultivars based on single parameter is difficult for a seedling as various plant parts have different response to the drought [9]. The summarized results were graphically represented as tree based on similarity matrix (UPGMA-Pearson's correlation coefficient) for tepary bean alone (Fig.1 Phenogram) and in comparison with other crops (Table 4) for their florescence values during water and heat stress.

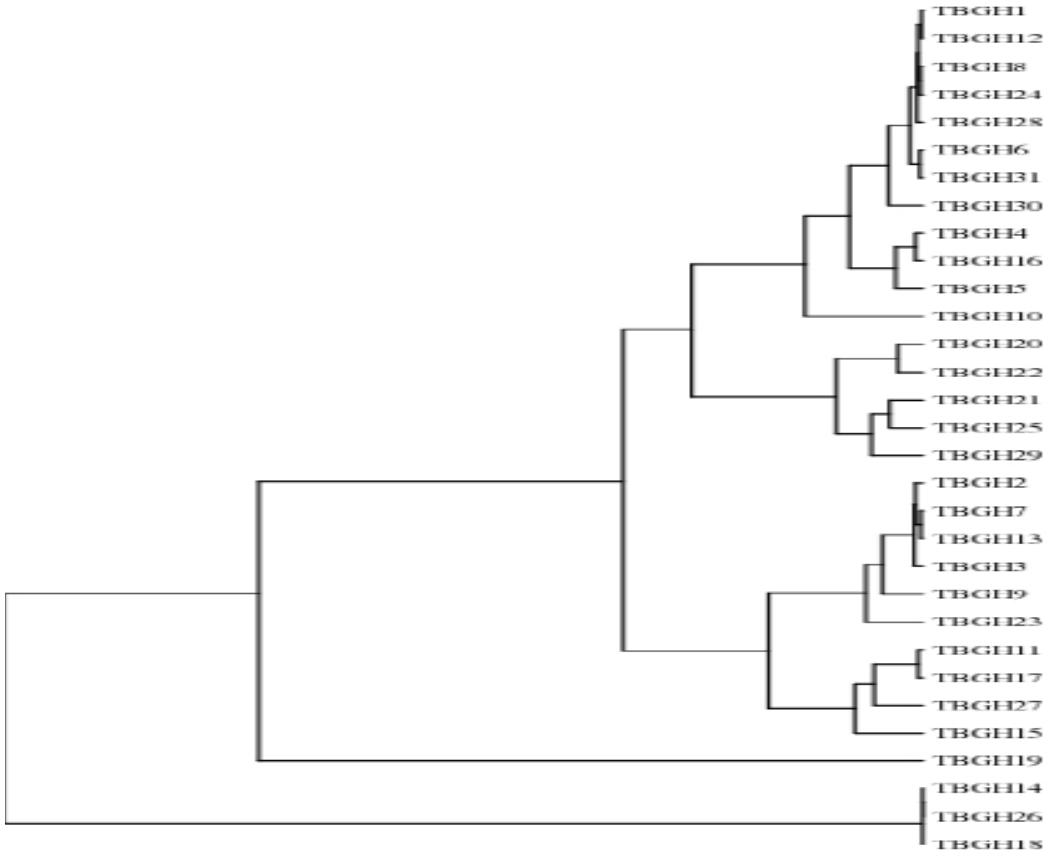

Fig 1: A Pheno gram of native lines based on six variables studied for tepary bean drought tolerance.

The photochemical efficiency is one of the useful indicators for tolerance to drought and varietal screening, besides other traits contributing to tolerance of a cultivar under water stress. In comparision, highly drought tolerant lines of tepary bean (0.80) were equally tolerant to soybean control (0.78), chickpea, pigeonpea (0.80) followed by mothbean (0.78) and mungbean (0.78). This indicates that the drought tolerant lines of available germplasm will be a resource for future germplasm improvements for seed nutritional quality with drought tolerance in these legume crops. Further, an observation revealed that high tolerance to drought was observed in brown and black color seeded tepary cultivars compared to white seeded cultivars which were supported by previous observations in pigeonpea.

CONCLUSION

Potentially drought tolerant and susceptible cultivars were selected for studies on genetics of drought tolerance. The selected lineswill be evaluated for major nutrients, resistance starch, antioxidants for future selections and to identify markers/QTLs for crop improvement for nutritional quality.Improvement of tolerance to stresswith high quantum yields will be useful to improve tepary bean and other food legume crops for rain fed agriculture. The cultivar's performance for quantum yield and nutritional traitsand the segregation of these traits due to ongoing crossing program will be useful to develop first genetic map for nutritional quality and drought tolerance in tepary bean.

ACKNOWLEDGEMENT

We like to acknowledge the funding agency USDA-NIFA-Capacity building grants program for providing us funds to conduct the research and VSU-ARS for providing the required facilities and equipment to conduct the research and assigning the ARS article series number 306 for publication of the findings. Authors like to acknowledge our soil science faculty Dr. Atlee and Mr. Broodie for providing the EC meter for analyzing samples for RI assay, Dr. Xu for providing facility to use hot air Owen, Dr. Hamama and Dr. Parry for providing chemicals and entomologist, Dr. Kraemer for kind support for providing insecticide. Authors like to acknowledge the new crops team (Mr. Bowen, Mr. Bates, Mr. Townes and Ms. Rocio) for supporting the research activity execution in greenhouse and high tunnel during the project period, and our student researchers Ms. Melissa, Ms. Davis, and Ms. Corey.

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