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YIELD, PHYSIOLOGICAL AND BIOCHEMICAL ASSESSMENTS OF DROUGHT RESPONSES IN SOME TEPARY BEAN LINES (*PHASEOLUS ACUTIFOLIUS*)

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ABSTRACT :

Among four lines of tepary bean (NE#5, NE#7, NE#8A and NE#19), comparatively, two produced high seed yield under drought conditions in the field. The high yielding lines (NE#8A and NE#19) exhibited potential dehydration-avoidance mechanisms based on physiological and morpho-physiological alterations of the root and leaves. NE#19 developed deep penetrating root that had the greatest mass in the deepest soil profile. This line is characterized by a balanced root: shoot growth pattern. Its leaves exhibited sensitive stomates in response to progressive drought. Except the less depth of the root penetration, NE#8A was closely similar to NE#19. However, it appeared to use less soil water than NE#19 suggesting earlier operation of its sensitive stomates. While NE#5 (low yielding line) exhibited less efficient dehydration-avoidance ability, it seemed having a more potential tolerance to withstand severe water dehydration. Primarily the dehydration tolerance of NE#5 to keep cellular functional integrity could be attributed to a prominent antioxidative role of peroxidase activity during the severe dehydration period. It is concluded that NE#8A and NE#19 may be useful in rationalizing the water use via prolonged irrigation frequency. Opportunity exists for bean breeders to employ diversity among lines in dehydration avoidance and tolerance towards enhancing drought resistance in *Phaseolus.* through intra- and interspecific breeding.

Abbreviations:

AA= ascorbic acid content, Chl a = chlorophyll a content, Chl b = chlorophyll b content, E = transpiration; F_0 = minimal fluorescence of dark-adapted leaves; F_m = maximal fluorescence of dark-adapted leaves; F_v/F_m = optimal quantum yield of* photosystem II (*PSII); g_s = stomata conductance; LA/R = leaf area / root dry mass; malondialdehyde (MDA) release, peroxidase (POD) activity, R/S = root-to-shoot ratio; tocopherols (TPH), RWC = leaf relative water content.

INTRODUCTION:

Tepary bean (*Phaseolus acutifolius*) is a pulse legume crop and one of nine economically important species of the genus *Phaseolus* (Allavena, 1984). It is a potential legume for human diet (Idouraine *et al.*, 1995; Miklas *et al.*, 1994), which is well adapted to arid environments (Miklas *et al.*, 1994). The seeds of tepary bean have similar composition as other legumes (Idouraine *et al.*,1995) while containing less proanthocyanidin (antinutritional factor) than common bean (*P. vulgaris* L.) (Miklas *et al.*, 1994). It is grown in the arid and semiarid areas of the southwestern USA, Central America, Mexico and Africa (Federici *et al.*, 1990). This *Phaseolus* species, as being an adapted to heat and drought and having potential for human diet, has been nominated for production in the arid regions of poor farming inputs in the developing world where its relative common bean (*P. vulgaris* L.) yields poorly if at all (Mohamed, 1996 and 2000; Morci and El-Murraba, 1960). Tepary bean, in addition, could be used to enhance stress resistance of common bean through interspecific breeding (McElory, 1985; Mohamed, 1990 and 1996). Therefore, there is a renewed interest among bean researchers in tepary bean (Federici *et al.*, 1990; Mohamed, 1991; Silbernagel, 1986).

Responses of tepary bean to drought were studied either focussing on its performance in yield trials (Federici *et al.*, 1990; Mohamed, 1996; Simon, 1993) or on mechanisms underlying its ability to postpone dehydration (Markhart, 1985; Yu and Berg, 1994). Identification of dehydration-avoidance mechanisms was assessed using one or few tepary bean lines and some of the studies focused only on few characteristics of the shoot or root genotype. Transfer of specific traits that are related to tepary potential to avoid dehydration into common bean genome has been suggested to enhance its drought resistance (Markhart, 1985; Mohamed, 2000). However, information is needed on the responses of the physiological and morpho-physiological traits related to dehydration-avoidance of tepary bean lines in connection with their seed yield under drought stress conditions. Different lines may have different combinations of dehydration postponing potentials (Hassan, 1995). When incorporating different potentials in a line, more durable and stable drought resistant may be obtained.

In spite of the potential for drought resistance of tepary, dehydration-avoidance type of mechanisms may not be effective in the plants especially at early developmental stages to withstand severe water deficit. Progressive elevated water-deficit could result in severe dehydration in the plant tissues. Such condition increases the free radicals formation of oxygen (Navari-Izzo and Rascio, 1999). This is because of the transit of the energized electrons from the photosynthetic process to oxygen under the conditions of limited water availability and the reduced utilization of light energy for carbon fixation. Subsequent mediated reactions lead to the degradation of the cellular and the membrane components and the loss of the membrane functions. Enzymatic and non-enzymatic antioxidants play a key role in the scavenging of the activated species such as superoxide and hydrogen peroxide (Sgherri *et al.*, 2000). Antioxidants, therefore, can provide the plants with tolerance to cope with severe water deficit that induces oxidative stress. However, information is lacking on such biochemical reactions in tepary. Understanding the physiological mechanisms of dehydration avoidance along with biochemical reaction-based tolerance would enable developing the

most precise procedure for screening potential germplasm. This also would assist to plan the most efficient strategy for interspecific breeding to improve drought resistance of common bean. The objective of the present study, therefore, was to asses seed yield, the physiological, morpho-physiological and biochemical responses of four tepary bean lines studied at different developmental stages to progressive water deficit.

MATERIALS AND METHODS:

A-Study of yield and dehydration postponing potentials:

1-Field trial:

Four tepary bean (*Phaseolus acutifolius)* lines (NE#5, NE#7, NE#8A and NE#19) were used in the present study. These lines were obtained originally from the Department of Horticulture, University of Nebraska (NE), USA. They were evaluated for seed yield in a field experiment conducted for two years under typical semiarid conditions in southern Egypt (the Research Station of the Faculty of Agriculture, Assiut University). The experiment was arranged as split-plots in a randomized complete-block design (RCBD). The whole plots contained drought and control treatments while the four lines were in the sub-plots. The whole plots of the drought treatment were surrounded by belts of 2 m wide ridges. Each sub-plot consisted of four rows (8.4 m^2). Seeds were planted at 25 cm on the northern side of 0.7 m wide and 3m long rows. All plants in this experiment were irrigated 3-4 days after emergence. Thereafter, the control plants were irrigated when 58% of the available soil water was depleted (Mohamed, 1996). The plants in the drought treatment were irrigated when 72% of the available soil water was depleted. The soil moisture was determined gravime-trically after drying to constant weight at 105°C. Soil samples were taken at 30-40 cm depth every 2-3 days. The average day temperature during the growing season (mid-May to mid-Sept.) ranged from 30-35°C. Data were recorded on shoot dry weight for the flowering plants (5-6 weeks after planting, using plant of one of the 4 rows) and the seed yield at the end of the growing season. Shoot dry mass was determined after drying at 80°C to constant weight.

2-Greenhouse experiment:

The four tepary lines were used in a morpho-physiological assessment of root and shoot traits related to the dehydration avoidance. Fifty-centimeter deep cuboid acrylic 3.3 liter containers were used. The container had a removable clear window on one of its 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 minimize 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 in a temperature-controlled greenhouse supplemented with artificial light providing, on average, 200 μmol m^{-2} s^{-1} PAR on the top of the plants. The average day/night temperature was 20-22/14-16°C and the relative humidity ranged between 55 to 65%. Seedlings were alternately watered with tap water and Hoagland nutrient solution every other day until the first trifoliate was fully expanded (about 15 days after seed planting). Subseq-uently, the containers were watered with the nutrient solution to the drip point. Thereafter, the water was withheld for 21 days. The experiment was randomized complete-blocks

(RCB). The half number (6 plants) was harvested before the water withholding and the other at the end of the water stress period.

The depth of the deepest root was observed through the clear windows of the containers. Then, the windows were removed and the roots were divided and harvested from each separate 10 cm of the soil profile. Also, soil samples were taken from each 10 cm of the profile. Subsequently, shoots were harvested and separated into leaves and stems. Leaf area was measured nondestructively 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) of the leaves was determined as follows: RWC =(Fw-Dw/Tw-Dw)x100. Leaves, stems and root sections were dried to a constant weight at 80 °C. The moisture content of the soil samples was determined gravimetrically. Both the soil samples and plant organs were allowed to cool down for 2to3 h before determining the dry weight.

3- Growth chamber experiment:

In this experiment, physiological alterations due to leaf genotype in response to drought were assessed. To minimize the differences in the water status of the leaves that could be affected by the variable root growth and penetration, seeds were planted in a limited root medium using small pots with a diameter of 5 cm and a height of 7 cm. The pots were filled with 0.1 liter of a dry peat moss/sand mixture (1:1, v/v). One seed was sown per pot. The seedlings were grown at 16/8h light/dark period under 400 µmol m^{-2} s^{-1} PAR on the surface of the unifoliate leaves in a controlled environment chamber. The day/night temperature was 22± 2°C/15±2°C and the relative humidity was about 60%. The experiment was split-plot in a RCBD with six replicates. Two water treatments (well-watered and water-stressed) were in the main plots. The sub-plots contained the four tepary lines. The pots of the water-stressed seedlings were covered with a clear plastic sheet on the surface of the soil around the seedling basal stems during the time period of water withholding. This was to reduce the water evaporation and thus allowing slow prog-ressive water depletion.

After complete emergence (about 6 days after seed planting) all pots were watered with Hoagland nutrient solution to the drip point. Subsequently, water was withheld from one set of plants. The other set was watered when needed. Four to six plants were sampled at different (0-4) days after starting the water stress treatment. Chlorophyll fluorescence parameters F_v/F_m, F_0 and F_m, transpiration rate (*E*) and stomata conductance (g_s) were determined. Chlorophyll fluorescence of the leaves was determined *in vivo* with a portable pulse amplitude modulation fluorometer (PAM 2000, Walz GmbH, Effeltrich, Germany) after dark adaptation for 30 min. Transpiration rate and stomatal conductance were also measured *in vivo* on the same leaves used for chlorophyll fluorescence. These gas exchange characteristics were obtained using a CIRAS-1 portable infrared gas analyzer connected to a broad Parkinson leaf chamber (PP System, Hitchin, Herts., England). Leaf area, relative water content (RWC) of the unifoliate, and the moisture content of the root medium were determined as indicated in the greenhouse experiment.

B-Study of biochemical potentials against severe dehydration :

Based on the yield and physiological study mentioned above, two most contrasting lines (NE#5 and NE#19) were selected. Seeds planting and seedling maintenance conditions were similar to that described above in the controlled environment chamber experiment. The following treatments were arranged in a randomized complete-blocks with four replicates: 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. These treatments were applied to 9-day-old seedlings after being watered to the drip point with the modified Hoagland nutrient solution. Severe water depletion treatment was subjected to 9 d of water withholding. Relative to the container capacity (water content few hours after watering to the drip point), the water depletion after the 9 d of water withholding is presented in Figure 5A. The pots of the water-stressed seedlings were covered with a clear plastic sheet on the surface of the soil around the seedling stems to allow slow progressive water depletion. The water-stressed seedlings were sampled 9 d after water withholding along with well-watered seedlings. The rewatered ones were harvested 24 h after the rewatering along with another set of the well-watered seedlings that to control the differences due to a possible age influence.

Chlorophyll *a* fluorescence parameters (F_0, F_m and F_v/F_m) were determined *in vivo*. Leaf relative water-content (RWC) and soil moisture were also determined. Leaf disks were prepared for the assays of chlorophyll (chl) *a* and *b*, carotenoid contents, tocopherols (TPH), malondialdehyde (MDA) release, peroxidase (POD) activity and ascorbic acid (AA) content. Chlorophyll *a* and *b* and carotenoid 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 was measured colorimetrically using the method of Heath and Packer (1968). The tocopherol was determined by HPLC-method.

For determining the ascorbic acid (AA) content, leaves were homogenized 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 (Anonymous 1995). 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).

C- Statistical 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. For

convenient presentation in the experiments that included the four tepary bean, NE#19 was considered a reference line as it was recommended for pulse legume production based on field trials in Assiut (Mohamed 1996 and 2000). Therefore, the data of this line was presented in Table (1) to indicate the effect of drought stress on the different studied parameters. The differences in NE#19 between the means for 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 (the greenhouse experiment) were idicated at 0.05 probability level. Data of the other three tepary lines were presented in form of ratios relative to NE#19 under control (C), drought stress (DS), and the difference between DS and C. The significance of the deviations for the relative means of the three lines from the unity (NE#19, ratio=1) was tested using Dunnett's Test at 0.05 probability level. On the other hand, data of the biochemical study were presented for measured values since it was conducted with only two selected lines. As devised by the coefficients of variation (C.V.) of the original data, the ANOVA for POD, AA, MDA and TPH assays was based on the square root transformed values. Means were compared using the "Least Significance Difference" ($LSD_{0.05}$) or Dunnett's Test at 0.05 probability level. For convenience, as the differences between the first well-watered sample (harvested along with the water-stressed seedlings) and the second well-watered sample (harvested along with the rewatered seedlings) were not significant, their average was presented as a one well-watered (control) treatment.

RESULTS:

Study of yield and dehydration postponing potentials

The relative dry weight of shoot from NE#5, NE#7 and NE#8A at the flowering stage under well irrigated conditions in the field did not differ form those of NE#19 (Fig. 1A). However, these lines varied in the seed yield. While NE#8A was closely similar to NE19 (reference line) in the potential seed yield, both of the NE#5 and NE#7 were significantly lower (40%) relative to NE#19. Under DS conditions, the shoot dry weight of NE#19 decreased significantly (46%) as compared with the control plants (Table 1A). The reduction in the seed yield was 42% in this line. Except the greater shoot dry weight in NE#8A relative to NE#19, the figure under the drought stress conditions (Fig. 1B) was the same as under well-watering conditions (Fig. 1A). The relative changes (Fig. 1C), however, indicated that the reduction in the shoot dry weight of all lines was relatively less than in NE#19. The lines NE#5 and NE#7 had a similar reduction to NE#19 in the potential seed yield. Nevertheless, NE#8A showed significantly less relative reduction of its potential seed yield.

Relative to NE#19 (Table 1B), there were no detectable differences in all the studied parameters of the three TP lines (Fig. 2A) in the greenhouse before the water withholding, except the high R/S ratio of NE#5. Three weeks after the water withholding (Fig. 2B), NE#7 showed significant decrease in the plant dry mass comparative to NE#19. This line showed also a reduction in the stem and the root dry masses, low R/S ratio and high values of the leaf surface area to the root dry mass (LA/R). NE#5 was similar to NE#19 in plant and shoot dry masses. However, it produced significantly less root dry mass and larger leaves. Therefore, its

R/S ratio appeared low while the LA/R value was high. NE#8A developed large leaves but also allocated great dry mass into the root. In contrast to NE#5 and NE#7, its R/S and LA/R were not, therefore, significantly different from NE#19.

Table (1): Effect of drought stress on the tepary bean line NE#19 grown in the field (A), in the greenhouse (B) and in a controlled environment chamber (C)[1].

(A) In the field[2]					
Drought treatment	SDM (g/plant)			TSY (kg/feddan)	
Non-stressed (C)	72.9			984.4	
Drought stressed (DS)	39.0			572.6	
Difference	*[3]			*	
(B) In the greenhouse[4]					
Drought treatment	PDM (g/plant)	SDM (g/plant)	STDM (g/plant)	LDM (g/plant)	LA (cm^2)
Before stress (C)	0.929	0.309	0.146	0.163	28.8
After Drought stress (DS)	4.384	0.820	0.415	0.405	86.2
Changes	3.455*	0.511*	0.269*	0.242*	57.4*
Drought treatment	RDM (g/plant)	R/S (Ratio)	LA/R	RD (cm)	RWC (%)
Before stress (C)	0.620	2.0	46.5	17.5	92.6
After Drought stress (DS)	3.580	4.4	24.1	45.7	85.4
Changes	2.960*	2.2*	-22.4*	28.2*	-7.2*
(C) In a controlled environment chamber[5]					
Drought treatment	LA (cm^2)	RWC (%)	E (mmol $H_2O.m^{-2}.s^{-1}$)	g_s (mmol $H_2O.m^{-2}.s^{-1}$)	
Non-stressed (C)	10.7	92.2	2.4	181	
Drought stressed (DS)	8.2	82.8	1.8	128	
Difference	*	*	*	*	

[1] SDM= shoot dry mass; TSY = total seed yield; PDM = plant dry mass; STDM = stem dry mass; LDM = leaf dry mass; RDM = root dry mass; R/S = root-to-shoot ratio; LA/R = the ratio of the leaf area to the root mass; RD= root depth; LA = leaf area; RWC = relative water content of the leaf; *E* = transpiration rate; g_s = stomatal conductance.

[2] Non-stressed and drought-stressed plants were irrigated when 58% and 72% of the available soil water was depleted, respectively.

[3] Significant at 0.05 level of probability.

[4] Plants were watered when needed for 2 weeks and then the water was withheld for 3 weeks.

[5] Nine-day-old seedlings either grown under well-watered conditions or the water was withheld for 4 days.

In spite of its root elongation (Fig. 2B), NE#5 had smaller root mass than NE# 19, especially, in the deepest levels (> 40) of the soil profile (Fig. 3A). NE#7 and NE#8A showed less root elongation in response to the water deficit (Fig. 2C) and developed shorter roots than NE#19 (Fig. 2B). The tepary NE#7 had less root mass in all depths of the soil profile than NE#19, except in the top 10 cm. However, the soil moisture around the roots of NE#5 and NE#7 was similar to NE#19 (Fig. 3B). These lines, therefore, used about the similar amount of the water from the top 40 cm of the soil profile. NE#8A developed great root mass in the top 10 cm and in the depth of 20-30 cm but apparently small root mass in the depth of 30-40 cm. Nevertheless, the soil moisture content was higher than NE#19 in the levels of the soil profile deeper than 20 cm. It seemed, therefore, to use less water than NE#19. In spite of lack of differences in RWC relative to NE#19 under DS conditions, RWC significantly decreased for the three lines due to the effected of the water deficit (Fig.2C). Distinctly, NE#7 had higher

RWC reduction than NE#5 and NE#8A ($P < 0.05$).

Seedlings of all tepary lines were similar for all the studied parameters in the growth chamber before starting of the water withholding (day 0, data not shown). Four days after water withholding, RWC of NE#19 (reference line) significantly reduced (10%) in comparison with the well-watered (control) seedlings (Table 1C). Also, the leaf area of this line significantly reduced (23%) and the changes in both the stomata conductance (g_s) and the transpiration rate (E) were significant in response of NE#19 to the water stress. The moisture content in the root medium 4 days after the water withholding as a percent of the control medium was, on average, 27 ± 0.7. There were no differences in the moisture content in the root medium for the different lines (data not shown). Relative to NE#19, also there were no detectable differences in the RWC of the leaves of all lines both under well-watered (C) (Fig.4A) and drought stress (DS) (Fig. 4B) conditions.

NE#5 and NE#7 developed larger leaf area under the control conditions. However, their leaves had similar area relative to NE#19 after 4 days of withholding the water. Contrary to NE#5 and NE#7, the leaves of NE#8A was similar in size to NE#19 in the control seedlings but significantly larger in the drought stressed seedlings. As indicated in Figure (4C), the leaf expansion of NE#5 and NE#7 was reduced in response to the water deficit more than NE#19. Therefore, in spite of their larger leaves than NE#19 under the well watering conditions, they appeared smaller when the water was withheld. Such relative changes in NE#7 was less ($P < 0.05$) than in NE#5. On the other hand, the relative changes in the leaf expansion for NE#8A due to the water deficit were significantly less than NE#19. Thus, the leaves of NE#8A appeared significantly larger than NE#19 under water deficit conditions.

The stomata conductance (g_s) and the rate of transpiration (E) in NE#5 were similar to NE#19 in the control seedlings (Fig. 4A). In contrast, high stomata conductance was shown by NE#7 and NE#8A and high rate of transpiration was detected in NE#8A. All the three studied lines were high in the leaf conductance and the rate of transpiration relative to NE#19 under drought stress conditions. As indicated in Fig. (4C) for NE#5 and NE#7, this was primarily due to the low changes for these parameters in response to drought conditions. However, NE#8A showed similar reduction in the stomata conductance and the rate of transpiration to NE#19. These parameters in NE#8A under drought conditions, therefore, mainly were affected by their high initial values under control conditions. The differences in RWC between drought stressed and control leaves were higher for NE#8A and lower for NE#5 and NE#7 than the reference line NE#19. There were no detectable changes of the chlorophyll fluorescence parameters F_v/F_m, F_0 and F_m (data not shown).

Study of biochemical potentials against severe dehydration

In NE#5, 82% of the water content of the container capacity was depleted while this was 73% for NE#19 (Fig. 5A). In contrast to the respective well-watered (control) seedlings (Fig. 5B), 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 wilt symptoms. After rewatering, the RWC increased to a level comparable to the control

seedlings. Except for the lower content of malondialdehyde (MDA) release (Fig. 6B) in NE#5, the leaves of the well-watered seedling of both tepary lines were similar in tocopherols (TPH), ascorbic acid (AA) content and peroxidase (POD) (Fig. 6A, C & D). Under the severe dehydration conditions, the TPH raised in both lines (Fig 6A). However, NE#5 had a lower amount of TPH than NE#19. After rewatering, the TPH content declined in both lines. The leaves of the rewatered seedlings of NE#5 contained a lower amount than the well watered ones. In contrast, the rewatered seedlings of NE#19 had a high TPH content compared with the respective control. Malondialdehyde (MDA) release did not show significant differences between the control and severe water dehydration treatments of both lines (Fig. 6B). Thus the MDA contents remained lower in NE#5 than NE#19. After rewatering, the MDA content increased in NE#19 while no significant change occurred in NE#5 compared with the respective control seedlings of each line.

The AA content, relative to the respective control seedlings, greatly decreased under the severe dehydration condition in both lines (Fig. 6C). However, the leaves of NE#19 contained significantly more AA under severe dehydration condition. The rehydrated leaves in contrast to those from well-watered (control) seedlings of line NE#19 showed an increased AA content. Although the content of AA after rewatering in line NE#5 elevated, it did not reach the control level. The rewatered seedlings of NE#19 distinctively, therefore, had a higher AA content than NE#5. POD activity significantly increased under the conditions of the severe dehydration in the seedlings of NE#5 while it remained unchanged in NE#19 (Fig. 6D). The former line, therefore, was higher in the POD activity than the latter. When the seedlings were rewatered, the activity of POD decreased to the control level in NE#5 while unchanged in NE#19.

No differences in the Chl *a*, Chl *b*, and carotenoid contents in the leaves of the control seedlings were detected between NE#5 and NE#19 (Fig. 7A, B and C). The severely dehydrated leaves of both lines exhibited significant decreases in Chl *a*, Chl *b*, and carotenoids compared to their respective control seedlings. In NE#5, Chl *a* and *b* elevated after rewatering reaching the level of the contents in the leaves of control seedlings. However, the decreases of Chl *a* and *b* in NE#19 remained after the leaf rehydration. The carotenoid contents in the severely dehydrated leaves of both lines, on the other hand, remained low after rewatering. There were no differences detected between the two tepary lines in the contents of Chl *a* and carotenoids in the severely dehydrated leaves. However, a higher amount of Chl *b* was found in the leaves of the severely dehydrated seedlings of NE#5 than NE#19. 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.7D). While it increased to the well-watered (control) level in NE#5 after rewatering, its value remained at a lower level in NE#19. F_0 showed no alteration due to severe dehydration or following rewatering in NE#5 (Fig. 7E). On the contrary, F_0 greatly increased in the severely dehydrated seedlings of NE#19. After rewatering, its value remained higher than the control seedlings of this line. The parameter F_m exhibited significant decrease in the severely dehydrated seedlings of both lines and returned to similar level as in the control seedlings after rewatering only in NE#5 (Fig. 7F).

Fig. (1): Shoot dry mass (SDM) and the total seed yield (TSY) of three tepary bean lines grown in the field either under well-irrigated condition (A) or subjected to drought (B) and the difference (C) between (A) and (B). Stars denote significant deviation from NE#19 (the cross line at the ratio 1) using Dunnett's test at 0.05 probability level.

Fig.(2):The leaf relative water content (RWC), the dry mass of the plant (PDM), shoots (SDM), stem (STDM), leaves (LDM) and root (RDM), the root-to-shoot ratio (R:S), the root depth in the soil profile (RD), the leaf area (LA) and the ratio of the leaf area to the root mass (LA/R) of three tepary bean lines grown in 50 cm deep containers in the greenhouse before (A) and after (B) 3 weeks of water withholding. In (C) the differences between (A) and (B) are shown. Stars denote significant deviation from NE#19 (the cross line at the ratio 1) using Dunnett's test at 0.05 probability level.

Fig. (3): Dry mass of the roots (A) and the soil moisture content (B) at different depths in the soil profile for three tepary bean lines grown in the greenhouse following 3 weeks of water withholding. Stars denote significant differences at the same depth of the soil relative to NE#19 (frame at the ratio 1, arrow) using Dannet's test (P < 0.05).

Fig. (4): The leaf relative water content (RWC), leaf area (LA), transpiration rate (*E*) and stomata conductance (g_s) of three tepary bean lines grown under 400 µmol.m^{-2}.s^{-1} PAR in a controlled environment chamber: A) non-stressed (control), B) four days after withholding the water and C) the difference between A and B. Stars denote significant deviation from NE#19 (reference line; the cross line at the ratio 1) using Dunnett's test at 0.05 probability level.

Fig. (5):Tepary bean lines (NE#5 and NE#19) subjected to severe dehydration and rewatering. A) the depleted water from the root growing medium of the seedling 9 days (severe water depletion) after water withholding relative to the water content few hours after watering to the drip point (container capacity), B) leaf relative water content (RWC) in the well-watered (WW) severely dehydrated (SD) and rewatered (RW) seedlings. Stars donate significant differences at 0.05 level of the probability between NE#5 and NE#19 when received the same water treatment.

Fig. (6):Tocopherols (TPH), malondialdehyde (MDA) release, ascorbic acid (AA) content and peroxidase (POD) activity in two tepary bean lines (NE#5 and NE#19) subjected to severe dehydration and rewatering.. A) to D) the determined values of these parameters for control (well-watered, WW), severely dehydrated (SD) and rewatered (RW) seedlings. Stars donate significant water treatment mean deviation within tepary bean line from the respective control at 0.05 level of the probability using Dunnett's test. The vertical arrows on the 'x' axes refer to significant differences at 0.05 level of the probability between the two bean lines when received the same water treatment using the "Least Significant Test" (LSD).

Fig.(7):Chlorophyll *a* (A) and *b* (B) and carotenoid (C) contents of the leaves, and chlorophyll *a* fluorescence parameters (F_0, F_M and F_v/F_M) (D to F) of the attached leaves in two tepary bean lines (NE#5 and NE#19) under well-watered (WW, control), severely dehydrated (SD) and rewatered (RW) conditions. Stars donate significant water treatment mean deviation within tepary line from the control at 0.05 level of the probability using Dunnett's test. The vertical arrows on the 'x' axes refer to significant differences at 0.05 level of the probability between the two bean lines when received the same water treatment using the "Least Significant Test" (LSD).

DISCUSSION:

One of main dehydration-avoidance mechanisms of tepary bean is the stomata sensitivity (Markhart, 1985). As indicated in our study in a controlled environment chamber, the four tested tepary bean lines exhibited

comparatively differential leaf reactions in response to the water deficit in the limited root medium (Fig. 4C). NE#19 and NE#8A (high yielding lines) showed appreciable reduction in the stomata conductance. NE#19, in addition, reduced its leaf expansion (23%). NE#8A exhibited relatively less reduction in the leaf area. Relative to NE#19 (reference line), apparently NE#5 (low yielding line) had a great reduction in the leaf expansion but negligible change in stomata conductance. The leaf genotype of the other low yielding line (NE#7) responded to the water deficit in terms of reducing leaf expansion and increasing the stomata resistance. However, the reduced leaf expansion was relatively less than NE#5 and the stomata resistance was lower than NE#19. Considering the relative changes in the RWC, the reduction of the transpiration surface (leaf area) seemed to be more effective than increasing the stomata resistance under the conditions of growing plants in a limited root medium.

The importance of the deeply penetrating roots as being a potential dehydration-avoidance mechanism has been noticed by several researchers in tepary bean (Markhart, 1985; Mohamed, 2000; Thomas and Waines, 1982). NE#5 and NE#19 had comparable root depth in the greenhouse. However, the difference was in the mass in the soil profile deeper than 40 cm. While the deeply penetrating roots would explore water in deep soil, the greater mass in the deeper zones could enable extracting the water from greater volume of the soil. It is noticeable, here, that the mechanism of leaf reduction was not potentially useful in NE#5 and NE#7 to sustain a better water status comparable to NE#19. This is because of the slower and gradual decrease of the moisture in the 50cm deep containers (3.1 liter) used in the greenhouse than in 7 cm deep pots (0.1 liter) that were employed in the growth chamber. A substantial leaf expansion occurred in the former case before the root could develop a recognizable drought signal to the leaf for reducing its expansion (Loveys, 1984).

The two high yielding lines (NE#8A and NE#19) were different in the root depth (Fig. 2B) and the root growth pattern (Fig. 3A). However, they had similar values for root-to-shoot and the transpiration surface-to-the water absorption mass. In spite of their comparable growth, NE#8A seemed to use less water (Fig. 3B). It is suggested that the sensitive stomates in NE#8A were operating towards the end of the drought period in the greenhouse experiment. Although the root of NE#8A is not as deep penetrating as NE#19, its great mass could enable the extraction of the water from large volume of the soil in the field. In the view of the shoot growth, there was a good agreement between the greenhouse (Fig. 2C) and the field (Fig. 1C) experiments where the plant samples were harvested at about the same age and at roughly comparable soil moisture in the depth 30-40 cm of the soil profile. All lines could use the available soil water to develop shoot growth similar or greater than NE#19 under the field conditions. However, the potential seed yield of NE#8A and NE#19 (two high yielding lines) suffered only either less or similar depression (Fig. 1 C) comparing with the two low yielding lines (NE#5 and NE#7). Therefore, these lines remained higher in seed yield than the two low yielding lines under drought stress (Fig. 1B). This observation suggests that the water availability during the seed development was the crucial factor affected the potential seed yield depression (Kimball and Idos, 1983). In this context, NE#19 has the potential traits of the leaf and the root to face the increasing needs for the water during the seed development. Thus, it could sustain high seed yield under

drought conditions in the field. NE#8A showed even less seed yield depression than NE#19. Bunce (1977) suggested that rapid stomata closure of soybean [*Glycine max* (L.)) Merr.] in response to water stress reduces transpiration more than photosynthesis. Therefore, stomata sensitivity could improve the photosynthetic water use efficiency. Noteworthy, it has been well documented that increasing stomatal resistance is affected by regulating signal(s) from the partially drying roots (Loveys *et al.*, 2000). Root of NE#19, in contrast to root of NE#8A, may not develop such recognizable drought signal as it could elongate and deeply penetrate into soil profiles of high moisture contents in the field.

Under condition of severe dehydration in the leaves as it was in the present biochemical study, alterations in the structural integrity of photosystem could occur. Wasteful quenching processes of singlet excited chlorophyll may proceed at increased rates resulting in formation of $^1O_2$ and other oxygen species (Navari-Izzo and Rascio, 1999). Due to the interaction between $^1O_2$ and polyunsaturated lipids, lipid hydroperoxids may be formed and react in a subsequent step to generate free radicals and decomposing membrane lipids in a chain reaction. These chain-propagating lipid peroxyl and lipoxyl radicals in membranes are scavenged by α-tocopherol, resulting in its tocopheroxyl radical (Fryer 1992) which can be reduced back to its original form by reacting with ascorbic acid (Hess 1993; Packer *et al.* 1979). Therefore, α-tocopherol expression increases in response to oxidative stress (Schmitz-Eiberger and Noga, 2001) and getting smaller under excessive $^1O_2$ formation (Boo and Jung 1999).

Tocopherol level was greatly higher in NE#19 than NE#5 (Fig. 6A) under severe dehydration. This was associated with decreased level of ascorbic acid in both lines than the control but NE#19 had a higher level than NE#5. This may indicate occurrence of excessive oxidation process under sever dehydration. Following the rehydration of the severely dehydrated-leaves, however, ascorbic acid content elevated in NE#19 (Fig. 6C). Also tocopherol, although it decreased after rehydration, remained higher than the respective control and about 40 times the level of rehydrated NE#5 (Fig. 6A). Increase in MDA (Fig. 6B) occurred 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 realized in Chl assays and fluorescence parameters for NE#19 (Fig. 7 D to F) especially F_o as a well established characteristic of chloroplast reaction to stress condition (Havaux *et al.*, 1988) that indicated a damage occurred in the photosynthetic system in NE#19. It seemed that the triggered antioxidative system of the oxygen radical scavenging compounds (ascorbic acid and corotenoids) in the leaves of NE#19 may protective against oxidative stress during the progressive water deficit but only to a limited extent. Apparently these non-enzymatic oxygen radical scavengers did not seem to play an important role in this tepary bean line when a certain level of severe dehydration is reached.

The present biochemical assessment suggests that the core potential difference in the antioxidative reaction to severe dehydration and subsequent rehydration between the two tepary lines was in the peroxidase activity which increased in NE#5 while decreased in NE#19 during the stress period (Fig. 6D). Although leaf Chl degradation occurred in both lines, only NE#5 that showed recovery after rehydration of the severely dehydrated leaves (Fig. 7 A and B). Dilution of the Chl content during the stress

period could lead to a decrease in the absorbance and trapping of photons (Misra *et al.*, 2001). However, the Chl recovery after rehydration and lack of change in MDA (oxidative degradation of lipids) (Fig. 6B) may be controlled by the peroxidase activity during the stress period (Fig. 6D). Our study showed a good consistence between the photosynthetic efficiency implied by the F_v/F_m parameter of the Chl *a* fluorescence (Fig. 7D) and the alteration of the leaf Chl *a* content due to the different water treatments. In contrast to NE#19, on the other hand, a prominent antioxidative role of peroxidase is indicated in NE#5 under severe oxidative stress of dehydration. Obviously, the tolerance of NE#5 to keep its cellular functional integrity under severe dehydration could be attributed to an antioxidative activity of peroxidase during the stress period.

The sum up of our study suggests that the deep penetrating root with greater mass and the sensitive stomates can clearly differentiate between the low and the high yielding lines of tepary bean. Also, it has been shown that different high yielding lines may relay on different strategies of resistance to drought. In particular, increasing stomata resistance seemed to play an important role in NE#8A while developing great mass of deep penetrating root characterized NE#19. The present study, additionally, provides new information over pervious reports (Markhart, 1985; Mohamed *et al.*, 2002) indicating that cellular biochemical reactions may constitute an important mechanism for tolerance to cope with severe dehydration. In this context, it is suggested that peroxidase plays a prominent antioxidative role. Several application impacts could be found useful for the production of pulse beans based on the present study. NE#8A and NE#19, due to their potential dehydration-avoidance mechanism, could be used in rationalizing the water use via prolonged irrigation frequency. However, NE#19 is not expected to sustain if severe dehydration occurred. In contrast, NE#5 (low yielding) would be expected to withstand exposure to a severe dehydration. Bean breeders may take into their account this germplasm variability to broaden the drought resistance by considering both dehydration avoidance and tolerance mechanisms. A fast Chl *a* fluorescence based-markers may be used to screen drought tolerant lines. Combining avoidance and tolerance mechanisms would be useful in breeding new elite tepary lines and also in improving drought resistance of the important relative species *P. vulgaris* L. (common bean).

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تقيم محصولى وفسيولوجى وبيوكيميائى لاستجابات الجفاف فى بعض سلالات فاصوليا التبارى (فاصيولس اكيتيفولس)

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شملت هذه الدراسة عن الجفاف تجارب حقلية محصولية وتجارب فسيولوجية بالصوبات وحجرات النمو وتجارب أخرى لتقديرات بيوكيميائية لنباتات بحجرات النمو، وذلك لأربع سلالات من فاصوليا التبارى (ان اى ٥ ، ٧ ، ٨أ ، ١٩). وقد أظهرت الدراسة إنتاج أعلى من المحصول البذرى لكل من السلالتين "ان اى ٨أ ، ١٩ " تحت ظروف الجفاف. وأوضحت تجارب فسيولوجيا تحليل النمو وفسيولوجيا النتح بالصوبات وحجرات النمو أن السلاله "ان اى ١٩" المرتفعة فى المحصول قد تميزت بنمو جذرى متعمق ذو كتله كبيره فى أعماق التربه، كما كان هناك توازن بين النمو الجذرى والخضرى بما يساعد على زيادة امتصاص الماء مع تقليل الفاقد منه بالنتح، كذلك فقد ظهر أن هذه السلالة تمتلك خاصية التحكم فى عملية فتح و غلق الثغور بما يشكل دعم آخر لتقليل فقد الماء تحت ظروف الجفاف. وقد كانت السلاله "ان اى ٨أ" الأخرى المرتفعة المحصول مشابهة للسلالة " ان اى ١٩ " فيما عدا أن جذورها اقل تعمقا فى التربة.

وقد وجد فى تجارب لتقديرات البيوكيميائية الخاصة بمضادات الأكسدة أن السلالة "ان اى ٥ " الأقل محصولا بذريا ورغم أن ليس لها امكانيات تقليل وتجنب الجفاف السابق ذكرها للسلالتين "ان اى ٨أ ، ١٩ " فإنها كانت أكثر تحملا عند تعرضها للفقد الشديد فى المحتوى المائى لأنسجة الأوراق نظراً لإنتاجها للبيروكسيديز.

وهذه الدراسة تؤكد أهمية ميكانيكيات تجنب الجفاف فى تعضيد الإنتاج المحصولى للتبارى كنبات يعتمد على تأخير فقد الماء من الأنسجة كوسيلة أساسيه فى توائمه للنمو والإنتاج تحت ظروف الماء الشحيح فى المناطق الجافة. والدراسة تضيف معلومات عن فعالية ميكانيكيات التحمل للجفاف (بما يحفظ الخواص الوظائفية للخلايا خاصة تلك المتعلقة بالتمثيل الضوئى) القائم على إنتاج مضادات الأكسدة لمواجهة الانخفاض الشديد فى المحتوى المائى لأنسجة الأوراق والذى يمكن أن يحدث عند اشتداد الجفاف بالدرجة التى لا يمكن تلافيه بواسطة ميكانيكيات تجنب الجفاف.

والدراسة يمكن أن يكون لها عائد تطبيقى مباشر فى استزراع فاصوليا النبارى بالمناطق التى تعتمد على الأمطار الموسمية الشحيحة سواء للاستخدام الآدمى أو كمراعى لتنمية الثروة الحيوانية، كما يمكن أن تساهم لدراسة فى تحسين الفاصوليا العادية بتوفيرها معلومات للمربى عن أصول وراثيه وإمكاناتها المختلفة لمقاومة الجفاف.