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Physiology of soybean as affected by PEG-induced drought stress*

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ABSTRACT

To evaluate the influence of drought stress on soybean physiology, a controlled-environment experiment was conducted at the Institute of Plant Sciences, University of Debrecen, Hungary. Two soybean cultivars, *ES Mentor* and *Pedro*, were subjected to four different levels of water deficiency elaborated by different polyethylene glycol (PEG) concentrations; 2.5, 5, 7.5 and 10 % starting from the post-germination phase. The measurements were made at four different stages; second node (V_2), fourth node (V_4), full bloom (R_2) and full pod (R_4). *ES Mentor* plants could not survive under 10 % PEG concentration at V_4 stage, and under both 7.5 and 5 % PEG concentrations at R_2 stage, and similar reaction was observed under 10 % PEG concentration at V_4 stage, and 7.5 % PEG concentration at R_2 stage for *Pedro*. For cultivar *ES Mentor*, increasing PEG concentration was accompanied by decreasing SPAD values at all stages, and *Pedro* followed a very similar trend except for a slight, insignificant increase in 2.5 % PEG treatment at V_2 stage as compared to control. However, differences were more measurable at later stages. Concerning chlorophyll content, Chl_a, Chl_b and Chl_{x+c} decreased as PEG concentration increased at all stages of *ES Mentor*; the reduction was insignificant at vegetative stages (V_2 and V_4 stages) and significant at reproductive stages (R_2 and R_4), whereas for *Pedro* 2.5 % PEG treatment had the best Chl_a and Chl_{x+c} contents at V_2 stage. However at the following stages, control treatment could maintain the best values, and the increase in PEG concentration was accompanied by a decrease in both contents. Chl_b, on the other hand, was significantly higher for 2.5 % PEG treatment than control at both vegetative stages, whereas in the reproductive stages it insignificantly decreased with increasing PEG concentration. Maximum photochemical efficiency of PSII (Fv/Fm) of both cultivars followed one trend throughout the studied stages; it decreased with increasing PEG concentration. Moreover, increasing PEG concentration was accompanied by a non-significant decrease in the actual photochemical efficiency of PSII (ΦPSII) of *ES Mentor* in all stages, whereas for *Pedro* 2.5 % PEG treatment resulted in better ΦPSII compared to control treatment at both vegetative stages, however, control was the highest at later stages and ΦPSII decreased with increasing PEG concentration. Significant differences were recorded for both cultivars in response of stomatal conductance to PEG application; increasing PEG concentration resulted in lower stomatal conductance in all stages (except for a slight increase in 5 % PEG treatment compared to 2.5 % PEG treatment at V_2 stage in *Pedro* plants). It could be concluded that drought stress had different effects on the physiology of the two cultivars; however, the negative effects were more obvious at the late stages of the plant's life cycle of both cultivars, which will presumably reflect on the yield component traits, and consequently, the expected yield.

1. Introduction

Soybean (*Glycine max* (L.) Merrill) is the most widely grown seed legume, providing an inexpensive source of protein [1,2], and is also the most widely grown oilseed crop worldwide [3]. Soybean is mostly sown under rain-fed irrigation scheme [4]. The global climate changes resulted in alterations in precipitation amounts and timings [5] and, consequently, have caused periods of drought stress which is considered as one of the most destructive abiotic stresses, especially with the fact that soybean is a drought-sensitive crop, particularly at certain growth stages [6]. The response to drought stress is a very complex process that involves multiple mechanisms on different levels [7,8]; for example, one of the physiological mechanisms includes water uptake maximization (by deep rooting for instance) and/or water loss minimization (for example by intense stomatal control) [9,10]. Flexas et al. [11] reported that drought stress level might be estimated by measuring stomatal conductance; if its value ≥ 0.2 mol H_2O m^{-2} s^{-1} then there is no drought stress, and if it falls between 0.1 and 0.2 mol H_2O m^{-2} s^{-1}

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then the plants are subjected to a moderate drought stress, and if it is ≤ 0.1 mol H_2O m^{-2} s^{-1} then severe drought stress is present.

Another mechanism is light absorption modification through changes in leaf's chlorophyll content [12]. Chlorophyll has a major role in light quantum's absorption and transmission, and chlorophyll content represents light use ability by plant [13]. Under drought conditions, chlorophyll pigments and photosynthetic electron transport system could be damaged, leading to reactive oxygen species (ROS) production [14] and causing fast diffusion across cell membrane and, eventually, cell death [15]. Under drought stress conditions, leaf photosynthetic performance can be inspected by observing the changes in the thylakoid membrane organization and function by measuring chlorophyll fluorescence. Chlorophyll fluorescence can also be considered as an indicator to the energy absorbed by chlorophyll being used by PSII photochemistry [16,17]. The quantum efficiency of PSII photochemistry (ΦPSII), on the other hand, can be considered as an indication of overall photosynthesis as it measures the proportion of light absorbed by chlorophyll associated with PSII that is used in photochemistry; this trait also can be altered under certain stress conditions like drought [16,18].

2. Materials and methods

The experiment was conducted in the Institute of Crop Sciences, University of Debrecen in 2018. Two soybean cultivars; *'ES Mentor'* and *'Pedro'* were surface-sterilized using 6 % (v/v) H_2O_2 for 20 min, rinsed extensively with deionized water and germinated geotropically between moisten filter papers at 22 °C. After germination, seedlings with good vigor were planted in 5 L pots. Each pot contained 10 seedlings. Each pot received 50 ml of dicot nutrient solution that consisted of the following substances: 2.0 mM $Ca(NO_3)_2$, 0.7 mM K_2SO_4, 0.5 mM $MgSO_4$, 0.1 mM KH_2PO_4, 0.1 mM KCl, 10 μM H_3BO_3, 0.5 μM $MnSO_4$, 0.5 μM $ZnSO_4$ and 0.2 μM $CuSO_4$. Iron was supplied in the form of 10^{-4}M Fe-EDTA [43], in addition to corresponding PEG solution. Nutrient solution of each pot was replaced with fresh alternative every 3 days. PEG 6000 (VWR International bvba Geldenaaksebaan, Leuven, Belgium) was used to induce water deprivation stress. PEG concentrations were as follows; 0, 2.5, 5, 7.5 and 10 % (0 % PEG, 2.5 % PEG, 5 % PEG, 7.5 % PEG and 10 % PEG, respectively). All measurements were made at 4 different stages of each cultivar [19]; second node (V2), fourth node (V4), full bloom (R2) and full pod (R4). Relative chlorophyll content (SPAD values) was recorded using SPAD-502Plus (Konica Minolta, Japan). Stomatal conductance (g_s) was measured using AP4 porometer (Delta-t devices, UK). Both SPAD and (g_s) were calculated by averaging 10 values per leaf of the most recently developed trifoliate.

Chl-fluorescence was determined on dark-adapted leaves (20 min of dark adaptation) by attaching light exclusion clips to the central region of each leaf. Chl-fluorescence parameters were measured using a portable chlorophyll fluorometer-*PAM-2100* (*WALZ,* Germany) as described by Schreiber et al. [44]. The fluorescence parameters included the minimal fluorescence (F_0) when PSII centres are open (open state) and increases the maximum fluorescence (F_m) when PSII centres are closed (closed state), the variable fluorescence (F_v), the potential photosynthetic capacity (F_v/F_o) which reflects the efficiency of electron donation to PSII and the ratio (F_m–F_0)/F_m, also known as F_v/F_m (potential/maximum photochemical efficiency of PSII) which is calculated from fluorescence values F_0 and F_m. The F_v/F_m ratio is one of the most common parameters used in fluorescence that reflects the capacity to trap electron by the PSII reaction centre. The actual photochemical efficiency of PSII (Yield) was also recorded. All of the fluorescence parameters were recorded from the last fully developed trifoliate of one seedling in every pot (replication).

Chlorophylls a and b and total carotenoids concentrations were calculated using the method described by Wellburn [20]; 50 mg of each leaf was blended with 5 ml *N,N*-Dimethylformamide (*N,N*-DMF). This solution was cooled down at 4 °C for 72 h and finally, the extract content of the pigment was determined using UV–VIS spectrophotometry (Metertech SP-830 PLUS, Taiwan) at three wavelengths; 480, 647 and 664 nm (Moran and Porath 1981). The following equations were used for quantifying chlorophyll *a* and *b* and total carotenoids contents [20]:

$$Chl_a\ (\mu g\ ml^{-1}) = (11.65\ A_{664} - 2.69\ A_{647})$$

$$Chl_b\ (\mu g\ ml^{-1}) = (20.81\ A_{647} - 4.53\ A_{664}).$$

$$Chl_{x+c}\ (\mu g\ ml^{-1}) = (1000\ A_{480} - 0.89\ Chl_a - 52.02\ Chl_b)/245$$

Each treatment had 3 replications in a split-plot design where the cultivar represented the main plots and PEG concentrations represented the sub-plots. The total number of pots was 30 (2 cultivars x 5 PEG treatments x 3 replicates). The statistical analysis (Multivariate General Linear Model) was made using SPSS (Ver. 25) software.

3. Results

Both cultivars could not survive after V2 stage in 10 % PEG treatment, moreover, both 7.5 % PEG and 5 % PEG treatments caused ES Mentor plants to die starting from the stage after V4, whereas only 7.5 % PEG treatment had a similar effect on Pedro plants.

3.1. Relative chlorophyll content (SPAD)

For ES Mentor and similar to the total chlorophyll content trait, PEG treatments resulted in lower relative chlorophyll content than control treatment did in all studied stages. Also for this trait, increasing PEG concentration was accompanied with decreasing SPAD values (Table 1) (Fig. 1A).

Cultivar Pedro followed a very similar trend except for a slight, insignificant increase in 2.5 % PEG treatment at V2 stage as compared to control. However, differences were more measurable at later stages; both 5 % PEG and 7.5 % PEG treatments were significantly lower than control treatment at V4 stage, and both 2.5 % PEG and 5 % PEG treatments were significantly lower compared to control treatment at both R2 and R4 stages (Table 1) (Fig. 1B).

3.2. Total chlorophyll content ($Chl_{a,b}$) (μg ml^{-1})

For cultivar ES Mentor, both Chl_a and Chl_b decreased as PEG concentration increased at all 4 studied stages (Figs. 2A and 3 A); the reduction was insignificant at both vegetative stages (V2 and V4 stages), however, the reduction was significant at reproductive stages (R2 and R4) (Table 1).

For Pedro, 2.5 % PEG treatment had the best Chl_a content at V2 stage, and 5 % PEG treatment was also better than control treatment. However at the following stages, control treatment could maintain the best Chl_a content, and the increase in PEG concentration was accompanied by a decrease in Chl_a content. All differences were insignificant (Fig. 2B). Chl_b, on the other hand, was significantly higher for 5 % PEG treatment than control at V2 stage; it was also higher for 2.5 PEG treatment, whereas 7.5 % PEG and 10 % PEG treatments resulted in the least Chl_b content at this stage. At V4 stage, 2.5 % PEG resulted in higher Chl_b content as compared to control treatment, and both 5 % PEG and 7.5 PEG treatments were significantly lower. In the following stages (R2 and R4), Chl_b content insignificantly decreased with increasing PEG concentration (Table 1) (Fig. 3B).

3.3. Total carotenoids (Chl_{x+c}) (μg ml^{-1})

For ES Mentor, control treatment had the highest Chl_{x+c} content at all studied stages compared to PEG treatments; the higher PEG concentration, the lower Chl_{x+c} was, however, the differences were insignificant at all stages except for at R4 stage where control treatment

Table 1
Chl_a, Chl_b, Chl_{c+x} and SPAD for the two studied cultivars in different PEG concentrations at different stages.

PEG Concentration	Stage	Chl_a (μg ml^{-1})		Chl_b (μg ml^{-1})		Chl_{x+c} (μg ml^{-1})		SPAD	
		ES Mentor	Pedro	ES Mentor	Pedro	ES Mentor	Pedro	ES Mentor	Pedro
0 %	V2	13.40^{a1}	14.54^{a1}	4.70^{a1}	4.35^{b1}	3.26^{a1}	4.28^{a1}	33.9^{a1}	33.7^{a1}
2.5 %		13.33^{a1}	15.93^{a1}	4.53^{a1}	5.26^{ab1}	3.25^{a1}	4.	33.0^{a1}	33.8^{a1}
5 %		12.77^{a1}	15.45^{a1}	4.37^{a1}	5.56^{a1}	3.20^{a1}	4.36^{a1}	33.0^{a1}	33.1^{a1}
7.5 %		12.16^{a1}	12.99^{a1}	3.73^{a2}	3.16^{b1}	3.10^{a1}	4.23^{a1}	32.7^{a1}	32.9^{a1}
10 %		12.13^{a1}	12.00^{a1}	3.06^{a1}	3.95^{b1}	2.61^{a1}	4.03^{a1}	29.3^{b2}	32.8^{a1}
0 %	V4	14.34^{a1}	15.40^{a1}	4.43^{a2}	5.35^{a1}	4.80^{a1}	4.62^{a1}	32.4^{a1}	35.3^{a1}
2.5 %		14.16^{a1}	15.24^{a1}	3.98^{ab2}	5.45^{a1}	4.22^{a1}	4.32^{a1}	31.9^{a1}	31.7^{ab1}
5 %		13.97^{a1}	14.84^{a1}	3.57^{bc1}	3.31^{b1}	4.06^{a1}	4.15^{a1}	31.8^{a1}	30.9^{b1}
7.5 %		13.96^{a1}	14.48^{a1}	3.10^{c1}	3.07^{b1}	3.87^{a1}	3.29^{a1}	31.7^{a1}	30.3^{b1}
0 %	R2	17.32^{a1}	12.95^{a1}	4.47^{a1}	4.94^{a1}	3.95^{a1}	2.75^{a1}	35.5^{a2}	39.5^{a1}
2.5 %		9.90^{b1}	11.65^{a1}	1.26^{b2}	4.36^{a1}	2.43^{a1}	2.25^{a1}	34.5^{a1}	35.6^{b1}
5 %		NA	11.53^{a}	NA	4.35^{a}	NA	2.10^{a}	NA	33.0^{c}
0 %	R4	15.05^{a1}	14.10^{a1}	6.49^{a1}	7.68^{a1}	4.84^{a1}	4.73^{a1}	36.9^{a1}	37.1^{a1}
2.5 %		8.80^{b1}	12.52^{a1}	4.14^{b1}	7.32^{a1}	2.55^{b1}	2.89^{a1}	31.8^{b1}	32.4^{b1}
5 %		NA	12.34^{a}	NA	5.108^{a}	NA	2.65^{a}	0.0	32.2^{b}

Same letter indicates no significant differences at .05 level among PEG concentrations within a cultivar.
Same number indicates no significant difference at .05 level between the two cultivars within a particular PEG concentration.

was significantly higher than 2.5 % PEG treatment (Table 1) (Fig. 4A).

For Pedro, both 2.5 % PEG and 5 % PEG treatments had higher Chl_{x+c} than control treatment (4.28 ± 1.4) at V2 stage, whereas Chl_{x+c} of both 7.5 % PEG and 10 % PEG treatments were lower. At the following stages, Chl_{x+c} content decreased as PEG concentration increased, but the differences were insignificant (Table 1) (Fig. 4B).

3.4. Maximum photochemical efficiency of PSII (F_v/F_m)

For both cultivars, F_v/F_m followed one trend throughout the studied stages; it decreased with increasing PEG concentration (Fig. 5A, B). Moreover, for ES Mentor, control and 2.5 % PEG treatments were not significantly different in all stages, however, 5 % PEG and 7.5 % PEG treatments were significantly less at V4 stage compared to control, whereas the difference was significant only in 7.5 % PEG treatment for Pedro (Tables 2 and 3).

3.5. Actual photochemical efficiency of PSII (ΦPSII)

Increasing PEG concentration was accompanied by a non-significant decrease in (ΦPSII) of ES Mentor in all stages (Table 2) (Fig. 6A). For Pedro on the other hand, 2.5 % PEG treatment resulted in better, yet not significant, (ΦPSII) compared to control treatment at both vegetative stages (V2 and V4), however, control was the highest at later stages and (ΦPSII) decreased with increasing PEG concentration (Table 2) (Fig. 6B).

3.6. Stomatal conductance (g_s) (mmol m^{-2} s^{-1})

Significant differences were recorded for both cultivars in response of stomatal conductance to PEG application; increasing PEG concentration resulted in lower stomatal conductance in all stages (except for a slight increase in 5 % PEG treatment compared to 2.5 % PEG treatment at V2 stage for Pedro) (Fig. 7A, B). Control treatment was significantly higher than all other PEG treatments, and 2.5 % PEG treatment was significantly better than higher PEG-concentration treatments for ES Mentor (Table 2).

As shown in Table 4, stomatal conductance showed significant negative correlation with drought application at all stages in both cultivars. SPAD value was also negatively affected by drought in both cultivars; the effect was more measurable at R4 stage. In additions, both cultivars showed reduced F_v/F_m value with increasing drought stress at all stages. However, both F_v and F_m were positively correlated with drought stress except at V4 stage in ES Mentor, whereas both traits were negatively affected by drought in Pedro, and the most negative effect occurred at R2 stage.

In ES Mentor all chlorophylls were more affected by drought at reproductive as compared to vegetative stages, whereas in Pedro Chl_a was most-negatively affected by drought at R2 stage, whereas both Chl_b and Chl_{x+c} were most affected at V4 stage.

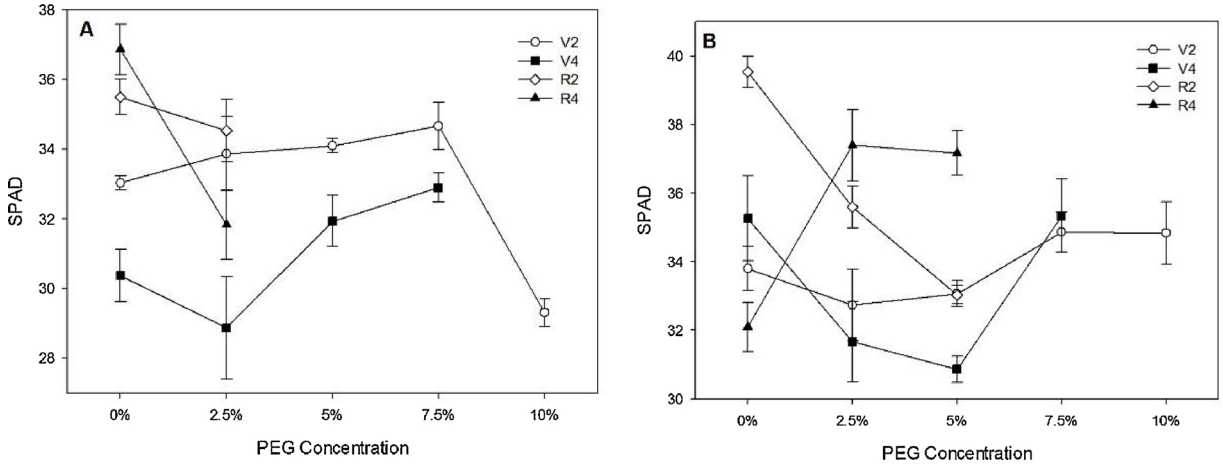

Fig. 1. SPAD of ES Mentor (A) and Pedro (B) in different PEG concentrations at different stages.

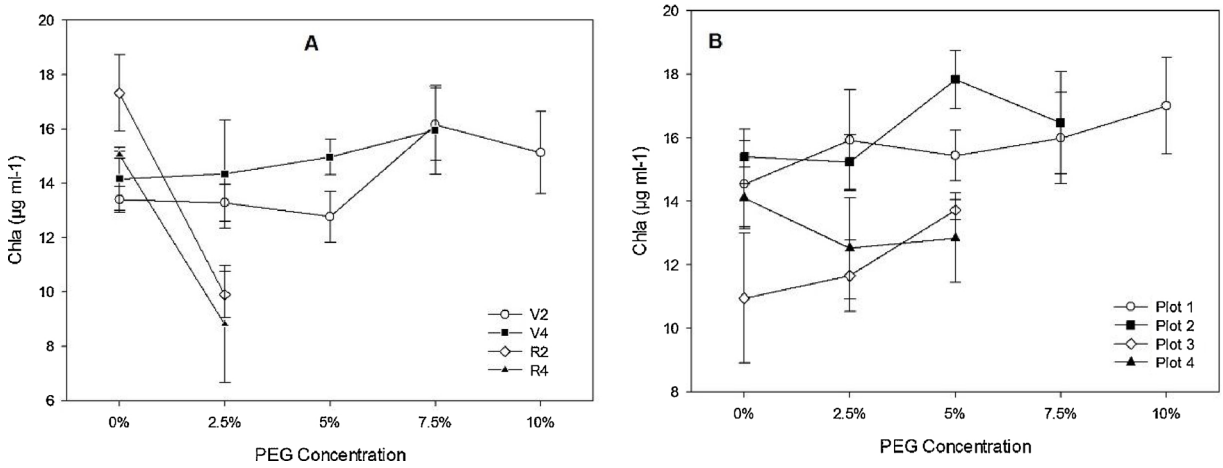

Fig. 2. Chl_a of ES Mentor (A) and Pedro (B) in different PEG concentrations at different stages.

Calculating the effect size also reflected the effect of PEG concentration on the different traits studied; except Chl_b at V4 stage and Fv/Fm at R2 stage, PEG concentration was higher and more significant compared to cultivar effect (Table 5).

4. Discussion

In our experiment, Chl_a and Chl_b decreased under drought stress conditions for cultivar ES Mentor at all stages, whereas relatively-moderate drought stress resulted in increased Chl_b content for cultivar Pedro at the early stages; however, it declined in the later stages. Similarly, Zhang et al. [21] concluded that Chl_a was significantly reduced under drought conditions compared to the non-drought counterpart, whereas Chl_b increased when plants suffered from water deficit. Hao et al. [1] reported significant decrease (by 32.2 %) in chlorophyll content as a result of drought stress. Mathobo et al. [22] subjected bean plants to drought stress for 24 days in different stages; the reduction of chlorophyll content was higher when drought occurred at later stages as compared to earlier stages, and control plants were always the highest in chlorophyll content; they suggested that the reduction in chlorophyll content might have resulted from leaves being damaged and turning yellowish due to drought stress. Previously, many papers reported a decrease in total chlorophyll content due to drought stress in other legumes like soybean [23], chickpea [24] and pea [25]. Moreover, Smirnoff [26] indicated that the decrease in total chlorophyll content is resulting from the damage to the chloroplasts caused by reactive oxygen species (ROS) as drought stress leads to the production of reactive oxygen species (ROS) such as O_2^- and H_2O_2, which lead to chlorophyll destruction [27]. Chlorophylls, as the main pigments of absorption, transport and conversion of light energy, its content is an important parameter indicating photosynthetic performance. ROS accumulated under environmental stresses will destroy chlorophylls, and chl_a is more sensitive to ROS than chl_b [28]. Exposing plants to water stress led to a significant decline in chl_{a+b} (from 19.5–13.0 mg g^{-1} DW), indicating the decreased capacity of absorbing and conversion of light energy [29]. Similar results were reported [30,31]. SPAD values significantly decreased from 35.48 to 22.38 under drought stress applied 30 days after R5.5 stage [32]. These results are in agreement with the general chlorophyll drops that occur when soybean plants are subjected to continuous water stress from early seed filling [33,34].

Total carotenoids were reduced as a result of drought stress application at all stages in ES Mentor, and at V4, R2 and R4 stages in Pedro. Carotenoid can protect chlorophylls from damage by dissipating excess light energy around PSIIthrough xanthophylls cycle [35,36]. Therefore, it is an important safeguard of photosynthetic mechanism, and its content can reflect the adaptive ability of plant to environment [29]. Previously, Zhang et al. [21] reported carotenoids content to be significantly reduced under drought stress conditions compared to the well-watered control, which was supported later by the conclusion that exposing plants to water stress led to a significant decline in carotenoid content (from 3.4 to 2.1 mg/g dry weight) [29].

F_v/F_m decreased as a result of drought stress in both cultivars, and ΦPSII showed similar trend in ES Mentor, whereas the slight drought stress resulted in better ΦPSII at vegetative stages in Pedro as compared

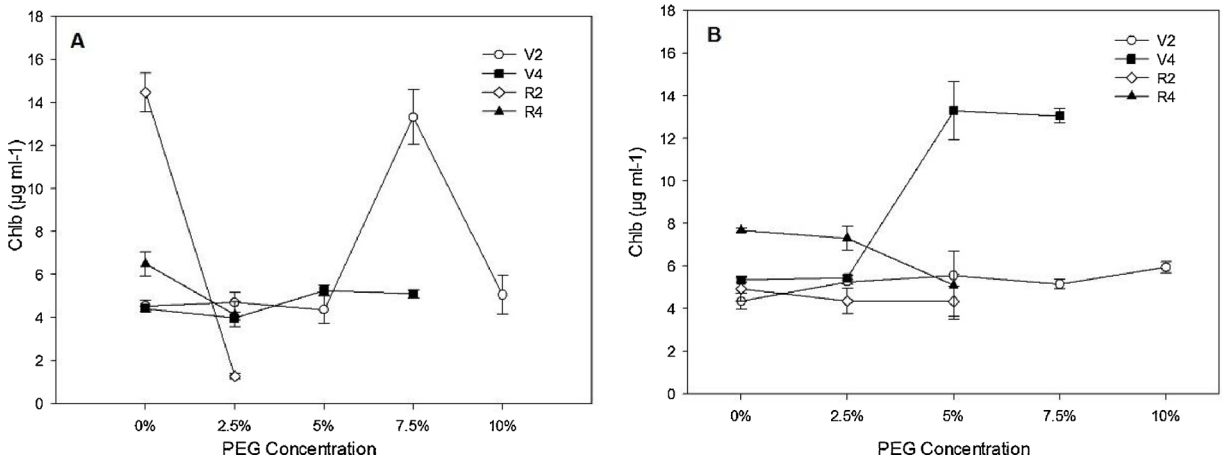

Fig. 3. Chl_b of ES Mentor (A) and Pedro (B) in different PEG concentrations at different stages.

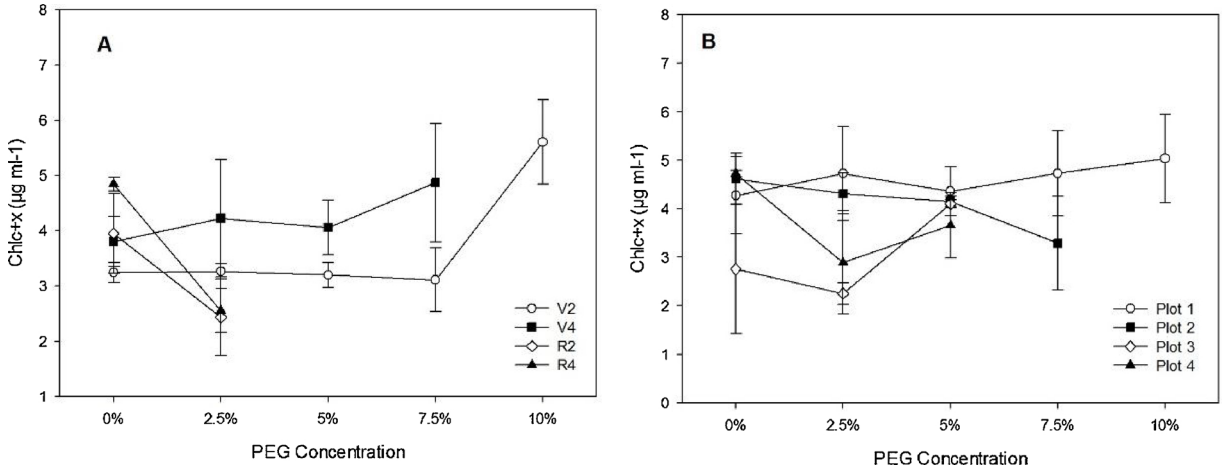

Fig. 4. Chl_{x+c} of ES Mentor (A) and Pedro (B) in different PEG concentrations at different stages.

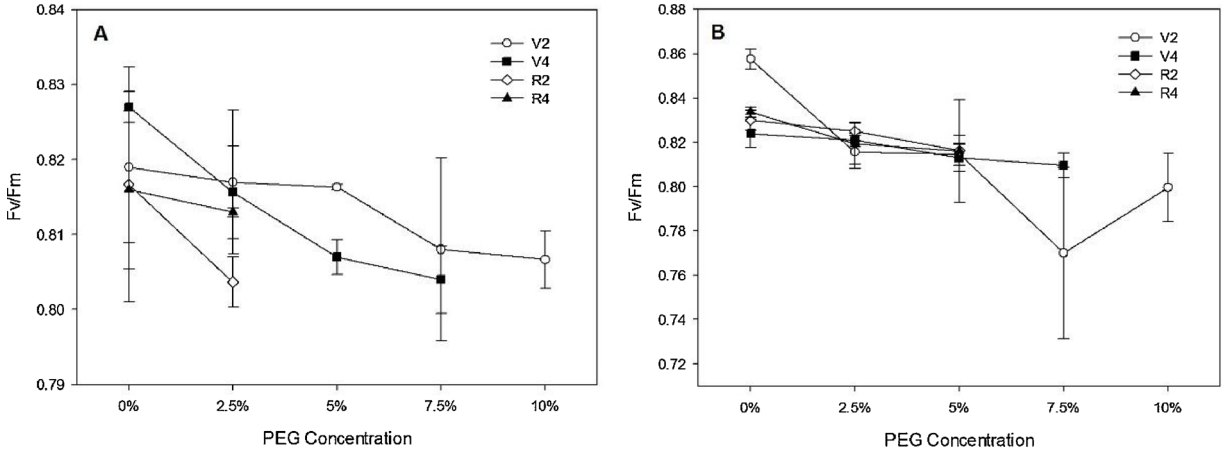

Fig. 5. F_v/F_m of ES Mentor (A) and Pedro (B) in different PEG concentrations at different stages.

Table 2
F_v/F_m, ΦPSII and stomatal conductance for the two studied cultivars in different PEG concentrations at different stages.

PEG Concentration	Stage	F_v/F_m		ΦPSII		g_s (mmol m-2 s^{-1})	
		ES Mentor	Pedro	ES Mentor	Pedro	ES Mentor	Pedro
0 %	V2	0.819^{a1}	0.821^{a1}	0.759^{a1}	0.762^{a1}	258^{a1}	131^{a2}
2.5 %		0.817^{a1}	0.816^{a1}	0.749^{a1}	0.769^{a1}	135^{b1}	82^{bc2}
5 %		0.816^{a1}	0.815^{a1}	0.748^{a1}	0.752^{ab1}	86^{c1}	83^{b1}
7.5 %		0.808^{a1}	0.770^{a1}	0.726^{a1}	0.727^{ab1}	82^{c1}	66^{bcd1}
10 %		0.807^{a1}	0.800^{a1}	0.725^{a1}	0.645^{b1}	76^{c1}	45^{d2}
0 %	V4	0.827^{a1}	0.824^{a1}	0.787^{a1}	0.788^{a1}	204^{a1}	129^{a2}
2.5 %		0.816^{ab1}	0.821^{a1}	0.778^{a1}	0.791^{a1}	106^{b1}	76^{b2}
5 %		0.807^{b1}	0.813^{a1}	0.772^{a1}	0.783^{a1}	34^{c2}	52^{cd1}
7.5 %		0.804^{b1}	0.810^{b1}	0.771^{a1}	0.782^{a1}	30^{c1}	34^{d1}
0 %	R2	0.817^{a1}	0.830^{a1}	0.787^{a1}	0.795^{a1}	145^{a1}	199^{a1}
2.5 %		0.804^{a1}	0.825^{a1}	0.762^{a1}	0.771^{a1}	106^{b1}	105^{b1}
5 %		NA	0.816^{a}	NA	0.749^{a}	NA	52^{c}
0 %	R4	0.816^{a1}	0.834^{a1}	0.782^{a1}	0.796^{a1}	112^{a2}	194^{a1}
2.5 %		0.813^{a1}	0.819^{a1}	0.766^{a1}	0.744^{a1}	47^{b1}	59^{b1}
5 %		NA	0.816^{a}	NA	0.734^{a}	NA	55^{b}

Same letter indicates no significant differences at .05 level among PEG concentrations within a cultivar.
Same number indicates no significant difference at .05 level between the two cultivars within a particular PEG concentration.

to control, however, it could not maintain that trend later at reproductive stages and it was reduced as a result of drought. Zhang et al. [21] reported maximum quantum yield of PSII (F_v/F_m) to be approximately 0.78–0.80 in control treatment, however, this parameter decreased in response to drought stress, but was not significantly different. Additionally, drought stress resulted in a reduction in quantum yield of PSII (ΦPSII) (from 0.53 to 0.13); they suggested that the reduced ΦPSII was a result of a decrease in the excitation energy trapping efficiency of PSII reaction centers. Similar conclusion was reported by Zlatev and Yordanov [37] in bean plants. Hao et al. [1] reported the decrease to be significant (from 0.83 to 0.66), whereas Mathobo et al. [22] concluded that the reduction was insignificant after 93 days of

Table 3
F_0, F_m, F_v, F_v/F_0 and F_m/F_0 for the two studied cultivars in different PEG concentrations at different stages.

PEG Concentration	Stage	F_0		F_m		F_v		F_v/F_0		F_m/F_0	
		ES Mentor	Pedro	ES Mentor	Pedro	ES Mentor	Pedro	ES Mentor	Pedro	ES Mentor	Pedro
0 %	V2	0.344	0.322	1.783	1.808	1.461	1.551*	4.263	4.818	5.199	5.616
2.5 %		0.320	0.348	1.767	1.788	1.443	1.458	4.547	4.203	5.558	5.149
5 %		0.313	0.329	1.715	1.779	1.400	1.449	4.472	4.418	5.478	5.421
7.5 %		0.343	0.339	1.795	1.547	1.451	1.199*	4.249	3.622	5.250	4.644
10 %		0.331	0.339	1.814	1.710	1.463	1.367	4.429	4.061	5.490	5.084
0 %	V4	.314	.314	1.818*	1.787	1.503*	1.473	4.794*	4.689	5.797*	5.688
2.5 %		.323	.328	1.756	1.841	1.432	1.512	4.447	4.601	5.450	5.604
5 %		.328	.285	1.697	1.554	1.370*	1.264	4.182*	4.428	5.181*	5.445
7.5 %		.309	.306	1.662*	1.698	1.336*	1.374	4.332	4.504	5.386	5.561
0 %	R2	.320	.329	1.651	1.880	1.351	1.560	4.239	4.753	5.175	5.725
2.5 %		.309	.308	1.687	1.806	1.355	1.489	4.400	4.853	5.475	5.881
5 %		NA	.311	NA	1.700	NA	1.388	NA	4.473	NA	5.476
0 %	R4	.314	.287	1.719	1.724	1.402	1.437	4.497	5.010	5.505	6.009
2.5 %		.330	.288	1.759	1.605	1.430	1.317	4.336	4.558	5.334	5.558
5 %		NA	.284	NA	1.563	NA	1.279	NA	4.605	NA	5.606

* Significant at 0.05 level among PEG concentrations within certain stage and cultivar.

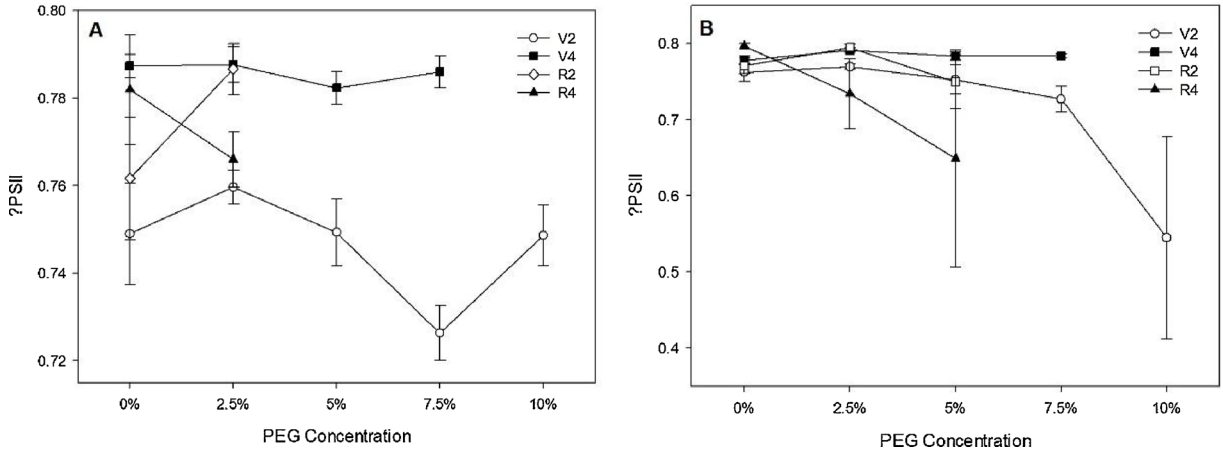

Fig. 6. ΦPSIIof ES Mentor (A) and Pedro (B) in different PEG concentrations at different stages.

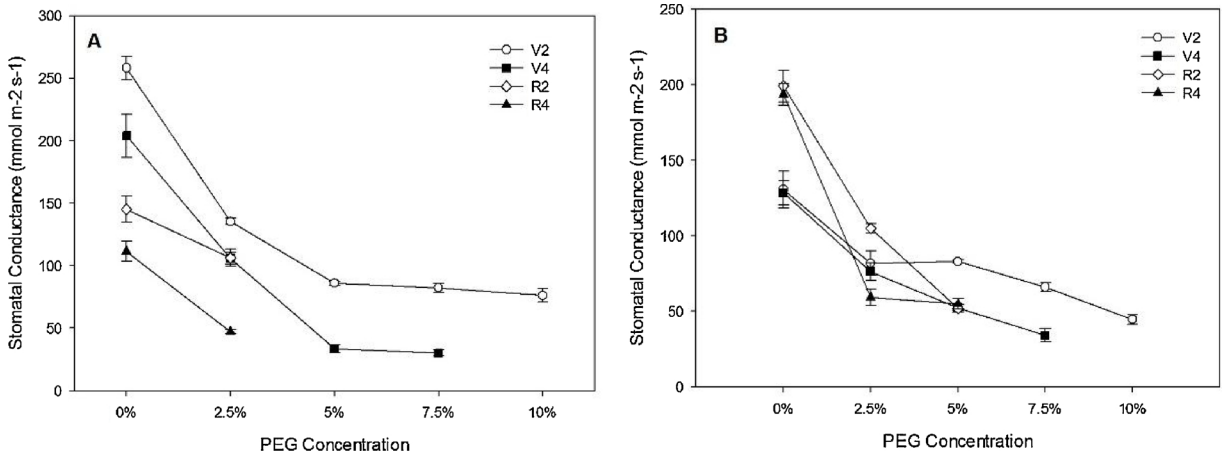

Fig. 7. Stomatal conductance of ES Mentor (A) and Pedro (B) in different PEG concentrations at different stages.

planting between control plants and plants suffered from drought stress for 24 days in early stages; however, later in the same experiment (100 days after planting) the difference was significant. Decrease in F_v/F_m was concluded to be an indication of down regulation of photosynthesis [38]. Liu et al. [39] also observed a decline in F_v/F_m ratio in drought stressed plants of two maize cultivars. This occurrence of chronic photoinhibition was justified as a result of photo-inactivation of PSII centers [37]. Compared with the control, water stress markedly decreased F_v/F_m (from 0.80 to 0.76) and ΦPSII (from 0.69 to 0.58) [29]. Consistently with our results, water stress treatment reduced total chlorophyll content and chl_a/chl_b, indicating the decreased capacity of absorbing and conversion of light energy, which may be the reason of reduced ΦPSII [29]. On the contrary, drought stress did not have an effect on F_v/F_m in dry bean [40].

Table 4
Correlation between the studied traits and PEG concentrations.

Cultivar	ES Mentor				Pedro			
Stage	V2	V4	R2	R4	V2	V4	R2	R4
Porometer	−.855**	−.924**	−.840*	−.969**	−.887**	−.946**	−.976**	−.873**
SPAD	−.449	−.591*	−.422	−.899*	−.430	−.027	−.964**	−.764*
F_v/F_m	−.366	−.814**	−.376	−.141	−.583*	−.594*	−.595	−.330
F_m	.145	−.783**	.188	.226	−.446	−.400	−.670-*	−.414
F_v	.023	−.841**	.022	.221	−.562*	−.439	−.759*	−.422
F_0	−.013	−.069	−.369	.282	.124	−.342	−.286	−.049
F_v/F_0	.014	−.651*	.190	−.306	−.493	−.360	−.413	−.274
F_m/F_0	.109	−.598*	.342	−.338	−.389	−.280	−.364	−.273
ΦPSII	−.316	−.170	.636	−.505	−.562*	.116	−.240	−.441
Chl_a	−.453	−.309	−.914*	−.823*	−.337	−.346	−.502	−.253
Chl_b	−.375	−.647*	−.991**	−.825*	−.697**	−.862**	−.256	−.584
Chl_{x+c}	−.559*	−.275	−.607	−.941**	−.179	−.478	−.408	−.344

* Correlation is significant at 0.05 level (2-tailed).
** Correlation is significant at 0.01 level (2-tailed).

Table 5
Effect size of PEG concentration, cultivar and PEG concentration*cultivar on the studied traits.

	SPAD	Chl_a	Chl_b	Chl_{c+x}	F_v/F_m	ΦPSII	g_s
V2							
PEG concentration	22	17.8	42.2*	21.6	31.2*	24.1	67.5*
cultivar	5.6	13.9*	4.5	13.7*	0.1	4.1	16.0*
PEG concentration*cultivar	61.5*	8.6	81.5*	14.8	25.4	32.3	86.6*
V4							
PEG concentration	32.7*	12.7	35.6*	0.5	50.2*	15.0	83.6*
cultivar	19.9*	10.4	39.1*	0.4	2.9	2.1	3.2
PEG concentration*cultivar	37.5	5.7	84.5*	15.8	7.1	14.6	74.3*
R2							
PEG concentration	54.4*	24.7	48.2*	26.4	7.9	27.6	83.8*
cultivar	4.9	5.3	12.6	0.3	20.5	0.2	0.5
PEG concentration*cultivar	38.8*	49.9*	90.8*	4.5	2.3	0.0	57.1*
R4							
PEG concentration	14.0	35.8	19.9	54.2*	5.2	22.3	75.7*
cultivar	4.4	4.2	14.2	0.1	5.0	4.6	4.2
PEG concentration*cultivar	79.1*	21.2	12.1	1.4	2.1	1.2	79.1*

* The effect size (Partial Eta Squared) is significant at 0.05 level.

Drought stress caused stomatal conductance to remarkably decrease as compared to control treatments in both cultivars; moreover, in most cases the more severe the drought, the more reduction percentage the stomatal conductance was. Previously, Ohashi et al. [41] reported that stomatal conductance of soybean plants significantly declined under water stress; similar results were reported by Zhang et al. [21] who found a decrease in g_s by 98.8 % under drought; they concluded that this decrease in g_s may be caused by the reduced open stomata ratio and stomatal aperture size in exposed water-stressed plants. Hao et al. [1] also reported a significant reduction in stomatal conductance from 0.25 to 0.10 mol H_2O m^{-1} s^{-1}. Mathobo et al. [22] justified the reduction in g_s in their experiment by the stomatal closure which prevented CO_2 from entering the leaf. A 70 % reduction of g_s after 22 days of drought stress was observed in dry bean [42]. Tang et al. [29] concluded that PEG-induced water stress significantly decreased g_s by 73 %.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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