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COMPARATIVE DROUGHT POSTPONING AND TOLERANCE POTENTIALS OF TWO TEPARY BEAN LINES IN RELATION TO SEED YIELD

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ABSTRACT

Tepary bean (*Phaseolus acutifolius* A. Gray) is a strategic germplasm for genetic improvement of the common bean *(Phaseolus vulgaris)* for resistance to various stresses including drought. However, its potential for this purpose has not been evaluated. It is imperative that the potential of various genotypes of tepary bean as genetic improvement materials is evaluated. In the present study, tepary line NE#19 had higher seed yield and greater seed size than NE#5 under well-watered conditions in the field at Assiut, Egypt. Drought reduced seed yield of both lines but NE#19 had higher seed yield, and its seed size was not affected. Both lines had comparable shoot dry mass and root depth under drought. However, line NE#19 developed fibrous roots of greater mass than NE#5 in response to drought, especially, in deep soil profile (40 to 50 cm). Root/shoot ratio was high and the portion of leaf area supported by the mass unit of root was small in NE#19. Additionally, the leaves of this line exhibited a reduced stomatal conductance in response to progressive drought. However, biochemical assessment indicated that NE#5 and not NE#19 can completely recover after rehydration of severe dehydrated leaves in terms of chlorophyll (*Chl*) contents and *Chl a* fluorescence parameters (F_0, F_M and F_V/F_M), reduced tocopherol and lack of alteration in malondialdehyde release. This was due to a prominent antioxidative role of peroxidase activity during the stress period. It is concluded that the potential drought postponing traits of NE#19 enabled it to sustain normal seed filling and to produce high seed yield. Line NE#5 was regarded as tolerant to severe drought and may not suffer great reduction in seed size and yield if given supplemental water during the critical phase of seed filling. Diversity in drought postponing and tolerance of the two lines may be of further interest to bean breeders.

Key Words: Chlorophyll *a*, oxidative stress, *Phaseolus acutifolius, Phaseolus vulgaris*

RÉSUMÉ

Le haricot Tepary (*Phaseolus acutifolius* A. Gray) est un germplasme stratégique pour l'amélioration génétique de l'haricot habituel pour sa résistance aux stresses incluant la sècheresse. Cependant, son potentiel pour cet objectif n'a pas encore été évalué. Il est impératif que le potentiel de divers génotypes d'haricot tepary comme matériels d'amélioration génétiques soit évalué. Dans cette étude, la lignée NE#19 du tepary avait le plus grand rendement en graines et larges graines que NE#5 sous des meilleurs conditions d'irrigation dans un champ à Assiut en Egypte. La sécheresse a réduit le rendement en graines de deux lignées mais NE#19 avait le rendement élevé et la dimension de ses graines n'était pas affectée. Les deux lignées avaient de masses de matière sèche de bourgeons et la profondeur des racines à la sécheresse comparables. Cependant, la lignée NE#19 développant des racines fibreuses de grande masse que NE#5 en réponse à la sécheresse, spécialement, en profile de sol profond (40 à 50 cm). Le taux de racines/bourgeons était élevé et la portion de la surface de feuilles supportée par la masse unitaire des racines était faible dans NE#19. En plus, les feuilles de cette lignée ont exhibé une conductance stomatale réduite en réponse à la sécheresse progressive. Cependant, l'évaluation biochimique a indiqué que le

NE#5 et le non NE#19 peuvent récupérer complètement après réhydrations des feuilles sévèrement déshydratées en termes des contenus chlorophylle *(Chl)* et des paramètres à fluorescence *Chl a* (F_0, F_M et F_V/F_M), le tocophérol réduit et la carence d'altération en libération de malondialdehyde. Ceci était dû au rôle antioxydatif proéminent de l'activité peroxydase durant la période des stresses. Il est conclu que l'ajournement du potentiel des traits de sécheresse de NE#19 a permis de soutenir le remplissage normal des graines et de produire le rendement en graines élevé. La lignée NE#5 était regardée comme tolérant à la sévère sécheresse et beaucoup n'ont pas souffert d'une grande réduction en dimension et rendement de graines si l'eau supplémentaire est donnée pendant la phase critique de remplissage de graines. La diversité en ajournement en sécheresse et la tolérance de deux lignées peuvent être d'intérêt supplémentaire aux reproducteurs d'haricot.

Mots Clés: Chlorophylle *a*, stresse oxydative, *Phaseolus acutifolius*, *Phaseolus vulgaris*

INTRODUCTION

Tepary bean (*Phaseolus acutifolius* A. Gray) is well adapted to grow under arid environmental conditions (Federici *et al.*, 1990) in the southwestern USA, Central America, Mexico and Africa. Due to its potential as human diet (Miklas *et al.*, 1994; Idouraine *et al.*, 1995), this legume specie has long been suggested for domestication in the arid regions of the developing world (Morci and El-Murraba, 1960; Mohamed, 1996, 2000). Because of crossability of tepary with common bean (*Phaseolus vulgaris*), it is interesting as breeding germplasm to improve common bean resistance to stresses including drought (McElory, 1985; Mohamed, 1990, 1996).

Physiological assessments suggested that drought resistance of tepary bean is mainly based on its dehydration postponing potential of the leaves and roots (Markhart, 1985; Yu and Berg, 1994; Mohamed *et al.*, 2002). However, other agronomic studies of drought resistance of tepary in the field showed differential growth and seed yield performance (Federici *et al.*, 1990; Simon, 1993; Mohamed, 1996). Different tepary lines may have different levels and/or combinations of the potential drought postponing mechanisms (Hassan, 1995). Incorporation of such mechanisms in a single line would result in a more durable and stable drought resistance. Combined assessment of physiological and yield potentials, therefore, may be the most useful approach. In addition to drought postponement, tolerance mechanisms enable plants to stand severe dehydration that may proceed when water deficit lasts for long. Severe dehydration in plant tissues increases the formation of free radicals of oxygen (Navari-Izzo and Rascio, 1999). This is because of the transit of the energised electrons from the photosynthetic process to oxygen under the conditions of limited water availability and the reduced utility of light energy for carbon fixation.

Subsequent mediated reactions can lead to the degradation of the cellular and the membrane components and the loss of the membrane functions (Scandalios, 1993). Enzymatic and non-enzymatic antioxidants play a key role in scavenging of the activated oxygen species such as superoxide and hydrogen peroxide (Sgherri *et al.*, 2000). Antioxidants, therefore, may provide the plants with tolerance to cope with severe dehydration that induces oxidative stress. However, information is lacking on the mechanisms of biochemical reactions inducing drought tolerance in tepary.

Understanding the physiological mechanisms of dehydration avoidance along with biochemical-based dehydration tolerance in relation to pulse yield would enable development of a precise procedure for screening of tepary lines. Also, the availability of elite tepary germplasm with defined differential mechanisms of drought resistance would assist to plan the most efficient strategy for improving drought resistance of common bean. The objective of the present study, therefore, was to assess pulse yield, physiological, morph-physiological and biochemical reactions of two tepary lines in response to progressive water deficit.

MATERIALS AND METHODS

Field trial for seed yield and its main components. Two tepary bean (*Phaseolus acutifolius* A. Gray) lines (NE#5 and NE#19) were evaluated for growth and seed yield in a field

experiment conducted under typical semi-arid conditions in southern Egypt at the Research Station of the Faculty of Agriculture, Assiut University, in Assiut, Egypt for two consecutive years of 1997 and 1998. The experiment was arranged as split-plots in a randomised complete-block design (RCBD) with four replicates. The whole plots contained drought and control treatments, while the two tepary lines were in the sub-plots. The whole plots of the drought treatments were surrounded by unplanted belts of 2 m wide ridges. Each sub-plot consisted of four rows.

Seeds were planted 25 cm apart on the northern side of 0.7 m wide and 3 m long rows. Three to four days after emergence (10-12 days after planting (DAP), seedlings were watered via furrow irrigation. Subsequently, the control plants were irrigated when about 58% of the available soil water was depleted (Mohamed, 1996). The plants in the drought treatment were irrigated at about 72%, available soil water was depleted. Accordingly, control plants were irrigated additionally, five times (24, 38, 52, 66 and 80 days after emergence). Plants of the drought treatment received irrigation once only at the flowering or early pod setting (40 to 42 days after emergence).

The soil texture of the site was clay loam and had a pH of 7.9. Soil moisture was determined gravimetrically after drying to constant weight at 105°C. Soil samples were taken at 30-40 cm depth every 2-3 days. The mean daily minimum and maximum temperatures during the growing season (mid-May to mid-Sept.) ranged from 30 to 35°C, respectively.

Shoot fresh and dry weights were determined at 5-6 weeks after planting, using one plant per 4 rows. Shoot dry mass was determined after drying to constant weight at 80°C. At the end of the growing season (80 to 85 days after emergence), the number of harvested pods, pod length, seeds/pod, percentage of seed set, 100-seed weight and the seed yield were recorded.

Greenhouse experiment for dry matter accumulation and partitioning. Fifty-centimeter deep cuboid acrylic, 3.3 liter containers were used. The containers had a removable clear window on one of their sides, which was covered with black plastic-sheet. Each pot contained 3.1 kg of clean washed sand covered with a layer of pebbles, 1 to 2 cm in diameter, to prevent surface encrustation and to minimise evaporation. Two seeds were planted in each pot that was then placed at an angle of about 60°. Seedlings were thinned to single plants 2 to 3 days after emergence. Growing plants were kept under natural daylight supplemented with artificial light using 200 µmol m^{-2} s^{-1} PAR on the top of the plants. The average day/night temperature was 20 to 22° C maximum and 14 to 16 °C minimum. Relative humidity ranged between 55 to 65%. Seedlings were watered with either tap-water or Hoagland nutrient solution (Hoagland and Arnon, 1950) every other day until the first trifoliate was fully expanded (about 15 days after seed planting). Subsequently, the containers were watered with the Hoagland nutrient solution to the drip point. Thereafter, the water was withheld 21 days. The experiment was laid out in a randomised complete-block design with six replicates. Half of the tested plants (6 plants) were harvested before water withholding, and the other half at the end of the water stress period.

The length of the deepest root was observed through the clear windows of the containers. Then, the windows were removed and the roots were separated and collected from each 10cm depth of the soil profile. Simultaneously, soil samples were taken from each 10cm depth of the culture column. Subsequently, shoots were harvested and separated into leaves and stems. Total leaf area was measured non-destructively with a portable leaf area meter (Model 100, LI-COR Inc. Lincoln, Nebraska, USA). Fresh weight (Fw) of leaf disks prepared from the first trifoliate was determined. Weight of fully turgescent leaf disks (Tw) was obtained by keeping them at 10 °C in the dark onto water-saturated filter papers in sealed petri-dishes until constant weight. Leaf disks were dried to a constant weight (Dw) at 80 °C. Relative water content (RWC) was determined gravimetrically. Leaves, stems and root sections were dried to a constant weight at 80°C.

Moisture content of the soil samples was also determined gravimetrically. Both the soil samples and plant organs were allowed to cool down for 2 to 3 h before determining dry weight.

Growth chamber experiment for photosynthetic properties and antioxidant reactions. Seeds were planted in a limited root medium using pots of 5 cm diameter and 7 cm height to minimise differences in water status of the leaves due to variable root growth and penetration. The pots were filled with 100 ml of a dry peat moss/sand mixture (1:1, v/v). Seeds were surface sterilised using calcium hypochlorite before planting. The cultures were maintained in a controlled environment chamber under 12 h photoperiod and photon flux density of 400 µmol $m^{-2}s^{-1}$ PAR on the surface of the leaves. The day/night temperature in the growth chamber was 24° C±0.5 / 15° C±0.5 and the relative humidity was about 70%. After emergence (about 6 days post seed planting), uniform seedlings were selected. Nine-day-old seedlings were watered to the drip point with Hoagland nutrient solution (Hoagland and Arnon, 1950).

The seedlings of each line were arranged in two plastic trays (30 cm by 60 cm). One tray was used for the treatment of mild water depletion and the other for severe water depletion. Mild water depletion was implemented by withholding the water for 4 days (Mohamed *et al.*, 2002). Severe water depletion treatment was subjected to 9 d of water withholding. Each tray contained four replicates of the following treatments arranged in randomised complete-blocks: (1) water-stressed, (2) well-watered (control-1, harvested along with the water stressed), (3) rewatered, and (4) well-watered (control-2, harvested along with the rewatered) seedlings. The pots of the water-stressed seedlings were covered with a clear plastic sheet on the soil surface around the seedling stems, during the period of water withholding. This was to reduce the water evaporation and thus, allowing slow progressive water depletion. The well-watered and the water-stressed seedlings were sampled 4 and 9 d after water withholding. The rewatered seedlings were sampled 24 h after the rewatering along with another set of the well-watered ones to control the differences due to a possible age influence.

Net photosynthesis (A), stomata conductance (g_s), intercellular CO_2 (C_i), transpiration rate (E) were measured *in vivo* for plants subjected to 4 water withholding using CIRAS-1 portable infrared gas analyzer connected to broad Parkinson leaf chamber (PP System, Hitchin, Herts., England). On the other hand, assay of these gas exchange parameters was not amenable for plants subjected to 9 d water withholding due to the severe dehydration of the leaves. Chlorophyll *a* fluorescence parameters (F_0, F_M and F_V/F_M) were determined with a portable pulse amplitude modulation fluorometer (PAM 2000, Walz GmbH, Effeltrich, Germany) after dark adaptation for 30 min. Leaf area was measured nondestructively with a portable leaf area meter (Model 100, LI-COR Inc. Lincoln, Nebraska, USA). Leaf disks were then prepared for the following assays: relative water content (RWC), Chl *a* and *b*, carotenoid contents, tocopherols (TPH), malondialdehyde (MDA) release, peroxidase (POD) activity and ascorbic acid (AA) content. Soil moisture was also determined.

Chlorophyll (*Ch*) *a* and *b* and carotenoid (*Car*) contents of the leaves were extracted in dimethyl sulphoxide and determined spectrophotometrically (Perkin-Elmer Lambda 5/15) as described by Wellburn (1994). Malondialdehyde (MDA) release as indicator of the structural integrity of the membranes was measured colorimetrically using the method of Heath and Packer (1968). Tocopherol (TPH) was determined by an HPLC-method described by Schmitz-Eiberger and Noga (2001). For determining the ascorbic acid (AA) content, leaves were homogenised with a dismembrator and extracted with potassium metaphosphate buffer. Two aliquots of each samples were measured, one sample containing ascorbic acid-peroxidase and the other without addition of the enzyme. Since AA is an easily oxidizable compound, it was necessary to add the reducing agent 2,3-dihydroxybutane-1,4-dithiol (DTE) during preparation. Analyses were performed according to a standardized procedure (Gary and Singh, 1971). The assay mixture for determination of peroxidase (POD) activity contained 0.77 ml 0.1 M potassium phosphate buffer (pH 6.0), 0.15 ml 20 mM guaiacol, 50 µl 0.6 M H_2O_2 and leaf extract in a total volume of 3 ml. Changes in light absorption at 420 nm were followed at 20-22°C to determine POD activity (Dai *et al.*, 1997; Schmitz-Eiberger and Noga, 2001). Protein analysis was

performed as described by Bradford (1976). The greenhouse and the growth chamber studies were conducted during 1999 - 2001.

Data analysis and presentation. Data of all experiments were subjected to separate and combined analyses of variance (ANOVA) as described by Gomez and Gomez (1984) relevant to experimental design used. The data of the two runs of each experiment were pooled based on the test of variance for the run and its interaction with the treatments. The differences between the means of the drought stressed and non-stressed (control) in the field and the growth chamber experiments or for the performance of before and after the drought stress in the greenhouse experiment were compared with the Least Significant Difference Test (LSD) at 0.05 probability level. As devised by the coefficients of variation (C.V.) of the original data in the growth chamber, the ANOVA for POD, AA, MDA and TPH assays was based on the square root transformed values. Means were compared using the LSD or Dunnett's Test at 0.05 probability level. For convenience, as the differences between the first well-watered sample (harvested along with the rewatered seedlings) were not significant, their average as presented as a one well-watered (control) treatment.

RESULTS

Seed yield and main components. Non-stressed plants of both NE#19 and NE#5 in the field had statistically similar (P>0.05) shoot water content, shoot dry mass, percentage of seed-set, number of seeds/pod and pod length (Table 1). Drought treatment induced comparable reduction in these traits in both tepary lines. Thus, the two lines remained similar in shoot water contents, shoot dry mass, percentage of seed-set, number of seeds/

TABLE 1. Growth and yield parameters of two the tepary bean line in response to drought stress under field conditions, Assiut [1]

Lines	Watering treatment		Change	Watering treatment		Change
	Non-stressed (control)	Drought - stessed		Non-stressed (control)	Drought - stessed	
	Shoot dry mass (g/plant)			Shoot water content (%)		
NE#5	71.1	36.8	- 34.3 *	79.1	71.2	7.9 *
NE#19	72.9	39.0	- 33.9 *	80.5	73.4	7.1 *
Difference	1.6 ns	2.2 ns		1.4 ns	2.2 ns	
	100-seed weight (g)			Seed set (%)		
NE#5	12.9	10.3	- 2.6 *	98	96	2 ns
NE#19	15.4	14.5	- 0.5 ns	97	94	3 ns
Difference	2.5 *	4.2 *		1 ns	2 ns	
	Number of seeds/pod			Pod length (cm)		
NE#5	4.7	4.6	-0.1 ns	6.7	6.5	-0.2 ns
NE#19	4.9	4.7	-0.2 ns	6.4	6.3	-0.1 ns
Difference	0.2 ns	0.1 ns		0.3 ns	0.2 ns	
	Number of pods/m^2			Total seed yield (g/m^2)		
NE#5	210	188	-22 *	145.6	87.1	- 58.5 *
NE#19	326	229	-97 *	246.1	143.2	- 102.9 *
Difference	116 *	41 *		100.9 *	56.1 *	

[1] Non-stressed and drought-stressed plants were irrigated when 58 and 72% of the available soil water was depleted, respectively; NS, * Non-significant and significant at 0.05 level of probability, respectively

pods and pod length under drought conditions. Under non-stressed conditions, however, NE#19 produced more pods, larger seeds and higher seed yield. The reduction in number of pods and seed yield in NE#19 due to drought treatment was 2 to 3-folds that of NE#5. Nevertheless, NE#19 appeared superior to NE#5. The drought treatment significantly ($P < 0.05$) reduced the 100-seed weight only in the line NE#5.

Dry matter accumulation and partitioning. There were no detectable differences in all the studied parameters of the two tepary lines (Table 2) in the greenhouse before water withholding. Three weeks after water withholding, similar values for RWC, shoot dry mass and root depth. However, line NE#19 showed greater plant dry mass than NE#5. Leaf area of NE#19 was smaller than that of NE#5. Consequently, R/S ratio was significantly ($P<0.05$) high, while the value of leaf area to root mass was significantly low in NE#19 compared with that of NE#5. Both lines exhibited differential distribution patterns in the soil profile (Fig. 1). Most interesting is the greater root mass of NE#19 than NE# 5 in the deepest points (> 40) of the soil profile. However, soil moisture around the roots of NE#5 was less than that around the roots of NE#19 in the depth range of 30-40 cm in the soil profile (Fig. 1B) in spite of their similar root mass. Line NE#5, therefore, used more soil water during the stress period than NE#19.

Leaf photosynthetic properties and antioxidant reactions. The depleted water from the limited root growing-medium of the seedling 4 days after water withholding in the growth chamber relative to the container capacity was about 56% (Fig.

TABLE 2. Dry matter accumulation and partitioning characters of two the tepary bean line in response to drought stress in the greenhouse[1]

Lines	Watering treatment		Change	Watering treatment		Change
	Before stress (control)	After drought stress		Before stress (control)	After drought stress	
	Relative water content RWC, %			Plant dry mass (g/plant)		
NE#5	90.7	81.1	-9.6 *	0.898	4.043	3.145 *
NE#19	92.6	85.4	- 7.2 *	0.929	4.400	3.471 *
Difference	1.9 ns	4.3 ns		0.031 ns	0.357 *	
	Shoot dry mass (g/plant)			Root dry mass (g/plant)		
NE#5	0.280	0.857	0.577 *	0.618	3.186	2.568 *
NE#19	0.309	0.820	0.269 *	0.620	3.580	2.960 *
Difference	0.090 ns	0.037 ns		0.002 ns	0.394 *	
	Root : Shoot (ratio)			Leaf area (cm^2/plant)		
NE#5	2.3	3.7	1.4 *	27.1	96.5	69.4 *
NE#19	2.0	4.4	2.4 *	28.8	86.2	57.4 *
Difference	0.3 ns	0.7 *		1.7 ns	10.3 *	
	Leaf area / Root mass			Root depth (cm)		
NE#5	43.7	31.3	-12.4 *	17.2	48.4	31.2 *
NE#19	46.5	24.1	- 22.4 *	17.5	45.7	28.2 *
Difference	2.8 ns	7.2 *		0.3 ns	2.7 ns	

[1] Plants were watered after emergence when needed for 2 weeks and then water was withheld for 3 weeks; NS, * Non-significant and significant at 0.05 level of probability, respectively

2A). The relative water content (RWC) (Fig. 2B and C), net photosynthesis (A) and intercellular CO_2 concentration (C_i) (Fig. 2 D and E) did not significantly change in the leaves of both tepary lines when compared to the well- watered (control) ones. Distinctively, line NE#19 exhibited decreased stomata conductance (g_s) (Fig.2F) and, consequently, a reduced transpiration rate (Fig. 2G). Photosynthetic water-use-efficiency was significantly ($P<0.05$) elevated in line NE#19 (Fig.1).

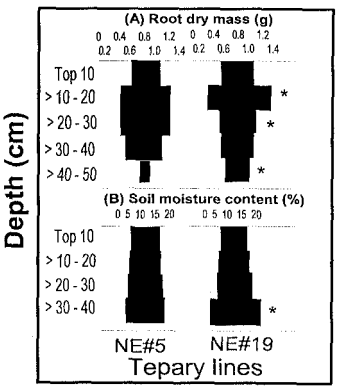

Figure 1. Root dry mass and the soil moisture content at different depths in the soil profile for plants of two tepary bean lines (NE #5 and NE #19) grown in the greenhouse following 3 weeks of water withholding. Stars denote significant differences between the two lines at the same depth of the soil ($P < 0.05$).

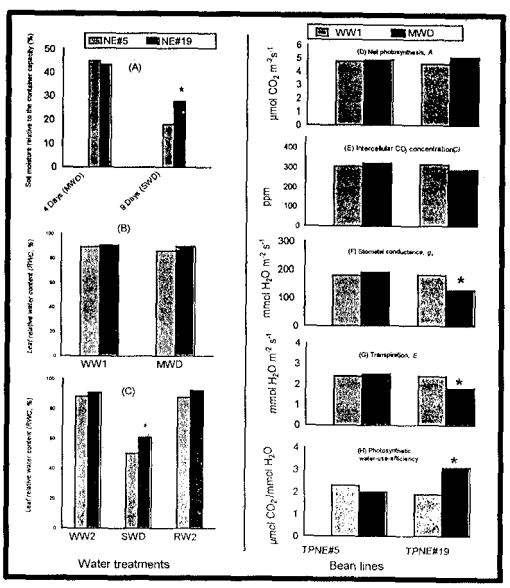

Figure 2. Soil water depletion (A), plant water status (B and C) and photosynthesis parameters (D to H) of two tepary bean lines (NE#5 and NE#19) grown in a growth chamber when subjected to water withholding. (A) Depleted water from the root medium is presented relative to the container capacity (water content few hours after watering to the drip point) 4 days (mild water depletion, MWD) and 9 days (severe water depletion, SWD) after water withholding. (B) Leaf relative water content (RWC) in the well-watered (WW1) and mildly dehydrated (MWD) seedlings, (C) RWC of severely dehydrated (SWD) and rewatered (RW2) seedlings. D - H) Photosynthesis parameters for WW1 and MWD treatments. Stars denate significant differences at 0.05 level between NE#5 and NE#19 when received the same water treatment in (A, B and C) and between water treatments for the same tepary line in (D to H).

The two tepary lines did not differ in the tocopherols (TPH) (Fig. 3A), malondialdehyde (MDA) (Fig. 3B), peroxidase (POD) (Fig. 3D), chlorophylla (Chl a) (Fig. 3E) and carotenoid contents (Car) (Fig. 3G) as determined in the leaves of control seedlings. However, AA (Fig. 3C) was higher in NE#19 than in NE#5 and the *vice versa* was detected for chlorophyll b (Chl b) (Fig. 3F). Four days of water withholding did not affect leaf contents of MDA, Chl *a* and Chl *b* (Fig. 3 B, E and F). However, TPH elevated in both lines more than in the control. Ascorbic acid (AA) and *Car* rose in stressed NE#19 while POD activity increased in stressed NE#5. Leaves of stressed NE#19 exhibited significantly greater AA than those in the stressed NE#5, but lower POD activity. Rewatering reduced TPH in the leaves of both lines against the control level. The level of MDA declined after rewatering to a level lower than the control in both lines. That for AA reduced while *Car* remained high in the rewatered seedlings of NE#19. Peroxidase (POD) activity elevated in the rewatered seedlings of NE#5. The values of *Chl a* fluorescence parameter F_v/F_m, in stressed leaves of NE#5 significantly increased more than the control leaves; yet no changes were detected in NE#19 (Fig. 3H). Parameter F_0 greatly decreased in NE#5 and increased in NE#19 (Fig. 3I). The alteration of F_M was not significant in both lines (Fig. 3J). Similar responses of *Chl a* fluorescence were detected in the leaves of rewatered NE#5. The increase in F_0 in the leaves of stressed seedlings of NE#19 dropped after rewatering, reaching similar values to those recorded in the control.

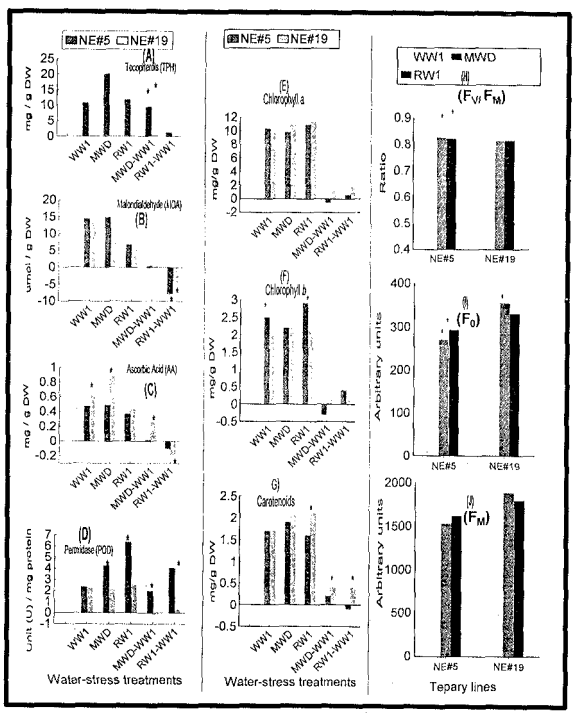

Figure 3. Effect of mild water depletion (MWD) and rewatering (RW1) for two tepary bean lines (NE#5 and NE#19) on tocopherols (TPH), malondialdehyde (MDA) release, ascorbic acid (AA) content and peroxidase (POD) activity (3A to 3D), chlorophyll *a* and *b* and carotenoid contents (3E to 3G) and chlorophyll *a* fluorescence parameters (F_0, F_M and F_V/F_M) of the attached leaves (3H to 3J). Stars donate significant water treatment mean deviation (based on the square root transformation in A to G) within tepary bean line from the respective control (MWD - WW1 and (RW1 - WW1) at 0.05 level of the probability using Dunnett's Test or differences (based on square root transformed data in A to G) between NE#5 and NE#19 receiving the same water treatment (WW1, MWD and RW1) using "Least Significant Differences, LSD) at 0.05 level of the probability.

Nine days after withholding water, 82% of the soil water content was depleted in NE#5, but only 73% for NE#19 (Fig. 2A). In contrast with the respective well-watered (control) seedlings (Fig. 2C), great decrease occurred in the leaf RWC of the two lines. Noticeably, the RWC of the leaves in NE#19 was about 11% higher than in NE#5. Nevertheless, both tepary lines were severely dehydrated and showed permanent above ground wilt symptom. After rewatering, the RWC increased to a level comparable to that of the control seedlings in both lines. Except for the lower content of MDA (Fig. 4B) in NE#5, the leaves of the well-watered seedling of both lines were similar in TPH (Fig. 4A), AA (Fig. 4C), POD (Fig. 4D), *Chl a* (Fig. 4E), *Chl b* (Fig. 4F) and *Car* (Fig. 4G).

Under the severe dehydration conditions, TPH rose while AA, *Chl a*, *Chl b* and *Car* decreased in both lines. The POD activity increased only in NE#5. The dehydrated leaves of NE#5 had significantly ($P<0.05$) lower TPH, MDA and AA but higher POD activity than those of NE#19. The rehydrated leaves, in contrast to those from well-watered (control) seedlings of line NE#19, showed increased TPH, MDA, and AA; and decreased *Chl a*, *Chl b* and *Car* content. Those rehydrated leaves of NE#5 exhibited low TPH and *Car* content over the control, while POD was similar to the control line NE#5 showed recovery of *Chl a* and *Chl b*. F_v/F_m parameter of the *Chl a* fluorescence significantly decreased in both lines when comparing the control with the severely dehydrated seedlings (Fig. 4H). Although F_v/F_m

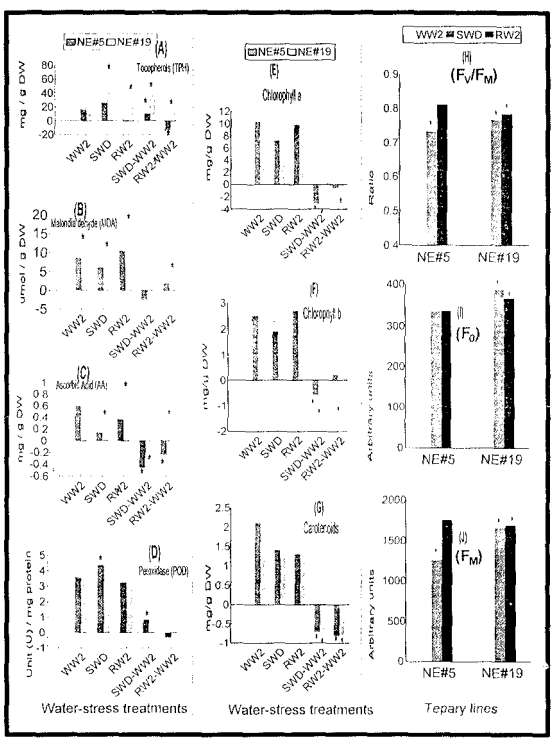

Figure 4. Effect of severe dehydration (SWD) and rewatering (RW2) as compared to the control (well-watered, WW2) for two tepary bean lines (NE#5 and NE#19) on tocopherols (TPH), malondialdehyde (MDA) release, ascorbic acid (AA) content and peroxidase (POD) activity (A to D), chlorophyll *a* and *b* and carotenoid contents (E to G) and chlorophyll *a* fluorescence parameters (F_0, F_M and F_V/F_M) of the attached leaves (H to J). Stars donate significant water treatment mean deviation (based on the square root transformation within in A to G) tepary bean line from the respective control (SWD - WW2 and (RW2 - WW2) at 0.05 level of the probability using Dunnett's Test or differences (based on square root transformed data in A to G) between NE#5 and NE#19 receiving the same water treatment (WW2, SWD and RW2) using "Least Significant Differences, LSD) at 0.05 level of the probability.

increased to the well-watered (control) level in NE#5 after rewatering, its value remained at a lower level in NE#19. For F_0 there was no alteration due to severe dehydration or following rewatering in NE#5 (Fig. 4 I). In contrast, F_0 greatly increased in the severely dehydrated seedlings of NE#19. After rewatering, its value remained higher than in the control seedlings of this line. For F_m a significant ($P<0.05$) decrease in the severely dehydrated seedlings of both lines was observed and returned to a similar level in NE#5 as in the control after rewatering (Fig. 4 J).

DISCUSSION

Reduction in shoot growth and water content of the two tepary lines by drought treatment was comparable in both lines at the flowering stage. This result was consistent for the field and the greenhouse grown plants (Table 1 and 2) where the plant samples were harvested at about the same age and at roughly comparable soil moisture in the 30-40 cm depth. The deeply penetrating roots as a potential dehydration-avoidance mechanism in tepary bean (Thomas and Waines, 1982; Markhart, 1985; Mohamed, 2000) did not represent differentiating factor between the two lines in this study. Leaf area (Table 1), root growth pattern (Fig. 1) and soil moisture content around the roots (Fig. 1), however, apparently varied in these lines. Line NE#19 reduced leaf area and allocated greater dry mass into the root than NE#5, especially in the deepest soil profile (40-50 cm). Consequently, this line had higher R/S ratio and decreased portion of the transpiration surface (leaf) to the absorption mass (root). Therefore, NE#19 appeared to use less water (Fig. 1).

Literature suggests that rapid stomata closure, in response to water stress, reduces transpiration more than photosynthesis in soybeans [*Glycine max* (L.) Merr.] (Bunce, 1977). Here, the increased stomatal resistance (Fig. 2 F), and consequently decreased transpiration rate (Fig. 2 G), was accompanied by no decrease in net photosynthesis (Fig. 2 D). As a result, an elevated water-use-efficiency (Fig. 2H) was found to substantiate the observed reduced water consumption by line NE#19 in the greenhouse (Fig. 1) and in the growth chamber (Fig. 2A and C). The morphological and physiological dehydration-avoidance responses, especially stomatal resistance are stimulated with recognisable transcription signals of partially drying roots during slow progressive water deficit in the soil (Loveys, 1984; Loveys *et al.*, 2000). It is suggested that dehydration-avoidance mechanism of line NE#19 operated most effectively later during the drought period after substantial water depletion occurred in the top 30 cm of the soil profile. Thus, the higher soil water contents around the root of line NE#19 was found in the deep points of the soil profile (>30 cm).

The potential of the dehydration-avoidance mechanisms possessed by NE#19 was most apparent during pod development and seed filling stages (Table 1), where neccessity for water availability crucially increases (Kimball and Idos, 1983). Noticeably, stressed NE#19 sustained normal seed filling of its genotype, producing seed size comparable to the well-watered plants. The actual seed yield of NE#19 under drought conditions in the field was 1.6 times that of NE#5. From the biological point of view, both NE#5 and NE#19 are considered of similar resistance level to drought since they produced almost the same proportion of their potential seed yield under drought conditions. However, line NE#19 is agronomically preferred when considering the unaffected seed filling and the actual harvested amount of seed yield.

It is noteworthy, that the severely dehydrated leaves of NE#19 maintained high levels of tocopherol after rehydration indicating existence of greater oxidative stress (Schmitz-Eiberger and Noga, 2001) than the respective control, and about 40 times the level of rehydrated NE#5 (Fig. 4A). These leaves also had more MDA (Fig. 4B), indicating oxidative degradation of lipids and presence of high level of poly-unsaturated fatty acids in the membrane lipids (Carpentier, 1999). Complete recovery was not realised for *Chl* contents and fluorescence parameters of NE#19 (Fig. 4 H, I and J), especially F_0 as a well-established characteristic of chloroplast reaction to stress condition (Havaux *et al.*, 1988) that indicated a damaged photosynthetic system. Therefore, triggered non-enzymatic antioxidants (TPH, AA and *Car)* in NE#19 may be effective against oxidative stress in the leaves during the

progressive water deficit to a level of dehydration, but certainly not when a severe water dehydration is reached.

Contrary to NE#19, the enzymatic type of antioxidants based on peroxidase activity was the predominant in NE#5 during the oxidative stress period. Severely, dehydrated leaves of NE#5 after rehydration, showed complete recovery for *Chl* content (Fig. 4E and F) and the photosynthetic efficiency implied by the F_V/F_M parameter of *Chl a* fluorescence (Fig. 4H). There was no change in MDA (oxidative degradation of lipids) (Fig. 4B) and no alteration in the F_0. The tolerance of NE#5 to severe water deficit could be attributed to a prominent antioxidative activity of peroxidase during the severe dehydration period. This is new information over previous reports (Markhart, 1985; Mohamed *et al.*, 2002), suggesting that cellular biochemical reactions may constitute an important mechanism for the drought tolerance of tepary bean.

According to the water depletion treatments imposed in the present field study, the stressed plants were irrigated after emergence and then at flowering/early pod-set. It is suggested that stressed NE#5 would not suffer a reduction in seed size if watered once more during the critical phase of active seed filling. Line NE#19 could be produced under limited water supply when grown in soils of a deep profiles. Introducing tolerance into this potential dehydration-avoidance line would sustain its yield under broad drought stress conditions. Diversity of mechanisms shown in NE#5 and NE#19 may be of further usefulness in enhancing drought resistance of the relative species *P. vulgaris* L. (common bean) *via* interspecific breeding.

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