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Antioxidative Reactions Inducing Tolerance to Severe Dehydration in Tepary Bean (*Phaseolus acutifolius*)

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Summary

Two tepary (TB) bean lines (NE#19 and NE#5) were subjected to progressive water depletion and rewatering (RW). Under a condition of mild water depletion (MWD), the leaf relative water content (RWC) was similar in both lines and did not significantly differ from the respective well-watered (WW) seedlings. Line NE#19 under MWD conditions significantly increased stomata resistance and reduced transpiration rate compared to WW seedlings. Also it showed tendency towards reduction in the intercellular CO_2 and elevated net photosynthesis. Its stem and root mass, specific leaf mass (SLM) increased while the portion of leaf area to 1 mg dry root mass (LA/RDM) decreased compared to the WW ones. NE#5 did not show such changes in gas exchange and plant growth parameters under MWD conditions when compared to the WW. Tocopherols (TPH) increased under MWD conditions but no changes occurred in malondialdehyde (MDA) and chlorophyll (Chl) content. Carotenoid and ascorbic acid (AA) contents in NE#19 and peroxidase (POD) activity in NE#5 increased. The rewatered (RW) seedlings showed a decline in TPH and MDA in both TB lines. AA decreased while carotenoid content remained high in NE#19. POD activity increased in NE#5. The fluorescence parameter F_v/F_m was higher under both MWD and RW than WW conditions for NE#5 but the change in NE#19 was not significant. When subjected to severe water depletion (SWD), the RWC significantly decreased and both TB lines suffered severe dehydration in spite of 11 % higher RWC in NE#19 than in NE#5. Compared to the respective WW, both lines had a significant sharp reduction in plant growth (leaf, stem and root) and SLM. However, NE#19 significantly exceeded the WW in LA/RDM and R/S ratio when subjected to SWD. TPH contents in severely dehydrated leaves were 2 (NE#5) to 3 (NE#19) folds the level in the WW ones. NE#5 showed an increase in POD activity but AA contents decreased. In contrast, POD activity in NE#19 did not elevate while AA significantly declined. Chl and carotenoid contents decreased in both lines. While fluorescence parameter F_v/F_m decreased in both TB lines, the F_0 showed stability only in NE#5. After the rehydration, NE#19 remained showing high level of TPH and MDA, a decrease in F_v/F_m and an increased F_0 value. This occurred in spite of the elevated AA content. Distinctively

Zusammenfassung

Einfluss einer defizitären Wasserversorgung auf das antioxidative Abwehrsystem von *Phaseolus acutifolius*. Zwei Tepary Bohnen-Linien (TB) (NE#19 and NE#5) wurden einem fortschreitenden Wassermangel ausgesetzt und danach wiederbewässert (RW). Unter moderatem Wassermangel (MWD) war der relative Wassergehalt (RWC) in beiden Linien ähnlich und unterschied sich nicht von den optimal bewässerten (WW) Pflanzen. In NE#19 stieg der stomatäre Widerstand unter moderatem Wassermangel (MWD) deutlich an, wobei die Transpirationsrate im Vergleich zu den optimal bewässerten Pflanzen reduziert war. Zusätzlich waren Tendenzen hinsichtlich einer Abnahme des interzellulären CO_2 und einer gesteigerten Nettophotosyntheserate zu erkennen. Stängel und Wurzelmasse, sowie die spezifische Blattmasse (SLM) stiegen an, während das Verhältnis der Blattfläche zu 1 mg Wurzeltrockenmasse (LA/RDM) im Vergleich zu den optimal bewässerten Pflanzen abnahm. NE#5 zeigte nicht solch starke Veränderungen in der Gasaustauschrate bzw. in den Wachstumsparametern unter moderatem Wassermangel. Der Tocopherolgehalt (TPH) stieg unter moderatem Wassermangel an, keine Unterschiede traten jedoch im Malondialdehyd- (MDA) bzw. im Chlorophyllgehalt (Chl) auf. Die Carotenoid- und Ascorbinsäuregehalte (AA) stiegen in der Linie NE#19 an, während die Peroxidase- (POD-) Aktivität in der Linie NE#5 stieg. Die wiederbewässerten Pflanzen (RW) zeigten eine Abnahme in TPH und MDA in beiden Linien. AA nahm ab, während der Carotinoidgehalt bei NE#19 auf einem hohen Niveau blieb. Die POD-Aktivität stieg in NE#5 an. Der Fluoreszenzparameter F_v/F_m war höher unter MWD und bei RW im Vergleich zu den optimal versorgten Pflanzen bei NE#5, jedoch waren die Veränderungen bei NE#19 nicht signifikant. Bei den Pflanzen unter starkem Wassermangel (SWD) nahm der RWC signifikant ab und beide Linien zeigten starke Dehydratationssymptome, wobei der RWC 11 % höher in NE#19 im Vergleich zu NE#5 war. Beide Linien zeigten eine signifikante Reduktion hinsichtlich der Wachstumsparameter und des SLM. NE#19 übertraf signifikant die WW hinsichtlich des LA/RDM- und des R/S-Verhältnisses unter SWD. Die TPH-Gehalte in stark dehydrierten Blättern lagen bis zu 2- (NE#5) oder dreimal höher (NE#19) im Vergleich zu den optimal bewässerten Pflanzen.

NE#5 showed a recovery after rehydration and it is regarded as a more tolerant to withstand severe water deficit than NE#19.

Key words. ascorbic acid – Chl a fluorescence – drought tolerance – germplasm – oxidative stress – peroxidase

NE#5 zeigte einen Anstieg der POD-Aktivität, während die AA-Gehalte abnahmen. Hingegen stieg die POD-Aktivität in NE#19 nicht an, während AA signifikant abnahm. Die Chl- und Carotenoidgehalte nahmen in beiden Linien ab. Während die Fluoreszenzparameter F_v /F_m in beiden TB-Linien sank, blieb F_0 in NE#5 stabil. Nach Wiederbewässerung, zeigte NE#19 hohe Gehalte in TPH und MDA, eine Abnahme im F_v /F_m und einen Anstieg des F_0-Wertes. NE#5 erholte sich stärker nach Wiederbewässerung und kann als die tolerantere Sorte gegenüber einem Wassermangel angesehen werden.

Introduction

Tepary bean (*Phaseolus acutifolius*) is a pulse legume crop and one of nine economically important species of the genus *Phaseolus* (ALLAVENA 1984). Its seeds have a 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 Arizona, New Mexico and Africa. Tepary bean, as being an adapted to heat and drought and having potential for human diet, has been nominated for production in the arid regions of the developing world (MOHAMED 1996 and 2000). The possible intercrossing of tepary and common beans has attracted an interest of bean breeders (MCELORY 1985; MOHAMED 1990 and 1991). Physiological investigations suggested that dehydration-avoidance, depending on the role of the root characteristics, are the main mechanism of the drought resistance of tepary bean (MARKHART 1985; MOHAMED et al. 2002). Seedling establishment is one of the important factors in crop production and dehydration avoidance mechanism may not be effective at early developmental stages to withstand unexpected severe dehydration stress. Therefore, it is important to understand the biochemical reactions of the tepary bean seedling when subjected to elevated severe water dehydration prior to assessing its utilization in interspecific breeding for drought resistance.

Elevated water deficit could increase the formation of free radicals 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 utility 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, there has been no report on the antioxidative reactions of this species to the severe water deficit. In this study, biochemical assays along with chlorophyll a fluorescence were used to assess antioxidative reactions of tepary bean seedlings grown under condition of progressive water deficit inducing severe dehydration.

Materials and Methods

Abbreviations

A: Net photosynthesis; Chl a: chlorophyll a content; Chl b: chlorophyll b content; F_0: initial Chl a fluorescence level; C_i: intercellular CO_2; E: transpiration rate; F_m: maximum Chl a fluorescence level; F_v /F_m variable: (the difference between F_m and F_0) to maximum Chl a fluorescence; g_s stomata conductance; RWC: leaf relative water content.

Plant material and growing conditions

Two tepary bean (*Phaseolus acutifolius* A. Gray) lines (NE#5 and NE#19) were used in the present study. Seeds for these tepary bean (TB) lines were surface sterilized using calcium hypochlorite. The sterilized seeds were planted in small, 100 ml, plastic pots with a diameter of 5 cm and a height of 7 cm filled with a mixture (1:1, v/v) of dry sand and peat moss. Before mixing, the sand was washed and both the clean sand and the peat moss were dried to a constant weight at 85° C in an electrical oven. Seed 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 24.0±0.5 °C/15.0±0.5 °C and the relative humidity was about 70 %.

Water stress treatments

After emergence (about 6 days post seed planting), uniform seedlings were selected from both TB lines. Nine-day-old seedlings were watered to the drip point with Hoagland nutrient solution. The seedlings of each line were arranged in two plastic trays. One tray was used for the treatment of a mild water depletion and the other for a 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. Relative to the container capacity (water content few

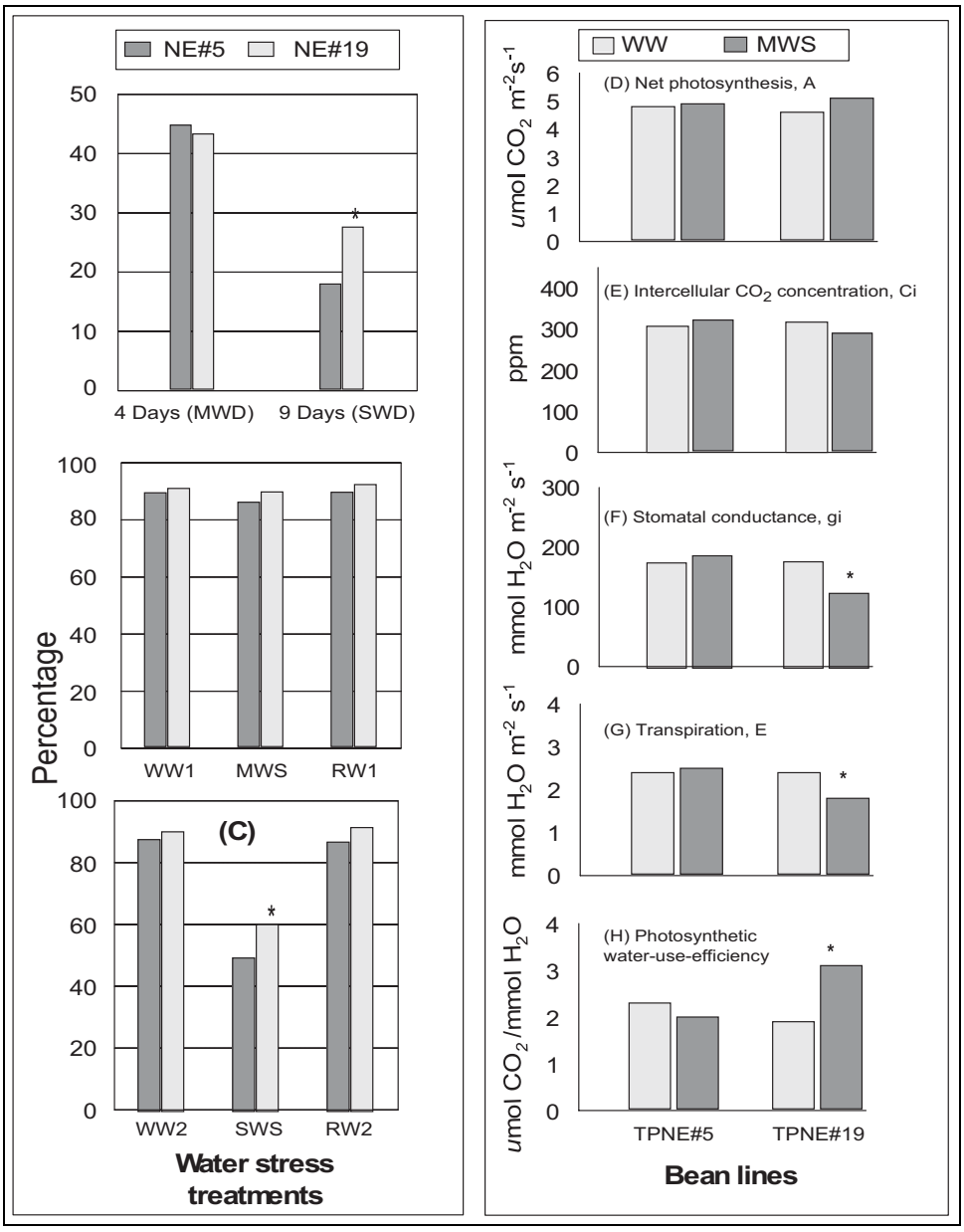


Fig. 1 Tepary bean lines (NE#5 and NE#19) subjected to water deficit conditions and rewatering. The presentation on the left side **(A)**: shows the depleted water from the root growing medium of the seedling after 4 days (mild water depletion, MWD) and 9 days (severe water depletion, SWD) of water withholding 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 (WW1), mildly water stressed (MWS) and rewatered (RW1) seedlings under MWD and **(C)**: the same as in **(B)** but under SWD conditions. Stars donate significant differences at 0.05 level of the probability between NE#5 and NE#19 when received the same water treatment. The presentation on the right side **(D** to **H)**: shows gas exchange parameters of seedlings from WW and MWS treatments of the two tepary bean lines. Stars donate significant deviation for mean of the mildly water stressed (MWS) treatment within each tepary line from the control (WW) at 0.05 level of the probability.

hours after watering to the drip point), the water depletion after 4 d and 9 d of water withholding is presented in Fig. 1A. Each tray contained four replicates of the following treatments arranged in a randomized complete-block experiment: 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 surface of the soil around the seedling stems during the time period of water withholding. This was to reduce the water evaporation and thus allowing slow progressive water depletion.

Measurements

The well watered and the water stressed seedlings were sampled 4 d 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* using CIRAS-1 portable infrared gas analyzer connected to broad Parkinson leaf chamber (PP System, Hitchin, Herts., England). 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.

Samples of the soil mixture were dried to a constant weight at 105 °C and their moisture contents were determined gravimetrically. Samples of roots, stems and leaves were also taken and dried at 70 °C to a constant weight. The soil, leaf, stem and root samples were allowed to cool down for 2 to 3 h before determining the dry weight. Relative water content of the leaves (RWC) was determined as $(W_f–W_d\ /W_t–W_d)$ x 100, where W_f is the fresh weight upon harvest, W_d is the dry weight after oven drying at 70 °C to a constant weight and W_t is the turgid weight obtained after overnight rehydration by keeping leaf disks at 5 °C in darkness on water-saturated filter papers in sealed petri dishes until constant weight.

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 as indicator of the structural integrity of the membranes was measured colorimetrically using the method of HEATH and PACKER (1968). Specific absorbance was determined at 532 nm and non-specific absorbance at 590 nm. In order to increase specificity, TBA-reactive compounds were further separated from interfering substances *via* RP-HPLC. Before injection, the sample pH was buffered with an equal volume of 0.5 M K-phosphate (pH 7.0). The method was calibrated with 1,1,3,3-tetraethoxypropane, which decomposes quantitatively to malondialdehyde during the TBA-reaction. The MDA content in calibration samples was analysed both, by spectrophotometry ($E_{532\ nm}$=155 mM^{-1} cm^{-1}) and HPLC analysis. HPLC conditions: Hypersil ODS column, 5 µm (250 mm*4 mm, 10 mm guard column); column temperature: 30 °C; mobile phase: A 5 % (v/v) acetic acid, B methanol; gradient program: 0–20 min from 30 % to 85 % B in A, 20–30 min 100 % B, injection volume 20 µl; flow rate: 1 ml min^{-1}; detection 532 nm; retention time of malondialdehyde: 3.2 min. Tocopherol was determined by an HPLC-method described by SCHMITZ-EIBERGER et al. (2002). Leaves were homogenized with a dismembrator and extracted with hexane/isopropanol. Extracts were chromatographed on a Lichrospher Si-60 column (5 µm, 4 mm*250 mm). The fluorescence signals of the tocopherols/tocotrienols were detected at 285 nm (excitation wave length) and 320 nm (emission wave length). ∝-,β-, γ- and δ-Tocopherols were characterized on the basis of the fluorescence emission of pure standards (Merck).

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). For preparing the leaf extract, freshly samples were weighed, immersed in liquid N_2 and ground to a fine powder in a buffer. Because some enzymes have membrane-bound isoenzyme forms, assays were performed with the crude leaf homogenates.

Data analysis and presentation

Combined analysis of variance (ANOVA) was conducted for the treatments in each water deficit condition (mild and severe water stress) over the tepary lines (GOMEZ and GOMEZ 1984). 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

Mild water-depletion and rewatering

The depleted water from the root growing-medium of the seedling that received mild water stress treatment relative to the container capacity was about 56 % (Fig. 1A). RWC did not significantly change in the leaves of the mildly water stressed seedlings of both tepary lines when compared to the well-watered (control) ones (Fig. 1B). However, seedlings of line NE#19 subjected to the mild water depletion exhibited increased stomata resistance (Fig. 1F) and consequently a reduced transpiration rate (Fig. 1G) compared to the control (well-watered) seedlings. Net photosynthesis in line NE#19 tended toward increasing (Fig. 1D) while the intercellular CO_2 tended to be lower (Fig. 1E) than the control. Significantly higher photosynthetic water-use-efficiency was found in the mildly water stressed seedlings of line NE#19 than in the well watered ones (Fig. 1H). There were no differences detected under well watering conditions in the measured and the derived growth parameters between NE#5 and NE#19 (Fig. 2A). Nevertheless, NE#19 exhibited significant increase in dry mass of stem and root and specific leaf mass while it reduced the leaf area portion to each mg of the root dry mass (Fig. 2B). Apparently NE#5 showed no appreciable changes in the all above-mentioned physiological and morpho-physiological characteristics.

Higher TPH was detected in the leaves of mildly water stressed seedlings than in those of the control treatment for both TP lines (Fig. 4A). After the rewatering process, the TPH declined to a level similar to its content in the leaves of the control seedlings. Both tepary lines did not differ in the TPH determined in the leaves of the control, mildly water stressed and rewatered seedlings. On the other hand, the amount of the MDA did not alter in the leaves of the mildly water stressed seedlings in both TP lines compared with the control

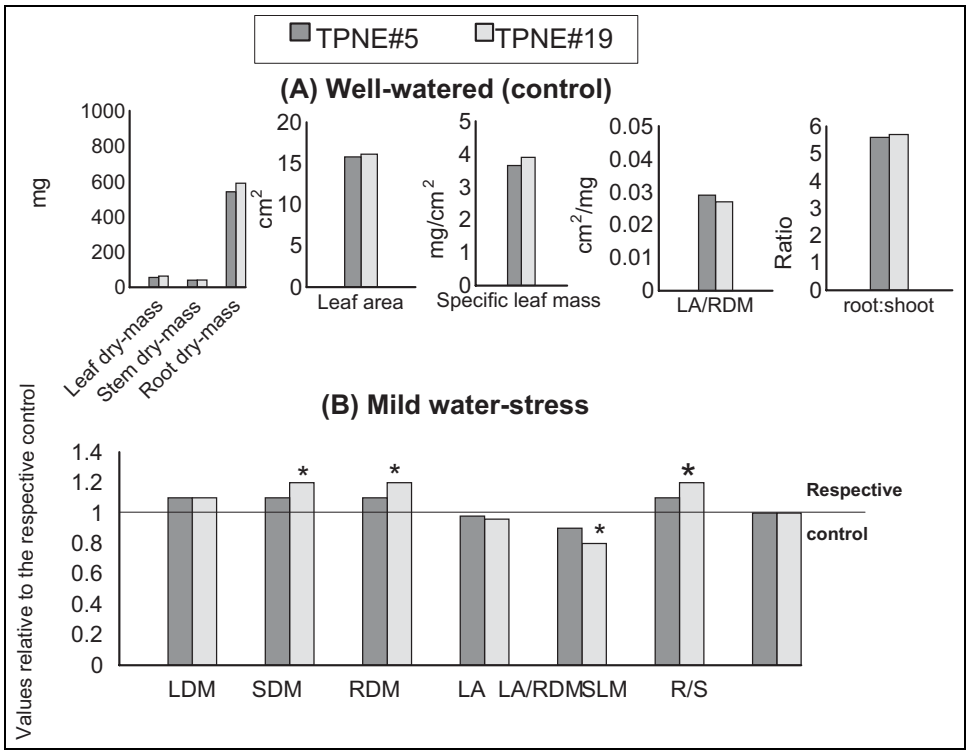


Fig. 2 The dry mass of leaves (LDM), stem (SDM) and root (RDM), the leaf area (LA), specific leaf mass (SLM), the leaf area (cm^2) portion to 1 mg of root dry mass (LA/RDM), the root-to-shoot ratio (R/S) of the control (well-watered, WW) seedlings of two tepary bean (NE#5 and NE#19) lines **(A)** and the values of these parameters for the mildly water stressed seedlings **(B)** relative to the respective WW tepary line (horizontal line intersects 'y' axis at 1). Stars denote significant deviation from the horizontal line (respective control) using Dunnett's Test at 0.05 probability level.

(Fig. 4B). The MDA declined after rewatering to a level lower than its content in the control seedlings.

A significantly greater amount of the AA was detected in control seedlings of NE#19 than those of NE#5 (Fig. 4C). However, the two lines were similar in their POD activity (Fig. 4D) in the control leaves. Under the mild water stress conditions, the POD activity elevated in the leaves of NE#5 while it remained unchanged in NE#19. The vice versa occurred for AA contents of the leaves. Thus, POD activity was higher in the mildly water stressed leaves of NE#5 than in NE#19. On the contrary, AA content was higher in the mildly water stressed leaves of NE#19 than in NE#5. After rewatering, POD activity increased in NE#5 but NE#19 showed no changes. The AA significantly decreased in NE#19 following rewatering.

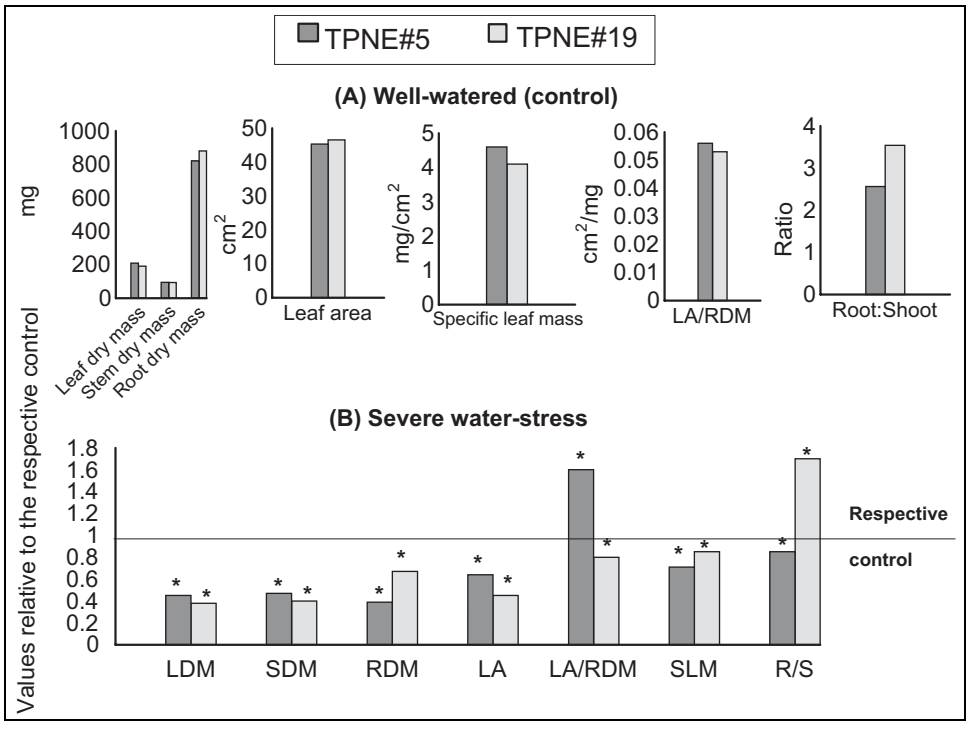


Fig. 3 The dry mass of leaves (LDM), stem (SDM) and root (RDM), the leaf area (LA), specific leaf mass (SLM), the leaf area (cm^2) portion to each 1 mg of root dry mass (LA/RDM), the root-to-shoot ratio (R/S) of the control (well-watered, WW) seedlings of two tepary bean (NE#5 and NE#19) lines **(A)** and the values of these parameters for the severely water stressed seedlings **(B)** relative to the respective WW tepary line (horizontal line intersects 'y'axis at 1). Stars denote significant deviation from the horizontal line (respective control)) using Dunnett's Test at 0.05 probability level.

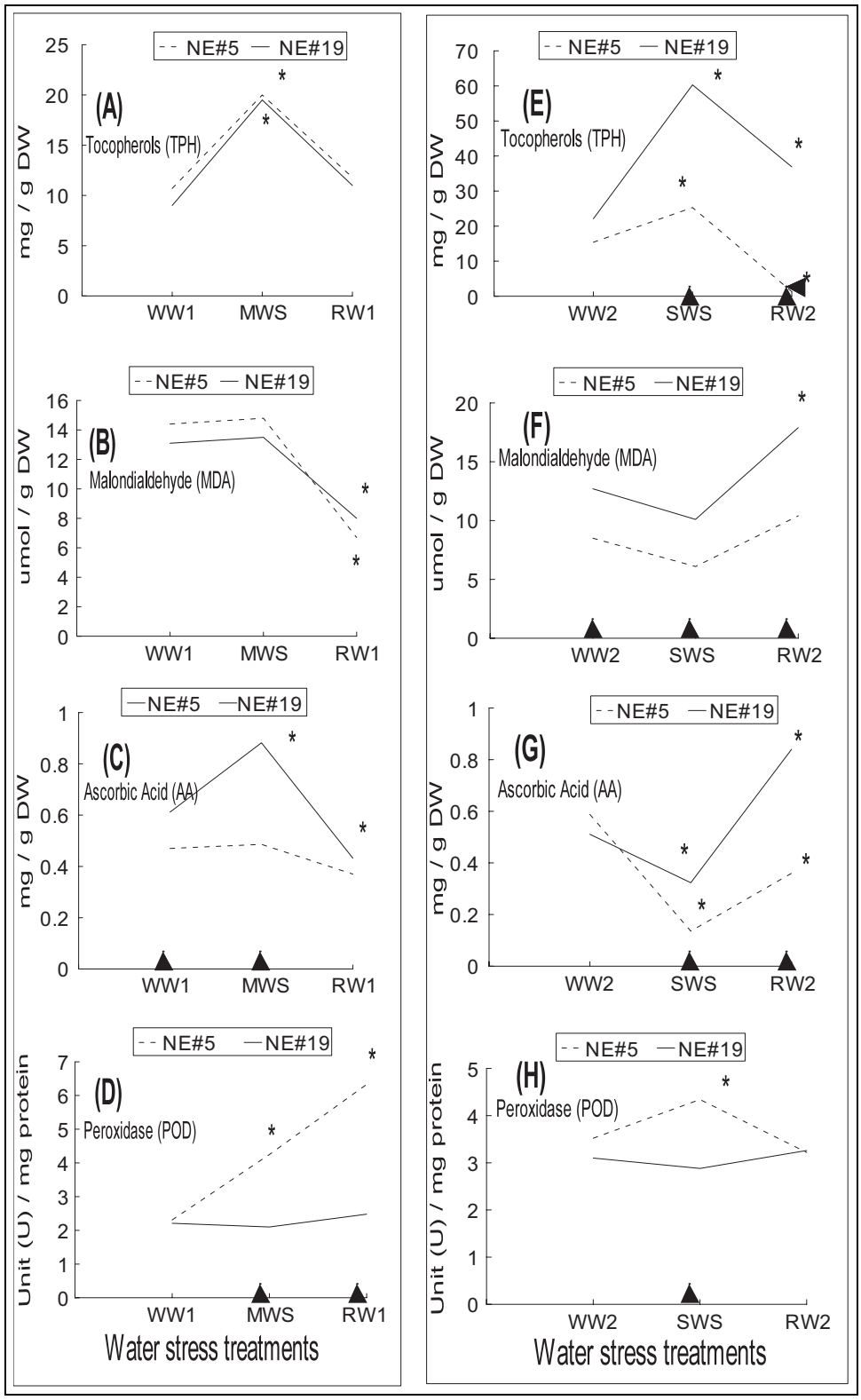


Fig. 4 Tocopherols (TPH), malondialdehyde (MDA) release, ascorbic acid (AA) content and peroxidase (POD) activity in two tepary bean lines (NE#5 and NE#19) subjected to water deficit conditions and rewatering. On the left side **(A** to **D)** the determined values of these parameters are presented for the control (well-watered, WW1), mildly water stressed (MWS) and rewatered (RW1) seedlings. On the right side **(E** to **H)** the determined values of these parameters are presented for control (well-watered, WW2), severely water stressed (SWS) and rewatered (RW2) seedlings. Stars donate significant water treatment mean deviation within tepary bean line from the respective control at 0.05 level of the probability. The vertical arrow heads on the 'X' axes refer to significant differences at 0.05 level of the probability between the two tepary lines when received the same water treatment.

The leaf contents of Chl a and Chl b were not affected at this mild water depletion level or after rewatering (Fig. 5A and B). Carotenoid contents, however, showed significant increases (Fig. 5C) in the mildly water stressed seedlings of only NE#19. The carotenoids remained at the same level in this tepary line after rewatering. No alteration occurred in the carotenoid content of the leaves in the mildly water stressed and rewatered seedlings of NE#5 as compared to the control ones. The values of the Chl a fluorescence parameter F_v /F_m detected in mildly water stressed leaves of NE#5 showed a significant increase compared with

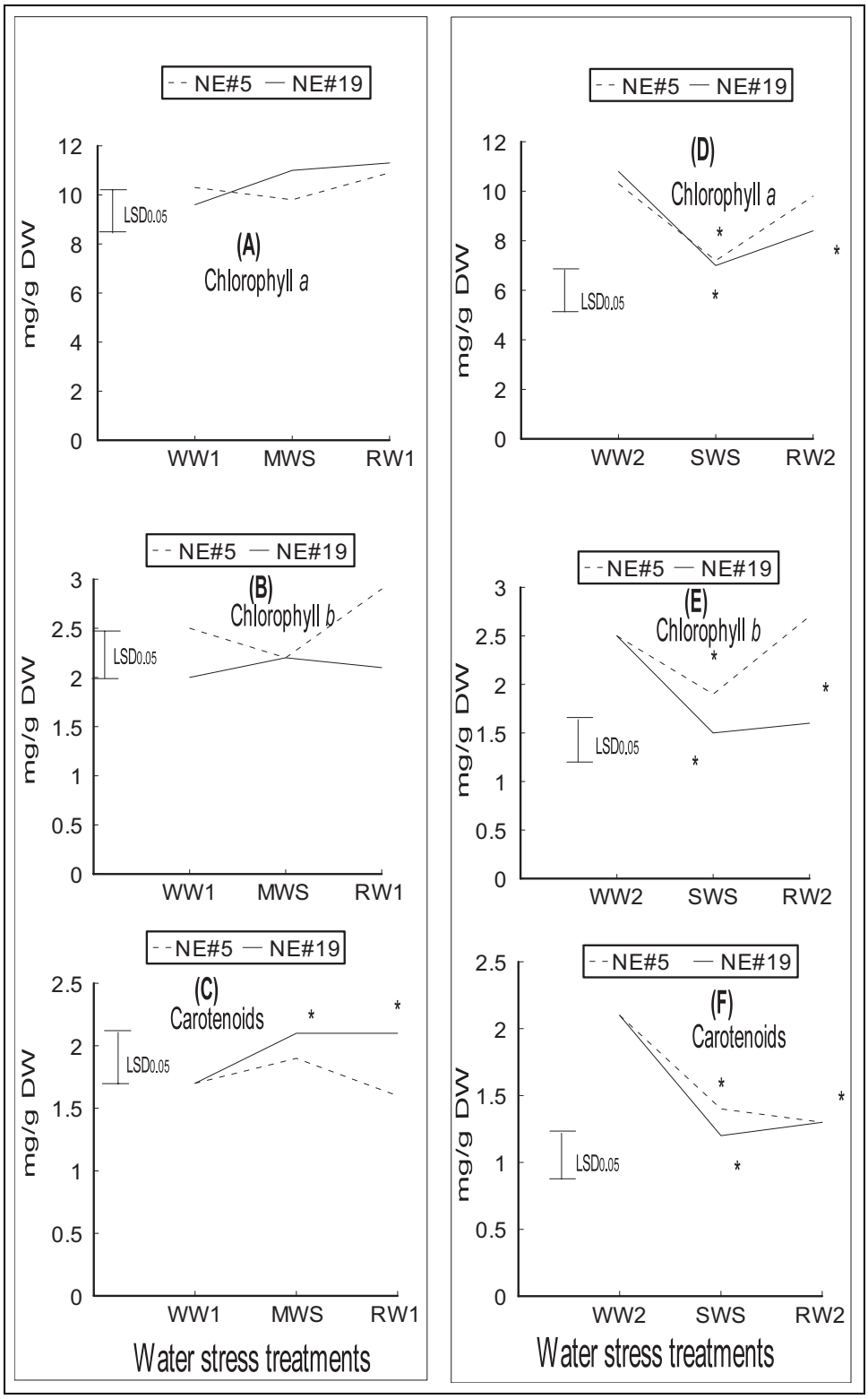


Fig. 5 Chlorophyll a and b and carotenoid contents in two tepary bean lines (NE#5 and NE#19) subjected to water deficit conditions and rewatering. On the left side **(A to C)** the determined values of these parameters are presented for the control (well-watered, WW1), mildly water stressed (MWS) and rewatered (RW1) seedlings. On the right side **(D** to **F)** the determined values of the parameters are presented for the control (well-watered, WW2), severely water stressed (SWS) and rewatered (RW2) seedlings. Stars donate significant water treatment mean deviation within tepary line from the control at 0.05 level of the probability. The vertical lines represent the values of the "Least Significant Difference" at 0.05 level of the probability ($LSD_{0.05}$) to separate differences between NE#5 and NE#19 receiving the same water treatment.

the control leaves (Fig. 6A). F_0 greatly decreased (Fig. 6B) while the alteration of F_M was not significant (Fig. 6C). Same responses of Chl a fluorescence were detected in the rewatered leaves of NE#5. In NE#19, there was a significant increase in F_0 of the mildly water stressed leaves compared to the control but F_v /F_m did not significantly deviate from the control. The leaves of rewatered seedlings of NE#19 gave similar F_0, F_m and F_v /F_m values to those recorded in the control.

Severe water-depletion and rewatering

In NE#5, 82 % of the water content of the container capacity was depleted while this was 73 % for NE#19

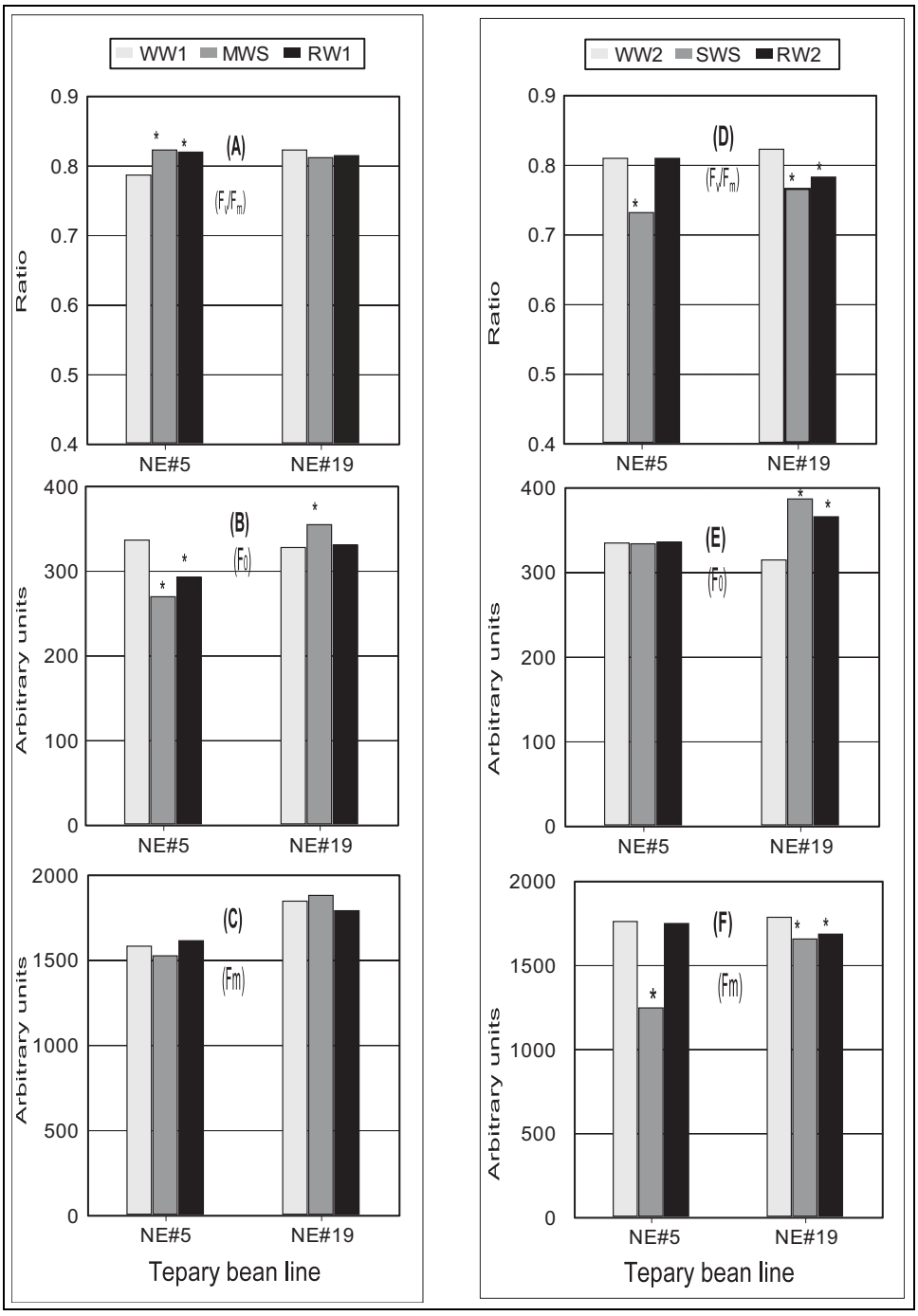


Fig. 6 Chlorophyll a fluorescence parameters (F_0, F_M and F_v /F_M) of the attached leaves in two tepary bean lines (NE#5 and NE#19) subjected to water deficit conditions and rewatering. On the left side **(A** to **C)** the determined values of these parameters are presented for the control (well-watered, WW1), mildly water stressed (MWS) and rewatered (RW1) seedlings. On the right side **(D** to **F)** the determined values of the parameters are presented for the control (well-watered, WW2), severely water stressed (SWS) and rewatered (RW2) seedlings. Stars donate significant water treatment mean deviation within TP line from the control at 0.05 level of the probability.

(Fig. 1A). In contrast to the respective well-watered (control) seedlings (Fig. 1C), 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. No differences were detected in the measured and the derived parameters of the well-watered (control) seedling growth in both tepary lines (Fig. 3A). Obviously, leaf, stem and root dry mass, leaf area and specific leaf mass decreased in the two tepary lines compared to the respective control ones (Fig. 3B). However, NE#19 had lower while NE#5 had higher portion of leaf area to 1 mg of root mass than the respective control. NE#19 showed also increased root:shoot ratio compared to the control.

Except for the lower content of MDA (Fig. 4F) in NE#5, the leaves of the well-watered seedling of both tepary lines were similar in TPH, AA and POD (Fig. 4E, G and H). Under the severe dehydration conditions, the TPH raised in both lines (Fig. 4E). 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. MDA did not show significant differences between the control and severe water dehydration treatments of both lines (Fig. 4F). Thus the MDA contents remained lower in NE#5 than NE#19. After rewatering, the MDA content increased in NE#19 while no 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 TP lines (Fig. 4G). 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 level of the control. 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. 4H). The former line, therefore, was higher in the POD activity than the latter. When the seedlings were rewatered, the activity of POD decreased 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. 5D, E and F). The severely dehydrated leaves of both TP 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. 6D). 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. 6E). On the contrary, 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. 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. 6F).

Discussion

Mild depletion of the moisture from the root medium stimulated physiological (Fig. 1F and G) morpho-physiological (Fig. 2B) and biochemical (Fig. 4C and D; Fig. 5C) alterations related to drought resistance mechanisms. Stomata resistance increased and consequently transpiration rate decreased in line NE#19. The net photosynthesis tended towards showing enhancement in line NE#19 while there was a tendency for reduction of the intercellular CO_2. Therefore, this line exhibited an elevated photosynthetic water-use-efficiency. The tendency of photosynthesis toward enhancement could be a result of increased specific leaf-mass (ZWACK et al. 1998). Significant partition of the accumulated dry matter was directed in line NE#19 to increase the mass of the absorption (root) and transport (stem) tissues while transpiration surface area did not change as compared to the control treatment (well-watered). Thus, portion of the transpiration surface area to the unit of water absorption mass significantly decreased. Line NE#19 is considered, therefore, a potential germplasm for dehydration-avoidance ability (MOHAMED et al. 2002). Line NE#5 did not appreciably show potential dehydration-avoidance physiological and morpho-physiological alterations.

Although NE#19 is considered to be more potential in dehydration-avoidance ability than NE#5, both lines were similar in the RWC of the leaves after being subjected to a mild water depletion (Fig. 1B). Nevertheless, RWC of the leaves was about 11 % higher in NE#19 than in NE#5 (Fig. 1C) when water deficiency progressed to a severe level (Fig. 1A). It is suggested that recognizable root signal regulating physiological and morpho-physiological responses of line NE#19 was developed only close to the end of the mild water stress treatment in this study (MOHAMED et al. 2002). Under such mild water-deficiency conditions stimulation of non-enzymatic (Fig. 4C and 5C) and enzymatic (Fig. 4D) antioxidative reactions were also developed before occurrence of a substantial change in leaf water status. At this point, no change in the Chl contents was detected in both TP lines (Fig. 5A and B). Also, there was no evidence indicating oxidative degradation of cellular lipids (Fig. 4B) or deterioration in the functional properties of the chloroplast in NE#19 (Fig. 6A to C).

In spite of the increased stomata resistance enhancing leaf RWC of NE#19 under severe water conditions, both lines eventually suffered a severe dehydration (Fig. 1C) due to the limited medium that restricted the role of root in water absorption. Tocopherol level that was similar in both lines under mild water stress (Fig. 4A) became greatly higher in NE#19 than NE#5 (Fig. 4E) under severe dehydration. In the former case, it was associated with increased level of ascorbic acid than the control treatment for line NE#19 and unchanged low level in NE#5. In the latter case, it was associated with decreased level of ascorbic acid in both lines than the control but NE#19 had a higher level than NE#5 (Fig. 4G). This may indicate that there was an excessive ascorbic acid in NE#19 than needed under mild water stress while it decreased due to excessive oxidation process existed under sever dehydration. Ascorbic acid production in NE#5 seemed to be enough under mild water stress while it was less than needed under sever dehydration.

Following the rehydration of the severly dehydrated leaves, however, ascorbic acid content elevated in NE#19 (Fig. 4G). Also tocopherol, although it decreased after rehydration, remained higher than the

respective control and about 40 times the level of rehydrated NE#5 (Fig. 4E). Increase in MDA (Fig. 4F) 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. 6D to F). It seemed that the triggered antioxidative system of the oxygen radical scavenging compounds (ascorbic acid and corotenoids) in the leaves of NE#19 may be protective against oxidative stress during the progressive water deficit only to a limited extent beyond the level of the mild water-deficiency treatment in this study. 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 water dehydration is reached.

Our assessment suggested that the core 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 unchanged in NE#19 during the stress period (Fig. 4H). Although leaf Chl degradation occurred in both TP lines, only NE#5 that showed recovery after rehydration of the severely dehydrated leaves (Fig. 5D and E). The Chl recovery after rehydration and lack of change in MDA (oxidative degradation of lipids) (Fig. 4F) may be controlled by the peroxidase activity during the stress period (Fig. 4H). A high correlations were detected between the photosynthetic efficiency implied by the F_v /F_m parameter of the Chl a fluorescence (Fig. 6D) and the alteration of the leaf Chl a content (Fig. 5D) due to the different water treatments in NE#5 ($r=0.99$, $P<0.02$) and in NE#19 ($r=0.98$, $P<0.03$). A fast Chl a fluorescence may, therefore, be used to screen drought tolerant tepary lines. Line NE#19 could be utilized in rationalizing the water use in irrigated lands by prolonging irrigation frequency up to certain mild water depletion level because it has a potential dehydration-avoidance mechanism. However, NE#5 would be expected to withstand exposure to a severe water-dehydration especially at the seedling stage. Bean breeders may make use of this differential dehydration-avoidance and tolerance mechanisms of this germplasm lines to produce an elite tepary line and to utilize it in improving drought resistance of the relative species *P. vulgaris* L. (common bean).

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