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Original Article

Comparison of leaf osmotic adjustment expression in wheat (*Triticum aestivum* L.) under water deficit between the whole plant and tissue levels

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ABSTRACT

This study compared osmotic adjustment (OA) expression and solutes involved in leaves of wheat with high OA capacity (cv. Hartog) under water deficit (WD) in the glasshouse (whole plant level) and laboratory (tissue level). WD was applied at the reproductive stage for the whole plant level and WD was induced at the tissue level using polyethylene glycol (PEG) 8000 as a non-permeating osmotic agent. In the whole plant Experiment, leaf OA was expressed at 16 days (0.26 MPa) and increased to 0.37 MPa at 37 days of treatment. In the tissue level experiment, exposure of leaf segments to PEG 8000 treatments of 0, −0.5, −1.0 and −1.5 MPa and sampling times of 0, 12, 24, 48 and 72 h showed that the maximum leaf OA (0.37 MPa) was expressed on PEG −0.5 MPa after 48 h of treatment. K^+, glycinebetaine and proline accounted for 21, 19 and 21% of OA in the glasshouse experiment. K^+ did not contribute to the OA, while Na^+ and proline only accounted for 5 and 1% in the laboratory experiment. Although OA was expressed in leaf segments of wheat subjected to WD under PEG -0.5 MPa, the laboratory-based PEG method with leaf segments could not substitute for the glasshouse experiment for screening germplasm for OA capacity.

Introduction

Crops that can capture more water for use in transpiration are required to increase yields when water supply is limited (Reynolds et al., 2005). Some characteristics related to soil water capture are the osmotic adjustment (OA) capacity, early vigor and a deep root system. The characteristic examined in this study was the OA capacity. OA is a common cellular response to water deficit (Zhang et al., 1999) and also an important characteristic of plant drought tolerance (Chandra Babu et al., 1999a). OA refers to the lowering of osmotic potential because of the net accumulation of solutes in response to water deficit (Condon, 1982; Zhang et al., 1999). OA occurs when new solutes are accumulated, not when existing solutes are concentrated due to water loss. Solute accumulation attracts water into the cell and tends to maintain cell turgor pressure, and thus OA is an acclimatized value as soil water potential declines (Chandra Babu et al., 1999b).

There are several advantages of OA at different levels of plant organization, such as for the whole plant and tissues that enables plants to maintain reasonable yield under water deficit. The benefits of OA at the whole plant level are enabling photosynthesis and growth of some parts of the plant to be maintained under water deficit (McNaughton, 1991), supporting the growth and survival of plants under severe water deficit (Pugnaire et al., 1999), extracting more water (1.3-fold) in wheat genotypes with higher OA capacity than those with lower OA capacity (Morgan and Condon, 1986) and maintaining leaf water status and productivity in rice during flowering (Lilley and Ludlow, 1996). An advantage of OA at the tissue level is increasing leaf tissue survival in non-irrigated rice so that leaf damage was half of irrigated plants at a leaf water potential of −4.0 MPa (Hsiao et al., 1984).

Some screening methodologies for assessing osmotic adjustment for crop improvement under water deficit (WD) can be

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carried out in field Experiments, controlled conditions (glasshouses and controlled-environment rooms) and in the laboratory. In this study, however, the focus was on comparing OA expression and the solutes involved in leaves of wheat with a high OA capacity (cv. Hartog) under WD in the glasshouse (whole plant level) and laboratory (tissue level). Wheat cv. Hartog was chosen to observe the leaf OA expression in this study, because it was reported to have a high OA capacity (Morgan, 2001). WD was applied at the reproductive stage for the whole plant level experiment and WD was induced at the tissue level using polyethylene glycol (PEG) 8000 as a non-permeating osmotic agent. PEG is suitable for imposing water deficit on plants because it blocks the pathway of water movement, reduces water absorption and causes desiccation of the plant (Lawlor, 1970).

Materials and methods

Experimental design and treatments

Experiment 1: leaf osmotic adjustment of cv. Hartog under water deficit at the whole plant level

Experiment 1 consisted of one cultivar grown independently in two different water regimes (WW and WD) where each plant was sampled six times with four replicates in a randomized block design. Each plant was placed in a separate container or Experimental unit. Cultivar Hartog was used because it was reported to have a high OA capacity (Morgan, 2001). The water regimes, WW and WD were obtained by withholding water from WD containers at the reproductive stage (treatment period of 37 d) also defined as the stage between ear initiation at the six fully-expanded leaves stage (40 d after sowing) and anthesis. The samples of WD treatment were collected initially and then after 9 (when leaves started to roll), 16, 23, 30 and 37 d of drying. The samples of WW (control) treatment were collected at 0, 16 and 37 d (Nio et al., 2011b).

Experiment 2: leaf osmotic adjustment in leaf segments of wheat cv. Hartog at the tissue level

Experiment 2 consisted of four treatments with PEG 8000 (0, −0.5, −1.0 and −1.5 MPa) and five sampling times (0, 12, 24, 48 and 72 h) with three replications in a randomized block design. Based on Michel (1983), PEG 8000 solutions of 0 (basal), −0.5, −1.0 and −1.5 MPa were formulated by adding 0, 198, 287 and 355 g, respectively, in 1 L basal solution. PEG treatments were imposed at each −0.5 MPa step, every 12 h. For treatment of −1.0 MPa, the osmotic potential of solution was lowered from −0.5 to −1.0 MPa after 12 h. For treatment of −1.5 MPa, the leaf segments were moved from PEG -0.5 to −1.0 MPa after 12 h and from −1.0 to −1.5 MPa after another 12 h (Nio et al., 2011a).

Cultural practices

Experiment 1: leaf osmotic adjustment of cv. Hartog under water deficit at the whole plant level

The experiment was conducted in containers (25 L) in a controlled-environment chamber with 10 h of light and 14 h darkness at 19/14 °C and relative humidity of 70%. The average irradiance in this room during the light-exposed duration was 463 µmol quanta $m^{-2}s^{-1}$ at plant height.

Richgro® potting mix (50% GigGin loam, 10% jarrah sawdust, 40% river sand, with limestone, gypsum, NPK, Mg, and Fe) was used. Before planting, 23 kg of soil in each container were supplied, with a basal fertilizer containing: 33.1 g superphosphate, 40 g ammonium nitrate, 5.5 g Richgro® complete micronutrients, 22 g calcium sulphate (gypsum), and 9 g potash (K_2SO_4), all together providing 0.4 g N, 0.06 g P and 0.07 g K for each kilogram of soil. The drained upper limit (field capacity) was 11% and the lower limit (wilting point) of plant available water for this soil was 3% on a volumetric basis.

Polyvinyl chloride (PVC) pipes (150 mm diameter, 800 mm height) split lengthwise for ease of root access, and then resealed with PVC tape, were used as containers for this study. Plastic mesh net was placed at the base of each container to retain soil but allow drainage.

Seeds of wheat were surface-sterilized with 2% commercial bleach for 2 min, washed with deionized water and placed on moist filter paper in Petri dishes in a cabinet in darkness at 18 °C. After 2 d, germinated seeds were sown at 15 mm depth. After sowing, to minimize evaporation, the top of each container was covered with plastic cling wrap and the cover was removed after emergence (about 3 d). At 7 d, the emerged seedlings were thinned to one per container, and 350 g of basalt gravel (10–18 mm) was added to the soil surface to minimize soil evaporation. Before imposing the treatment, containers were watered to field capacity by weight every second day.

The nutrient solution used for watering contained 0.1% Phostrogen® to provide a diluted fertilizer in water that contained 1.27 mM N, 0.38 mM P, 2.22 mM K, 0.12 mM Mg, 0.11 Ca, 0.001 mM Fe, 0.0006 mM Mn, 0.002 mM B, 0.0002 mM Cu, 0.0001 mM Zn, 0.00008 mM Mo and 0.42 mM S.

When the treatments commenced, water containing additional nutrients was withheld from WD containers, whereas WW containers were watered to field capacity every second day with tap water only without additional nutrients (Nio et al., 2011b).

Experiment 2: leaf osmotic adjustment in leaf segments of wheat cv. Hartog at the tissue level

Segments of fully-expanded flag leaves (FEFL) of wheat cv. Hartog were used in this Experiment. The plants were grown in 12 L containers in a controlled-environment room with the conditions given earlier. Richgro mix was used as described earlier.

Seeds of wheat were surface sterilized in the same manner as was carried out in Experiment 1. Containers were watered to field capacity by weight every second day. The nutrient solution contained 0.1% Phostrogen® to provide a diluted fertilizer in water that contained 1.27 mM N, 0.38 mM P and 2.22 mM K as well as the other nutrients listed above in Experiment 1.

Flag leaves were detached from 81-day-old plants, that is in the middle part of the linear phase of grain growth. Leaves were rinsed with 0.5 mM $CaSO_4$ and immersed into basal solution. The composition of basal solution was based on being 0.1-strength for macronutrients and 0.25-strength for micronutrients of the nutrient solution used by McDonald et al. (2001) to grow Triticeae species. The basal solution (mM) contained 0.4 N, 0.02 P, 0.4 K, 0.25 Ca, 0.05 Fe, 0.04 Mg, Ca 0.25, S 0.3, Cl 0.3, B 0.00625, Mn 0.0005, Zn 0.0005, Ni 0.0003, Na 0.000125, Cu 0.000125, Mo 0.000125 and MES (buffer). The pH of the solution was 6.5 at the commencement of the experiments and 6.6–6.9 at the end of the experiments.

Leaves were cut into 1–2 cm segments and immediately transferred to 125 mL Erlenmeyer flasks (sealed with cling wrap plastic) and incubated in 100 mL basal solution and PEG 8000 solution depending on the treatment. All flasks were put on a rotary shaker (100 rpm) under continuous light (121 µmol quanta m^{-2} s^{-1}, PAR) in a 20 °C room (Nio et al., 2011a).

Measurements

Plant level

The same measurements were taken in Experiments 1 and 2, that is the water content (WC), relative water content (RWC),

osmotic potential (OP) and the concentration of inorganic and organic solutes in the water of plant leaves.

In Experiment 1, the youngest fully expanded leaf (YFEL) was collected at each sampling for measurement of the WC, RWC, OP and the concentration of inorganic and organic solutes. For each replicate, the YFEL of the main stem and tiller 1 (4 cm from the middle area of these leaves) on two plants was collected for the OP and solute measurements. The YFEL of tiller 2 of two plants was collected for the WC and RWC measurements. For the measurements of the leaf WC and RWC as well as leaf sap OP in Experiment 2, the leaves were rinsed for 2 min using 0.5 mM $CaSO_4$ and mannitol that had the same osmotic potential of expressed sap (OP) as the respective PEG treatment, followed by 0.5 mM $CaSO_4$ for 5 s. The leaf WC and RWC as well as leaf sap OP were measured using the same method as in Experiment 1.

The leaf WC and RWC were measured by weighing the fresh mass of excised leaf segments, floating the samples on 0.5 mM $CaSO_4$ for 24 h in darkness, weighing the turgid mass, drying in an oven for 48 h at 70 °C, and re-weighing the dried sample. $CaSO_4$ (0.5 mM) was used during the floating period to obtain the turgid weight, as it can maintain membrane integrity and minimize solute leakage into the apoplast (Meidel and Kirby, 1979). The WC (milliliters per gram dry mass) was calculated as (fresh mass - dry mass)/dry mass. The RWC (%) was calculated as 100 × (fresh mass − dry mass)/(turgid mass − dry mass).

For the OP measurement, the leaf tissues were placed in cryovials, frozen in liquid N_2, and kept in the freezer at −20 °C until analysis. Sap from samples (thawed while still in sealed vials) was squeezed using a simple press and 10 µL was analyzed using a Fiske one-ten osmometer (Fiske Associates; MA, USA). For measuring the concentration of inorganic and organic solutes, the leaf tissues were wrapped in aluminum foil, frozen in liquid N_2 and freeze-dried.

In Experiment 1, osmotic adjustment (OA) was calculated as the difference between the measured OP in leaves of the WD plants and the estimated OP as a result of concentration-effect of any change in the tissue WC in WD plants. The concentration-effect on OP was the proportional decrease in the leaf OP due only to any reduction in the WC under the WD and was equal to $OP_{WW} \times (WC_{WW}/WC_{WD})$. In Experiment 2, OA was calculated as the difference between the measured OP and the estimated OP as a result of any concentration-effect of any change in the tissue WC in the PEG treatments. The concentration-effect on OP was the proportional decrease in the leaf OP due only to the reduction in WC under the PEG treatments and was equal to $(WC_{control}/WC_{PEG}) \times OP_{control}$ (Ma et al., 2006; Ma and Turner, 2006). Although the RWC can also be used in this calculation, the WC was used to calculate the OA, because using the RWC was problematic in that the amount of water entering the apoplast/intercellular spaces of floating leaf tissues is uncertain (Ma et al., 2006; Ma and Turner, 2006; Boyer et al., 2008).

To calculate the contribution of solutes to the OA, the difference in concentration of individual solutes between the WD and WW (extra solutes) was expressed on a molar basis of the amount of water present in leaf samples and then calculated as the OP of extra solutes using the equation: $OP_{extra\ solutes} = M \times R \times T$, where M is mol L^{-1}, R is the gas constant and T is the absolute temperature (Chang, 1981). The contribution of extra solutes to OA (%) = 100 × ($OP_{extra\ solutes}$/calculated OA). As the WC and OP in the WW plants were not measured at days 9, 23 and 30 in Experiment 1, the OA values at these days were estimated by interpolation using a power function fitted to values of OA at days 0, 16 and 37. To calculate the contribution of each solute to the OA at days 9, 23 and 30, values of the WC, OP and the concentration of each solute in the WW plants were estimated by interpolating between measured values assuming a linear trend. This was consistent with the measured values for the WD treatment. The values at day 9 were estimated using a linear equation from days 0–16 and the values at days 23 and 30 were estimated using a linear equation from days 16–37.

K^+, Na^+ and Cl^-, were measured in freeze-dried leaf tissue samples. Dried samples were ground to a fine powder and the ions were extracted from 100 mg in 10 mL 0.5 M HNO_3 (exact amounts of ground tissue and acid were recorded). After 0.5 M HNO_3 was added to all samples, the samples in the vials were shaken at 30 °C in a dark room for 48 h. Diluted extracts were analyzed for K^+ and Na^+ (Jenway PFP 7 flame photometer; Sherwood Scientific Ltd; Cambridge, UK) and for Cl^- (Buchler-Cotlove chloridometer; Buchler Instruments Division Nuclear-Chicago; Fort Lee, NJ, USA). Analyses were confirmed by taking a certified reference tissue through the same procedures (Nio et al., 2011a, 2011b).

Glycinebetaine, proline and sucrose were measured in the freeze-dried leaf tissues. Approximately 100 mg of lyophilized powder was accurately weighed into a 50 mL centrifuge tube. Three mL of ice-cold 5% (v/v) perchloric acid was added and mixed using a vortex mixer before being centrifuged at 15,000 rpm for 30 min (Fan et al., 1993). The supernatant was collected and stored in a sealed glass vial on ice. The pellet was extracted a second time in 3 mL of ice-cold 5% (v/v) perchloric acid, as before. The supernatants were combined and the pH was adjusted to between 3.0 and 3.5 using K_2CO_3 to precipitate the perchlorate. The sample was again centrifuged and the supernatant collected and the volume measured. HPLC was used to measure compounds (glycinebetaine, proline and sucrose), using the following protocol: extract was filtered (0.22 µm) before injection into an HPLC (600 E) pump and 717 plus autoinjector and 996 photodiode-array [PDA] detector (Waters; Milford MA, USA) equipped with a Sugar-Pak column as described by Naidu (1998).

Statistical analyses

The data were analyzed using the Genstat for Windows 10th Edition software (VSN International; Hemel Hempstead, UK). Analysis of variance was used to observe significant differences and interactions among treatments (where $p < 0.05$, unless otherwise stated). The standard errors were determined using Microsoft Office Excel 2003 (Microsoft Office Software, Las Vegas, USA).

Results and discussion

Changes in leaf water relations during WD

Generally the WC, RWC, and OP in leaves of wheat cv. Hartog decreased under water deficit (WD) conditions in Experiments 1 and 2. The leaf WC decreased to 1.6 mL/g DM in Experiment 1 (Table 1), but it declined to only 2.9 mL/g DM in Experiment 2 (Table 2). The leaf RWC decreased exponentially with time to 50% in Experiment 1 and to 81% in Experiment 2. The lowest leaf OP was −3.8 and −1.3 MPa, respectively in Experiment 1 and Experiment 2. The lowest WC, RWC and OP in Experiment 1 were observed after 37 d of the WD treatment (Table 1). The lowest WC and RWC values in Experiment 2 were observed in PEG −1.5 MPa at 72 h (Table 2). The leaf OP in −1.5 MPa was not measurable as the samples would 'time out' before the freezing point was reached. The leaf OA was expressed in both experiments. In Experiment 1, the leaf OA was expressed at 16 d (0.26 MPa) and increased to 0.37 MPa after 37 d of treatment (Table 1). In Experiment 2, the leaf OA in −0.5 MPa PEG (Table 2) was significant at 24 h (0.15 MPa) and peaked at 48 h (0.37 MPa).

Table 1
Water content (WC), relative water content (RWC), measured and estimated osmotic potential (OP), and calculated osmotic adjustment (OA) for youngest fully-expanded leaf (YFEL) of wheat cv. Hartog at 0, 16 and 37 d of well-watered (WW) and at 0, 9, 16, 23 30 and 37 d of water deficit (WD) at 19/14 °C (10 h light/14 h darkness). Values are mean ± SE (n = 4). Modified from Nio et al. (2011b) with permission from the publisher.

Treatment	Day	WC (mL/g DW)	RWC (%)	Measured OP (MPa)	Estimated OP (MPa)	OA (MPa)
WW	0	5.09 ± 0.10	92.97 ± 0.51	−1.45 ± 0.05		
	16	5.39 ± 0.08	87.98 ± 0.74	−1.58 ± 0.05		
	37	2.89 ± 0.02	93.13 ± 0.18	−1.88 ± 0.04		
WD	0	5.09 ± 0.10	92.97 ± 0.51	−1.45 ± 0.05	−1.45 ± 0.05	0 ± 0
	16	3.45 ± 0.23	61.81 ± 5.52	−2.73 ± 0.20	−2.47 ± 0.19	0.26 ± 0.03
	37	1.56 ± 0.08	50.26 ± 3.40	−3.77 ± 0.27	−3.40 ± 0.32	0.37 ± 0.02

Note: WC, RWC, LWP and OP values at days 16 and 37 are significant differently between WW and WD ($p < 0.05$).

Table 2
Water content (WC), relative water content (RWC), measured and estimated osmotic potential (OP), and calculated osmotic adjustment (OA) for segments from the fully-expanded flag leaf (FEFL) of wheat cv. Hartog in four PEG 8000 treatments (0, −0.5, −1.0, and −1.5 MPa) at 0, 12, 24, 48 and 72 h. Treatments were applied as −0.5 MPa steps every 12 h. Values are mean ± SE (n = 3). Modified from Nio et al. (2011a) with permission from the publisher.

Treatment (MPa)	Hour	WC (mL/g DW)	RWC (%)	Measured OP (MPa)	Estimated OP (MPa)	OA (MPa)
0	0	3.38 ± 0.12	93.68 ± 0.22	−1.51 ± 0.01		
	12	4.35 ± 0.07	99.71 ± 0.02	−1.44 ± 0.02		
	24	4.73 ± 0.09	99.37 ± 0.36	−1.32 ± 0.07		
	48	5.14 ± 0.08	98.51 ± 1.21	−1.35 ± 0.17		
	72	4.89 ± 0.14	95.20 ± 2.73	−1.35 ± 0.31		
−0.5	0	–	–	–		
	12	3.96 ± 0.09	98.79 ± 0.15	−1.49 ± 0.01	−1.58 ± 0.01	−0.09 ± 0.00
	24	4.44 ± 0.05	96.75 ± 0.66	−1.50 ± 0.03	−1.35 ± 0.10	0.15 ± 0.04
	48	4.55 ± 0.08	95.67 ± 0.02	−1.71 ± 0.10	−1.34 ± 0.02	0.37 ± 0.07
	72	4.00 ± 0.10	88. 27 ± 7.48	−1.77 ± 0.02	−1.53 ± 0.08	0.24 ± 0.02
−1.0	0	–	–	–		–
	12	–	–	–		–
	24	3.79 ± 0.04	91.36 ± 0.45	−1.40 ± 0.03	−1.59 ± 0.06	−0.19 ± 0.02
	48	3.42 ± 0.04	84.54 ± 1.83	−1.75 ± 0.15	−1.74 ± 0.04	0.01 ± 0.00
	72	3.08 ± 0.32	82.81 ± 7.48	−1.94 ± 0.03	−1.81 ± 0.03	0.13 ± 0.01
−1.5	0	–	–			
	12	–	–			
	24	–	–			
	48	3.18 ± 0.20	84.38 ± 2.73			
	72	2.88 ± 0.30	80.[illegible] ± 5.93			

Note: 1) WC values are significantly different among 0, −0.5, and −1.0 MPa at 24 h after treatment ($p < 0.05$) and significantly different among 0, −0.5, −1.0 and −1.5 MPa at 48 and 72 h after treatment ($p < 0.05$).
2) RWC values are significantly different among 0, −0.5, and −1.0 MPa at 24 h after treatment ($p < 0.05$) and significantly different among 0, −0.5, −1.0 and −1.5 MPa at 48 h after treatment ($p < 0.05$).
3) OA values are significantly different between −0.5 and −1.0 MPa at 24, 48 and 72 h after treatment ($p < 0.05$).

Concentrations of K^+, Na^+, Cl^-, glycinebetaine, proline and sucrose as related to leaf OA under WD

Among the inorganic solutes, K^+ and Na^+ accounted for the leaf OA under the WD treatment, resulting in a higher leaf K^+ concentration from 9 to 30 d of treatment in Experiment 1 and it peaked at 16 d of treatment (Table 3). On the other hand, PEG-induced-WD treatment decreased the leaf K^+ concentration in Experiment 2 (Table 4). The leaf Na^+ concentration did not differ between the WW and WD plants (Experiment 1, Table 3); however, the Na^+ concentration in −0.5 MPa PEG at 48 h (124 μmoL/g DM) was 48% higher than in ze[illegible]EG (Experiment 2, Table 4). The WD treatment did not result in a higher leaf Cl^- concentration than in the WW treatment (Experiment 1) as well as in Experiment 2 where the leaf Cl^- concentration in −1.5 MPa was lower than −0.5 MPa at 72 h. The extra leaf K^+ concentration in WD plants contributed 21% of the OA in Experiment 1. The contribution of 21% was calculated from 16 or 37 d because at 16 d the extra K+ was the highest whereas the OA at 37 d was the highest. The extra leaf Na^+ concentration accounted for 5% of the OA in Experiment 2.

Table 3
Concentrations of K^+, Na^+, Cl^-, glycinebetaine, proline and sucrose in youngest fully-expanded of wheat cv. Hartog during well-watered (WW) and water deficit (WD) conditions. Values are means ± SE (n = 4). Modified from Nio et al. (2011b) with permission from the publisher.

Treatment	Day	[K^+] (μmol/g DM)	[Na^+] (μmol/g DM)	[Cl^-] (μmol/g DM)	[Glycinebetaine] (μmol/g DM)	[Proline] (μmol/g DM)	[Sucrose] (μmol/g DM)
WW	0	1164.9 ± 11.5	71.3 ± 0.3	225.6 ± 3.7	50.5 ± 2.1	0 ± 0	28.6 ± 0.8
	16	1175.1 ± 26.8	79.9 ± 0.9	516.2 ± 44.8	40.0 ± 3.4	0 ± 0	113.9 ± 0.6
	37	736.9 ± 12.0	44.6 ± 0.9	232.9 ± 14.7	77.2 ± 2.5	0 ± 0	95.9 ± 8.8
WD	0	1164.9 ± 11.5	71.3 ± 0.3	225.6 ± 3.7	50.5 ± 2.1	0 ± 0	28.6 ± 0.8
	16	1297.6 ± 40.4	79.6 ± 2.3	481.5 ± 41.3	85.4 ± 8.4	19.0 ± 1.5	48.5 ± 1.0
	37	731.4 ± 39.[illegible]	45.4 ± 1.8	100.6 ± 9.5	160.1 ± 2.1	103.8 ± 10.6	62.7 ± 3.6

Note: 1) [K^+] values at d[illegible]s are significantly different between WW and WD ($p < 0.05$).
2) [Cl^-] values at day 37 are significantly different between WW and W[illegible] < 0.05).
3) [Glycinebetaine], [proline] and [sucrose] values at days 16 and 37 are significantly different between WW and WD ($p < 0.05$).

Table 4
Concentrations of K^+, Na^+, Cl^-, glycinebetaine, proline and sucrose in segments from the fully-expanded flag leaf of wheat cv. Hartog in four PEG 8000 treatments (0, −0.5, −1.0, and −1.5 MPa) at 0, 12, 24, 48 and 72 h. Treatments were applied as −0.5 MPa steps every 12 h. Values are mean ± SE (n = 3). Modified from Nio et al. (2011a) with permission from the publisher.

Treatment (MPa)	Hour	[K^+] (μmol/g DM)	[Na^+] (μmol/g DM)	[Cl^-] (μmol/g DM)	[Glycinebetaine] (μmol/g DM)	[Proline] (μmol/g DM)	[Sucrose] (μmol/g DM)
0	0	1165.7 ± 14.1	91.1 ± 3.4	242.6 ± 2.0	105.6 ± 5.3	0 ± 0	30.9 ± 0.3
	12	885.3 ± 24.3	90.7 ± 0.9	225.3 ± 0.3	99.9 ± 4.1	0 ± 0	19.4 ± 0.5
	24	805.3 ± 12.1	87.2 ± 0.1	186.3 ± 4.3	110.6 ± 8.4	0 ± 0	22.6 ± 2.1
	48	770.2 ± 10.8	87.9 ± 1.6	139.9 ± 4.2	78.4 ± 3.9	20.4 ± 1.5	24.5 ± 3.6
	72	815.1 ± 61.7	85.6 ± 5.3	110.7 ± 0.5	71.3 ± 10.1	20.1 ± 0.7	19.9 ± 1.1
−0.5	0	–	–	–	–	–	–
	12	809.3 ± 0.2	104.1 ± 0.1	208.7 ± 0.0	100.5 ± 1.6	4.6 ± 0	19.0 ± 0.6
	24	784.3 ± 29.7	123.8 ± 1.4	195.6 ± 1.4	106.2 ± 3.7	0 ± 0	19.7 ± 2.7
	48	697.1 ± 7.3	130.4 ± 0.0	132.3 ± 10.6	63.9 ± 4.6	29.9 ± 2.3	20.2 ± 4.0
	72	652.4 ± 20.9	141.8 ± 12.3	102.4 ± 4.2	82.7 ± 5.7	15.1 ± 0.1	24.4 ± 1.5
−1.0	0	–	–	–	–	–	–
	12	–	–	–	–	–	–
	24	488.4 ± 0.8	120.9 ± 6.3	129.2 ± 1.6	45.7 ± 6.5	6.8 ± 1.4	0 ± 0
	48	400.8 ± 37.2	97.1 ± 17.4	73.1 ± 7.7	29.5 ± 9.8	10.0 ± 2.7	0 ± 0
	72	390.1 ± 1.6	100.1 ± 13.5	35.4 ± 1.9	34.3 ± 6.7	8.1 ± 1,0	0 ± 0
−1.5	0	–	–	–	–	–	–
	12	–	–	–	–	–	–
	24	–	–	–	–	–	–
	48	205.6 ± 3.8	86.3 ± 2.8	50.3 ± 5.4	18.8 ± 5.2	10.8 ± 3.2	0 ± 0
	72	122.8 ± 16.7	71.4 ± 7.3	16.7 ± 0.8	22.4 ± 9.1	4.9 ± 0.1	0 ± 0

Note: 1) [K^+], [glycinebetaine], [sucrose] values are significantly different among 0, −0.5, and −1.0 MPa at 24 h after treatment ($p < 0.05$) and significantly different among 0, −0.5, −1.0 and −1.5 MPa at 48 and 72 h after treatment ($p < 0.05$).
2) [Na^+] and [Cl^-] values are significantly different between 0 and −0.5 MPa at 12 h after treatment ($p < 0.05$), significantly different among 0, −0.5, and −1.0 MPa at 24 h after treatment ($p < 0.05$) and significantly different among 0, −0.5, −1.0 and −1.5 MPa at 48 and 72 h after treatment ($p < 0.05$).
3) [Proline] values are significantly different among 0, −0.5, −1.0 and −1.5 MPa at 48 and 72 h after treatment ($p < 0.05$).

The contribution of organic solutes to the leaf OA was clearly observed in Experiment 1 (Table 3), but it was not significant in Experiment 2 (Table 4). The leaf glycinebetaine and proline concentrations in the WD plants were higher than in the WW plants in Experiment 1. The extra leaf glycinebetaine concentration in the WD plants accounted for 8% of the OA at 16 d and 19% at 37 d of drying. The contribution of extra leaf proline concentration was 4% and 21% of OA, respectively, at d 16 and 37, respectively. However, in Experiment 2, the leaf proline concentration in −0.5 MPa PEG was higher than in zero PEG at 48 h and this extra proline concentration contributed only 1% of OA.

Leaf OA expression at the whole plant and tissue levels

The leaf OA occurs in cv. Hartog at whole plant levels at the reproductive stage in the controlled-environment (Nio et al., 2011b) and at tissue level in the laboratory (Nio et al., 2011a). Wheat cultivars with a high OA capacity, such as Hartog (Morgan, 2001), can continue physiological processes, such as photosynthesis and water extraction in spite of some dehydration (Chandra Babu et al., 2001). OA is regarded as beneficial as it can help plants to extract more water (Morgan and Condon, 1986).

The mechanisms of OA expression and solute accumulation are different between the whole plant (Nio et al., 2011b) and leaf segments (Nio et al., 2011a). Osmotic adjustment occurred in whole plants subjected to 2–4 wk of WD as well as in leaf segments exposed to PEG 8000-induced WD for 2 d. These results showed that the mechanism of OA could be triggered quickly in leaf segments. K^+ was the major solute involved in OA in leaves of intact plants (that is at the whole plant level), but not in excised leaf segments. This difference indicated that roots or and other tissues or both, such as dying leaves of the intact plant influenced the development of OA in wheat. Root-to-shoot and leaf-to-leaf signaling systems were unavailable (Davies and Zhang, 1991) in excised segments, compared with intact plants. The root-to-shoot signaling system via the xylem under WD includes abscisic acid (ABA), cytokinin, strong ion difference, anions and cations, and changing pH in the xylem sap. Leaf-to-leaf signaling system distributes ABA from older to younger leaves for turgor control (Davies and Zhang, 1991). The duration and level of organization in the plant affected the mechanism of OA expression and solute accumulation during WD.

In this study, OA was expressed and the patterns of solutes that contributed to OA under controlled-environment and laboratory conditions were investigated. The whole plant level in the controlled-environment (Experiment 1) and the tissue level in the laboratory conditions (Experiment 2) expressed the same level of OA (0.37 MPa). Although the laboratory-based PEG method with leaf segments had some advantages such as shorter duration, under well-controlled conditions and repeatability (Gibon et al., 2000), this simpler method cannot substitute for a controlled-environment when screening germplasm for OA capacity. The system with leaf segments was osmotically active over hours in the control solution without PEG (0 MPa) and the OA peaked at 0.36 MPa after 48 h incubation in the solution. Between these experiments, soil-grown plants in the controlled-environment condition was an effective model for screening germplasm for OA and the solutes that contribute to it in wheat.

The same level of OA was measured in the whole plant and tissue levels; however, the contribution by solutes to the leaf OA was very small at the tissue level. The solutes contributing to the leaf OA in the controlled-environment were K^+ (21%), glycinebetaine (19%) and proline (21%) and those contributing to the leaf OA in the laboratory-based PEG method were Na^+ (5%) and proline (1%). Further experiments need to measure solutes other than K^+, Na^+, Cl^-, glycinebetaine, proline and sucrose that contributed to the leaf OA in the whole plant and tissue levels. The inorganic and organic solutes include free amino acids in addition to proline, organic acids such as malate, citrate, other sugars (fructose, glucose), other soluble carbohydrates such as fructans, and other inorganic ions such as NO_3^-, NH_4^+, Mg_2^+ and Ca_2^+ (Morgan, 1984; Munns et al., 1979; Ma and Turner, 2006). In addition, the uptake, influx and efflux of nutrient ions by leaf segments should also be measured to explain the increase and decrease in solute

concentrations in leaf segments related to the OA in response to PEG-induced WD. The availability of O_2 in the PEG solution should be examined as the O_2 concentration was inversely proportional to the PEG concentration, as the O_2 concentration was higher in PEG 6000 than in PEG 6000 when the PEG concentration was beyond 5% in the solution (Mexal et al., 1975). The availability of O_2 may have influenced metabolism processes in the leaf tissue, such as respiration.

Leaf OA was expressed in wheat cv. Hartog under WD conditions both at the whole plant level (soil-grown plants) in the controlled-environment conditions and at the tissue level (leaf segments) in the laboratory conditions. K^+, glycinebetaine and proline were contributors to the leaf OA under WD at the whole plant level, whereas Na^+ and proline were contributors to the leaf OA during WD under −0.5 MPa at the tissue level. The results of this study still need to be validated with further evaluation of leaf OA expression within and between wheat genotypes and under different experimental conditions.

Conflict of interest

The authors declare that there are no conflicts of interest.

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