PEG-MEDIATED OSMOTIC STRESS RESPONSES OF WHEAT-BARLEY ADDITION LINES

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Abstract: Photosynthetic responses of three wheat-barley addition (add) lines exposed to PEG-induced drought stress and under rewatering period were investigated in order to improve wheat drought tolerance by the help of barley chromosomes. The wheat-barley disomic addition lines (2H, 3H, 4H) the wheat line (Triticum aestivum L. cv. 'Mv9kr1') were found to have better responses to osmotic stress relative to the parental barley cultivar (Hordeum vulgare L cv. 'Igrí'). Addition lines with 2H and 4H chromosome from barley used similar strategy of acclimation to osmotic stress. These lines were able to avoid drastic water loss as well as exhibiting only a slight decrease in stomatal conductance ($g_s$) in contrast to barley. At the same time, photosynthetic processes in 4H addition seemed to be more sensitive to the decreased relative water content (RWC) of leaves caused by 21% PEG resulting reduction in stomatal to non-stomatal limitation ratio and impaired recovery ability. 3H addition line could be characterized as the most dehydration tolerant among the examined lines on the basis of water wasting responses shown by high $g_s$, decreased intrinsic water use efficiency and more successfully sustained shoot biomass production in contrast to root. Changes in Y(II) parameters were moderate in the addition lines indicating that the electron transport processes were not damaged by osmotic stress. Our results suggest that wheat line also avoided being dehydrated similar to 2H and 4H add but the relatively high RWC under severe water deficit was primarily due to the pronounced stomatal closure. Changes in shoot-root ratio and net CO$_2$ assimilation rate ($P_N$) was also similar to those in 4H add. Although the maintained root growth and strong decreased $g_s$ may be the indicators of drought avoidance in barley, in spite of these traits low RWC was observed which contributed to the significantly impaired $P_N$ primarily limited by the non-stomatal processes. Considering to drought sensibility, we concluded that barley genotype Igrí is not the most suitable gene source for improving water stress tolerance of wheat but 2H addition line seemed to be more resistant to osmotic treatments than wheat and could be used in wheat breeding programs in the future.

Keywords: barley, biomass production, drought avoidance, drought tolerance, leaf gas exchange, wheat

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INTRODUCTION

Water shortage, increased requirement for food production and/or less and less arable land are thought to be the main problems of the annual agricultural which demand to develop crop plants with an acceptable productivity (Tardieu 2012). It is estimated that wheat (*Triticum aestivum* L.) global grain yield must be doubled (Rajaram 2001) even under unfavourable environmental effects such as limited water availability. Improvement for adaptation to drought can be achieved by transposition of genomic regions controlling the survival of plants under water deficit. Several quantitative trait loci (QTLs) associated with drought-related traits have been determined in barley therefore it could be a potential gene source for development of drought tolerance in wheat. For example, QTLs affecting osmotic adjustment, relative water content and water soluble carbohydrates were mapped on 2H, 3H and 4H barley chromosomes (Teulat *et al.* 2002, Diab *et al.* 2004). In malting barley, QTLs for photochemical activity of PSII are also located on the 2H, 3H and 4H barley chromosomes (Wójcik-Jagła *et al.* 2013). Selecting plants which have efficient water saving strategy may be a way to minimise the water consumption of agriculture (Condon *et al.* 2004). Genomic regions controlling the rate of water-use-efficiency (WUE) have been also determined in the barley genome (Chen *et al.* 2012).

The first reaction of a plant stressed by drought may be a decline in water loss through the decrease of stomatal aperture (Henson *et al.* 1989). Stomatal conductance ($g_s$) makes the determination of an important factor associated with drought tolerance, called intrinsic water use efficiency ($WUE_i$) possible (Ehdaie and Waines 1993, Molnár *et al.* 2007). This value is controlled by photosynthetic CO$_2$ assimilation processes and stomatal resistance to water loss (Sinclair *et al.* 1984); therefore it can be determined as the ratio of $P_N$ and $g_s$, as described by Martin and Ruiz-Torres (1992). It has been reported that closed stomata result increased WUE through the inhibition of transpiration more than CO$_2$ diffusion into the chloroplasts at the initial stage of water deficit (Chaves *et al.* 2009). Not only the WUEi but the biomass productivity of crop cultivars may be important criteria for drought tolerance (Blum 1993), but WUEi is not always positively correlated to crop yield (Tuberosa 2012). Through decreased $g_s$
which may occur quickly as a short-term adaptation mechanism, plants are able to moderate water loss under water scarcity (Chaves 1991, Cornic 1994, Molnár et al. 2004), thereby relative water content (RWC) goes down very little. Despite of this fact stomata also could play role as a limiting factor against the diffusion of CO₂ into the chloroplasts of mesophyll cells which is termed as stomatal limitation ($L_s$), followed by parallel declining in the net photosynthetic rate ($P_N$) under water stress (Cornic 2000, Lawlor and Cornic 2002, Medrano et al. 2002). Not only the value of $P_N$ but also many other photosynthetic parameters such as carboxylation efficiency or electron transport rate may show a strong correlation with $g_s$ even more than water status itself (Medrano et al. 2002). Some studies describe $g_s$ as an important determining factor in the change of $P_N$ even at severe water deficit and the role of metabolic factors may be unconsidered (e.g. Cornic and Fresneau 2002), while $g_s$ and metabolic factors (RuBP and ATP) could limit together the assimilation even under moderate drought according to others (Tezara et al. 1999, Medrano et al. 2002). Brodribb (1996) described a biphasic model according to the changes in the intercellular CO₂ concentration ($C_i$) under increasing drought. In the first phase, a substantial reduction in $C_i$ was noticed as $g_s$ decreased. In the second non-stomatal phase, increment in $C_i$ and irreversible photoinhibition was detected while $g_s$ reached a minimum level.

Plant biomass is a crucial parameter but not necessarily correlated with grain yield in wheat under drought stress (Paul et al. 2016). At the same time the asymmetric growth of root and shoot makes it possible to compensate negative effects of unfavourable water supply. More intense root dry biomass production compared to shoot dry matter may contribute to higher relative water content (RWC) in plants which avoid being water stressed (Morgan 1984). The higher root biomass could also result an increased drought tolerance (Hoffmann and Burucs 2005) since low RWC affects particular processes of photosynthesis negatively (Chaves 1991, Cornic 1994). Therefore, preservation of water status may become significant to maintenance assimilation capacity and growth (Akram 2011).

Under water deficit photochemistry declines and energy dissipation shows higher value (Guo et al. 2013) resulting imbalance between energy capture and metabolism (Lawlor and
Tezara 2009). Under drought conditions when the CO$_2$ assimilation is impaired it is important to protect chloroplast against extra harmful reduction force by terminal dissipation of excess light energy from the PSII reaction centre (Ruban and Horton 1995, Horton et al. 2005). This rapidly activated regulatory mechanism could be detected as non-photochemical quenching (NPQ) (Horton et al. 2005). When lack of water becomes scarce, the quantum yield of non-photochemical quenching (YNPQ) and the amounts of the products of xanthophyll cycle (zeaxanthin and antheraxanthin) increased significantly (Tambussi et al. 2002) thereby may be minimize the damage of PSII. If plants are high sensitive to water deficit, damages may occur in PSII reaction centres (Murata et al. 2007) resulting intensification in the quantum yield of nonregulated energy dissipation (Y(NO)).

The goal of this study was to compare photosynthetic responses to the polyethylene-glycol (PEG) induced water deficit in three wheat-barley addition lines in relation to the wheat and barley parental genotypes. The measured parameters were employed as a selection system for sorting wheat-barley introgression lines that have better drought tolerance and/or recovery capacity than the wheat genotype. The basis of our selection is the values of relative water content, biomass production, gas exchange and fluorescence induction parameters characterizing the ability of drought resistance suggested by literature data.

**MATERIALS AND METHODS**

**Plant materials**

*Triticum aestivum* L. cv. ‘Mv9kr1’ wheat line, *Hordeum vulgare* L. cv. ‘Igri’ barley cultivar and Mv9kr1-Igri wheat-barley disomic addition lines 2H, 3H and 4H (2H add, 3H add and 4H add, respectively) (developed by crossing Mv9kr1 wheat with Igri barley) were investigated. Genotypes were produced in the Agricultural Research Institute of Hungarian Academy of Sciences (Martonvásár). The disomic addition lines carry the full genome of wheat and one extra homologous chromosome pair of barley genotype ‘Igri’ 2H, 3H or 4H (Molnár-Láng et al. 2000).
Culture condition and induction of osmotic stress
Seedlings were grown in 1500 cm$^3$ pots containing half-strength modified Hoagland nutrient solution (Nagy and Galiba 1995) in growth chambers with normal CO$_2$ concentration at 20/25°C. The light intensity for growth was 200 μmol (photon) m$^{-2}$ s$^{-1}$ and circadian illumination 12 h dark/12 h light was applied. Water deficit was induced in 4-week old plants by increasing the osmotic pressure of the hydroculture medium through the addition of polyethylene glycol (PEG 8000, Sigma, St. Louis, MO). Measurements were made after the 7-day treatment with 15% and 21% PEG and after 2 and 7 days of rewatering. The applied PEG concentrations resulted in osmotic potentials of −0.7 MPa and −1.75 MPa. All the experiments were performed on intact leaves or leaf segments of wheat cultivars and the hybrid lines.

Determination of RWC and dry matter production
Drought response of plants was monitored through determination of relative water content (RWC) and dry matter production of roots and shoots. The RWC was determined as RWC = (FW − DW) x 100 / (SW − DW), where FW is the fresh weight, SW is the water-saturated weight and DW is the oven dry weight for 12 h at 105°C. Dry matter productions were estimated by harvest method. The shoot and root dry mass (g/plant) was determined on 7-week old plants at the end of whole experimental period and data were compared with the values for control plants of same age, grown in Hoagland solution without PEG.

CO$_2$ gas exchange and chlorophyll fluorescence measurements
The CO$_2$ assimilation of intact leaves was measured with an infrared gas analyser (Analytical Development Co. Ltd., United Kingdom) with 6.25 cm$^2$ assimilation surface. The net CO$_2$ assimilation rate ($P_N$), stomatal conductance ($g_s$) and intercellular CO$_2$ concentration ($C_i$) were calculated in the light-saturated state of photosynthesis using the equations of von Caemmerer and Farquhar (1981) at 360 ppm CO$_2$ levels under 1,000 μmol(photon) m$^{-2}$ s$^{-1}$ light intensity. $L_s$ and $L_{ns}$ parameters were obtained from $P_N$ versus $C_i$ curves as described by Lawlor (2002), between 0–1,000 ppm CO$_2$ at light saturated state of photosynthesis using a gas.
diluter. The intrinsic water use efficiency (WUE$_i$) will be calculated as $P_N/g_s$ as described by Martin and Ruiz-Tores (1992).

Changes in fluorescence efficiency were measured with a pulsed-amplitude modulation fluorometry (PAM 101-103, Heinz Walz Effeltrich, Germany). The minimal fluorescence yield of the dark-adapted state ($F_0$) was detected after 15 min dark adaptation. The maximal fluorescence yield of the dark-adapted state ($F_m$) and maximal fluorescence yield of the light-adapted state ($F_m'$) were determined by applying saturating flashes ($8,000$ μmol m$^{-2}$ s$^{-1}$) lasting 0.8 s. Photosynthesis was induced by continuous illumination (actinic light) of leaves at $1,000$ μmol m$^{-2}$ s$^{-1}$ light for 15 min. The fluorescence parameters were calculated as described by van Kooten and Snel (1990) and Klughammer and Schreiber (2008a) on the basis of the following equations: effective quantum yield of PS II, $Y_{II} = \frac{(F_m' - F)}{F_m'} = \frac{\Delta F}{F_m}$; quantum yield of regulated energy dissipation, $Y_{NPQ} = \frac{F}{F_m} - \frac{F}{F_m'}$, quantum yield of non-regulated energy dissipation, $Y_{NO} = \frac{F}{F_m}$.

**Statistics**
The results are the means ± LSD5% of five measurements on different plants per treatment for CO$_2$ gas exchange, chlorophyll fluorescence and RWC parameters and of eight measurements per treatment for the biomass parameters.
RESULTS AND DISCUSSION

Impact of PEG-induced water stress on relative water content, dry matter production and gas-exchange parameters

The alterations of some processes in photosynthesis could be partly attributed to the changes in RWC under different water conditions (Chaves 1991, Cornic 1994) hence RWC can be considered as a sensitive indicator of drought stress (Clavel et al. 2005). The reduction in RWC was much more pronounced in Igri (barley) compared to the other lines when the 15% PEG was applied (Figure 1A). Under more severe drought (21% PEG), 3H add also reacted remarkable drop while 2H add, 4H add and Mv9kr1 (wheat) showed RWC of approximately 90%. At the same time, RWC values of barley and 3H add recovered rapidly after the second day of re-watering. The high RWC in wheat could be attributed to significant closure of stomata at 21% PEG contrast to 2H and 4H add lines (Figure 1B) which have retained hydration of their tissues even with a higher stomatal conductance ($g_{as}$) in contrast with 3H add line. 3H add also did not closed its stomata under the treatments but this reaction reflected in lower RWC. When the reduction in $g_s$ is more intensive, than that in assimilation rate ($P_N$) results improved intrinsic water use efficiency (WUE$_i$). The stomatal control led to an increase in WUE$_i$ in wheat and barley under 21% PEG in relation to moderate stress (Figure 1C). In the case of wheat, the better WUE$_i$ was primarily attributed to the significant drop in $g_s$ and less to the changes in $P_N$ (Figure 2A). 3H and 4H add showed the lowest WUE$_i$ value at severe water deficiency, primarily caused by higher $g_s$ and not by a large reduction in $P_N$. Treatment with 15% PEG significantly reduced $P_N$ in all genotypes but the decrease was the most prominent in barley. Raising the PEG concentration (21% PEG) similar changes in $P_N$ were represented by wheat and 4H add, it means approx. 34% and 37% loss of control value while the impairing was milder (approx. 23% loss) in 2H and 3H add line. Usually, drought tolerant barley cultivars show successfully maintained RWC of leaves when water availability is limited (Matin et al. 1989). In our research $P_N$ was strongly correlated with RWC in wheat ($R^2=0.99$) and in barley ($R^2=0.97$) under osmotic treatments so the assimilation associated processes are more sensible to the loss of water content in parental lines but less in the
addition lines, especially in 3H add. Inhibition of $P_N$ was the most pronounced in the case of barley with 67% loss compared to its own control but responded fast with only 26% loss to the recovered RWC under the re-watering period. None of the examined lines showed full recovery of $P_N$ but the most prominent result was detected in 3H add with less than 8% loss of control. 15% PEG induced significantly higher intercellular CO$_2$ concentration ($C_i$) in the case of all genotypes excepting 2H add (Figure 2B). Further substantial increment was observed only in barley. If $C_i$ shows increase at low $g_s$ not only stomatal resistance but also impaired metabolic processes may be responsible for the inhibited photosynthesis (Brodribb 1996, Zhou et al. 2007). Consequently, metabolic limitation became significant in barley at the maximum water deficit indicated by higher $C_i$ and strong stomatal closure. Although 21% PEG also significantly enhanced $C_i$ in 3H and 4H add relation to their control level but it was noticed under substantially less decrease of stomatal aperture than in the barley line.

It is also essential whether the stomatal or non-stomatal limitation is dominated in the impaired CO$_2$ assimilation. At mild and moderate water stress stomata closure is the primal inhibitor of photosynthesis and less affected by biochemical processes (Bota et al. 2004), but at stronger water deficit the role of impaired metabolic processes may intensify at low $g_s$ (Flexas and Medrano 2002), the latter is termed as non-stomatal limitation of photosynthesis ($L_{ns}$). $L_{ns}$ reached higher values compared to stomatal limitation ($L_s$) in the examined lines even under slight osmotic stress (Figure 2C, D). When water deficit intensified, further decreased in the $L_s/L_{ns}$ ratio was noticed in 4H add and barley with different stomatal response. In contrast to this observation, 2H add was the only line in which $L_s$ exceeded $L_{ns}$ value under 21% PEG indicating better drought tolerance. In the beginning of regeneration period $L_s$ became the dominant factor in relation to $L_{ns}$ in wheat contrast with Igri, 4H add and 3H add. Although $L_{ns}$ was not abolished by 7-day rewatering in the case of any lines at the same time barley showed the most substantial reduction in relation to its value under 21% PEG indicating prominent ability to recover after water deficit.

The root and shoot dry weight of wheat and 4H add were equally depressed by osmotic treatments contributing to the
unchanged shoot/root ratio (Table 1) The root dry mass production of 2H add and 3H add was also negatively affected by deficient water availability (approx. 32% loss) more than shoot growth resulting raised shoot/root ratio especially in case of 3H add. Although barley maintained root growth more successfully than the other lines the shoot production was the most limited by water deficit resulting significantly reduced shoot/root ratio. More intense root growth compared to shoot could result higher RWC (Morgan 1984) through maximizing water uptake. Not only the asymmetric growth but the closure of stomata is the main feature of plant following drought avoidance strategy. Although the root growth of barley and the stomatal responses are indicative of drought avoidance in spite of these features barley was not able to sustain its water status indicating water stress sensitivity. Moreover, the decreased shoot/root ratio under drought conditions may contribute to yield loss due to the reduced assimilating area (Hoffmann et al. 2009).

Impact of PEG-induced water stress on chlorophyll a fluorescence parameters
Chlorophyll fluorescence measurement can be applied to discriminate between drought tolerant and sensitive wheat (Sayar et al. 2008). The effective quantum efficiency of PSII photochemistry (Y(II)) is directly related to the assimilation rate (Edwards and Baker 1993) therefore YII was utilised to examine perturbation of photosynthesis performance. Disturbance of CO₂ fixation and/or the damaged PSII contribute to the reduced Y(II). The latter may be caused only by stronger stress whereby PSII damages structurally (Tambussi et al. 2005). Y(II) began to decrease significantly in 4H add and in the parental lines even at 15% PEG concentration but no further noticeable reduction was observed when drought became more pronounced (Figure 3A). In the case of 2H add drought events did not cause significant depression on Y(II) moreover it was noticed no considerable changes in chlorophyll a fluorescence parameters were measured under the whole experiment reflected by negligible L₉₅ too. In the end of re-watering period addition lines could recover successfully their Y(II) while this parameter did not reach the value that of control in barley and wheat.
Under drought conditions when CO$_2$ availability is limited by depressed $g_s$, absorbed light energy can exceed that energy which is needed for Calvin cycle thus may result increment of the photoprotective processes (Chaves et al. 2009) such as NPQ and down regulation in Y(II) (Demmig-Adams et al. 1996). Y(NPQ) intensified substantially in parental lines when 15% PEG was applied parallel with the downregulation of Y(II) but further changes were not measured under severe water deficit. (Figure 3A, B). In the case of 3H add and 4H add, 15% PEG resulted higher Y(NPQ) but it was diminished to the control level by 21% PEG in 3H add and remained constant in 4H add. Induction of Y(NPQ) was not necessary in 2H add during the limited water availability indicating a positive effect of maintained $g_s$ against the over-reduction of photosynthetic electron transport rate. The 7-day rewatering caused a reduction in Y(NPQ) for the most genotypes especially for barley in relation to value under 21% PEG.

If plants are highly sensitive to water deficit, damages could be detected in PSII reaction centres (Murata et al. 2007) as it reflects by the increment in the quantum yield of nonregulated energy dissipation (Y(NO)) in barley already at 15% PEG level (Figure 3C). At the same time only slight alterations were detected in Y(NO) for the addition lines and wheat under stress conditions. Moreover, this difference in Y(NO) values between the barley and the other lines showed further increment at maximum water deficit. High Y(NO) in barley could contribute to the insufficient recovery of Y(II) under favourable water supply while it was attributed to Y(NPQ) rather than PSII damages in the case of wheat. These results suggest that drought treatments had no noticeable effect on the capacity of primary charge separation in wheat and addition lines. These plants were able to compensate the effects of excess light mostly through the photoprotective regulated dissipation mechanisms while the intensified Y(NPQ) seems to be not enough efficient to avoid PSII damages in barley.
CONCLUSIONS

We compare photosynthetic and physiological responses to two different degrees of water deficit and rewatering in wheat-barely derivatives with the parental lines. On the basis of parameters, it could be determined which hybrid could be suitable to increase the tolerance of wheat against drought stress.

Drought tolerance is a complicated trait involving several physiological, morphological and biochemical processes. Stomatal closure is the most efficient way to reduce water loss allowing impaired WUE (Chaves et al. 2009) but keeping high $g_s$ even under drought conditions has been considered a mode of drought resistance trait (Johnson et al. 1987). 2H add and 4H add not only kept open their stomata but also were able to retain their water status more successfully in contrast to barley and 3H add. Moreover, 2H add and 4H add maintained their RWC at a level similar to that of wheat without intense stomatal closure. This suggests that an active osmoregulation mechanism may exist in these lines contributing to efficient water uptake. In 2H add the CO$_2$ assimilation and the photosynthetic electron transport processes were slightly influenced by osmotic stress and shoot/root ratio showed increment but the biomass production was more depressed than that of wheat (Table 1). Although 2H and 4H add responded similar to osmotic treatments according to their $g_s$, 4H add seems to be more sensitive the loss in RWC. This sensitivity was shown by the decreased $L_s/L_{ns}$ ratio at stronger water deficit and the impaired ability to restore its $P_N$ value largely due to $L_{ns}$. 3H add could be characterized as the more dehydration tolerant genotype among the examined lines on the basis of water wasting responses shown by opened stomata, low WUEi and increased shoot/root ratio. This line showed the most promising shoot biomass production under the water deficit. Despite of significant decreased RWC 3H line seems to be less sensitive to water loss than barley according to their $P_N$ and $L_{ns}$, $Y$(II) and $Y$(NO). Although treatment with 15% PEG was accompanied by significant drop in $P_N$ in the case of all genotypes under stronger osmotic treatment wheat line was able to produce a satisfactory assimilation level in spite of low $g_s$ and after all its WUE$_i$ value elevated to the control level. It seems that wheat avoids being dehydrated by the lower $g_s$ at more severe osmotic effect. This response might be essential to
preserve water content of leaves which were strongly correlated with $P_N$ under the PEG treatments. The less limited CO$_2$ fixation and increased Y(NPQ) may contribute to protect PSII from damages but recovery of Y(II) and $P_N$ were not fully. At the same time, among the examined lines root and shoot biomass production of wheat was the most pronounced. From our results, barley seems to be the most drought sensitive genotype, as regards RWC, $P_N$, $L_{ns}$ and Y(NO) parameters in spite of the maintained WUEi and root growth. The change of Y(II) in barley was similar to its $P_N$ indicating a close correlation ($R^2=0.89$) under the whole experiments. Consequently, the decreased Y(II) may be attributed to the significant disturbance of CO$_2$ assimilation indicated by elevated $L_{ns}$ and suggests low tolerance to water deficit in Igri. In spite of the unfavourable responses of barley, its regeneration ability is prominent as far as RWC and $P_N$ are concerned. It may the consequence of the less retention of root growth under water deficit.

Finally, the results suggest that, photosynthetic responses of 2H add were less sensitive to water stress than the parental wheat genotype and could be useful genetic material in wheat breeding programmes. At the same time wheat also showed significantly better drought tolerance than barley therefore the examined barley genotype is not the most suitable gene donor for improving drought stress tolerance of bread wheat.
Figure 1. Effects of increasing osmotic stress followed by 7 days of regeneration on relative water content (RWC) (A), stomatal conductance \( (g_s) \) (B), intrinsic water-use-efficiency (WUEi) (C) under 1,000 \( \mu \text{mol (photon)} \text{ m}^{-2} \text{ s}^{-1} \) light intensity. Vertical bars represent ± SD.
Figure 2. Effects of increasing osmotic stress followed by 7 days of regeneration on net photosynthetic rate ($P_N$) (A), intercellular CO$_2$ concentration ($C_i$) (B), stomatal limitation ($L_s$) (C), nonstomatal limitation ($L_{ns}$) (D) under 1,000 μmol (photon) m$^{-2}$ s$^{-1}$ light. Vertical bars represent ± SD.
Figure 3. Effects of increasing osmotic stress followed by 7 days of regeneration on effective quantum yield of PS II photochemistry (Y(II)) (A), quantum yield of regulated energy dissipation (Y(NPQ)) (B), quantum yield of non-regulated energy dissipation (Y(NO)) (C) under 1,000 μmol (photon) m⁻² s⁻¹ light intensity. Vertical bars represent ± SD.
Table 1. The shoot/root ratio and biomass production of root and shoot (g/plant) expressed in terms of dry matter for 21% PEG-treated (stress) and control plants of similar age grown in nutrition solution without PEG (control).

| Genotypes          | Control | Stress | Control | Stress | Control | Stress |
|--------------------|---------|--------|---------|--------|---------|--------|
| Shoot/Root Ratio   | 2.76    | 3.187  | 2.76    | 3.187  | 2.76    | 3.187  |
| Shoot dry weight (g) | 0.047±0.031 | 0.034±0.022 | 0.047±0.031 | 0.034±0.022 | 0.047±0.031 | 0.034±0.022 |
| Root dry weight (g) | 0.024±0.019 | 0.016±0.011 | 0.024±0.019 | 0.016±0.011 | 0.024±0.019 | 0.016±0.011 |
| Shoot/root ratio   | 1.198   | 1.857  | 1.198   | 1.857  | 1.198   | 1.857  |

PEG: Polyethylene glycol
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