# Osmotic potential at full turgor: an easily measurable trait to help breeders select for drought tolerance in wheat

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## Abstract

This study investigated the relationship between osmotic potential at full hydration ($\pi_{100}$) and turgor loss point ($\Psi_{TLP}$) in wheat (*Triticum aestivum*) to determine the potential of using $\pi_{100}$ to predict $\Psi_{TLP}$ under well-watered (WW) and drought (WS) conditions. Two methods for determining $\pi_{100}$ were tested: pressure–volume (PV) analysis and freezing point osmometry. The study also measured $\pi_{100}$ in a range of 38 field-grown wheat cultivars to determine whether there is genetic variation in $\pi_{100}$ under field conditions. $\pi_{100}$ correlated with $\Psi_{TLP}$ using both methods under both water treatments, particularly WS. Genetic variation of $\pi_{100}$ in the field, under rainfed conditions, was greater than controlled conditions and ranged from −0.94 to −1.95 MPa. Overall, the evidence supports development of $\pi_{100}$ as a novel tool for plant breeders to screen large populations of wheat and identify genotypes with lower $\Psi_{TLP}$, an integrative trait that is related to drought tolerance.

**Key words:** *Triticum aestivum* — water deficit — phenotyping — permanent wilting point — turgor loss point — osmotic adjustment

Drought tolerance, in an agricultural context, is defined as the ability to adapt to water-limited conditions while managing to produce a profitable yield (Blum 1996, Passioura 1996). Mechanisms of drought tolerance in wheat are characterized by drought avoidance and endurance strategies. Drought avoidance includes management practices such as sowing earlier and planting earlier maturing varieties so that the sensitive reproductive and grain filling stages occur before the onset of terminal drought (Turner and Nicolas 1987). Drought avoidance traits also include minimizing water loss via stomatal closure and diverting carbon allocation to non-photosynthetic organs and defence molecules (Chaves et al. 2002). Consequently, this limits $CO_2$ diffusion into the leaf, decreasing carbon assimilation and ultimately biomass and yield (Chaves et al. 2002, McDowell 2011).

Drought endurance can be considered as true drought tolerance and involves changes in cellular biochemistry and physiology that assist in maintaining cell function under drought conditions (Shao et al. 2008). This includes regulation of plant water status through metabolic changes and modification of leaf functional traits (Chaves et al. 2002). Turgor maintenance under water stress is a key mechanism conferring drought tolerance (Hsiao et al. 1976). One strategy to maintain turgor under drought is through a lower turgor loss point ($\Psi_{TLP}$), which is the leaf water potential ($\Psi_{leaf}$) when turgor reaches zero. When leaves dehydrate during transpiration and under drought, cell volumes shrink so bulk leaf turgor pressure decreases. At the $\Psi_{TLP}$, bulk leaf turgor pressure is equal to zero, and $\Psi_{leaf}$ is equal to the osmotic potential ($\pi$) (Turner 1981). Lower $\Psi_{TLP}$ can be achieved by: (i) increasing internal solute concentration to decrease osmotic potential at full hydration ($\pi_{100}$), (ii) increasing cell wall elasticity (decreasing bulk elastic modulus [Ɛ]) and (iii) increasing the apoplastic water fraction ($a_f$) by redistributing symplastic water outside of the cell walls (Bartlett et al. 2012a). $\pi_{100}$ has been identified as the main driver of $\Psi_{TLP}$, while Ɛ was found to have no direct role in drought tolerance (Lenz et al. 2006, Bartlett et al. 2012a). However, these relationships were identified from associative analyses, but have not been tested under controlled conditions.

$\Psi_{TLP}$ has been well studied in an ecological context (Lenz et al. 2006, Mitchell et al. 2008, Bartlett et al. 2014, Meinzer et al. 2014), but there is little information about this trait in crop species. This may be because it was generally assumed that all crops stopped functioning at the permanent wilting point (Briggs and Shantz 1912), the term used in agriculture for $\Psi_{TLP}$. Moreover, the permanent wilting point was assumed to be the same for all crops (−1.5 MPa) (Slatyer 1957). Although limited studies indicate that $\Psi_{TLP}$ and $\pi_{100}$ vary between wheat genotypes (Gunasekera and Berkowitz 1992, Johnson et al. 1984, Quarrie 1983, Rascio et al. 1988), high genetic variation is likely because of breeding in contrasting environments. Moreover, osmotic adjustment (OA) may add to the inherent variation in $\pi_{100}$. OA occurs through active accumulation of ions and organic compounds under severe drought conditions, as opposed to the passive decrease in $\pi$ through dehydration (Zhang et al. 1999). Hence, OA is generally quantified as the difference in $\pi_{100}$ between well-watered and water-deficient conditions (Hsiao et al. 1976) and is considered to be the main driver of drought-induced changes in $\Psi_{TLP}$ (Bartlett et al. 2012a). Genetic variation for OA in wheat is well known (Morgan et al. 1986), and because of its relationship with increased yields in water-limited environments (Richards 2006) and stability across environments (Blum et al. 1999), OA has been emphasized as a desirable trait to include in crop breeding programmes (Kusaka et al. 2005). Because $\Psi_{TLP}$ integrates OA, Ɛ and other individual water relations traits, it would be more desirable to select for $\Psi_{TLP}$ in breeding programmes aimed at improving drought tolerance if a rapid measurement method was available.

$\Psi_{TLP}$ has traditionally been determined from pressure–volume (PV) curves (Tyree and Hammel 1972, Tyree and Richter 1982), which are time-consuming and only a limited number of samples (typically eight to ten leaves) can be measured in a day. This precludes the PV method from use in extensive phenotyping

programmes. However, the more efficient method of determining $\pi_{100}$ through osmometry measurements indicates that osmometer-determined $\pi_{100}$ could potentially be used as a rapid and inexpensive predictor of $\Psi_{TLP}$ (Bartlett et al. 2012b). Measuring $\pi$ of cell sap with a vapour pressure osmometer takes between 30 s for expressed sap (Turner 1981) to 10 min for a killed sample of leaf tissue (Bartlett et al. 2012b). Thus, several hundred samples could potentially be measured per day.

The relationship between $\pi_{100}$ and $\Psi_{TLP}$, and the ability to predict postdrought $\Psi_{TLP}$ have not been examined in wheat or any other crop species. Therefore, the main goal of this study was to determine the potential of predicting $\Psi_{TLP}$ of wheat using osmometer-determined $\pi_{100}$ under well-watered and drought conditions. The relationship between $\Psi_{TLP}$ and $\pi_{100}$ was further examined by PV analysis of two wheat cultivars contrasting in OA capacity, to determine whether drought-induced changes in $\Psi_{TLP}$ were due to OA. The second aim of this was to identify whether genetic variation in $\pi_{100}$ exists in field-grown wheat genotypes that reflect a broad range of breeding development.

## Methods

**Plant material and growing conditions:** Eight spring wheat cultivars (*Tritium aestivum* L.), with variation in physiological parameters and leaf functional traits (Table 1), were grown in a natural-light glasshouse with day/night temperatures of 25/15°C, respectively. Five seeds were sown directly into each pot and thinned to one seedling upon germination. Pots (polyvinyl chloride, 90 mm diameter × 400 mm depth) contained a bottom layer of 0.5 kg of gravel for drainage and 2.5 kg of reddish-brown loamy clay soil that had been oven-dried at 65°C for 10 days and was sourced from the University of Western Australia's research farm 'Ridgefield' (Pingelly, Western Australia). The gravimetric soil moisture content at 100% pot water-holding capacity was 24.8% of soil dry weight. Two weeks after seedling emergence, 1.25 g of slow-release, granular fertilizer (N-P-K 13.8-2.0-15.8, Hortico® All-Purpose Compound Fertiliser, Dulux Australia, Clayton, Vic., Australia) was spread over the top soil layer of each pot. To minimize soil evaporation, 40 g of white polyethylene beads was also added.

**Experimental design:** A randomized complete block design was implemented for the glasshouse layout, with four replicates of each cultivar per treatment of well-watered (WW) and water-stressed (WS) conditions. After seven weeks of growth, between mid-booting and first

awns emerging (Zadoks growth stage GS45 – 49, [Zadoks et al. 1974]), water was withheld from plants in the WS treatment while WW plants were maintained at 80% pot water capacity (19.8% soil moisture content). Watering was withheld until permanent wilting symptoms were observed, which was defined as leaf wilting, leaf rolling and cessation of stem elongation. Then, individual WS replicates and the corresponding WW replicate were harvested to measure leaf water status parameters.

A separate set of plants for PV determinations were grown under the same conditions and treatments as those described above. The plants comprised of four replicates per treatment of the cultivars 'Hartog' and 'Kukri' that were selected based on contrasting osmotic adjustment capacity and leaf functional traits under water stress (Ryan 2013).

**Depletion in plant-available water (PAW):** Pots were weighed daily during the drought treatment. Depletion in plant-available water (PAW) during the drought treatment was determined from differences in consecutive daily pot weight and normalizing to maximum (pot water capacity) and minimum (visually determined point of permanent wilting for each cultivar) soil moisture contents.

**Plant water relations:** Flag leaves were sampled at the visually determined point of permanent wilting, when leaf water potential ($\Psi_{leaf}$), relative water content (RWC) and $\pi_{100}$ were measured. Because turgor pressure at the permanent wilting point is assumed to be zero, $\Psi_{leaf}$ at the permanent wilting point = $\Psi_{TLP\_visual}$ to distinguish it from $\Psi_{TLP\_PV}$ determined from PV analysis. $\Psi_{TLP\_visual}$ was measured using a Scholander-type pressure chamber (Model 600, PMS Instrument Company, Albany OR, USA), using the protocol outlined in Turner (1981). After the determination of $\Psi_{TLP\_visual}$, leaves were split lengthwise and weighed immediately. One leaf segment was oven-dried at 70°C for 7 days and then weighed again for the determination of RWC (Smart and Bingham 1974), while the other leaf segment was placed into a prelabelled vial, with the base in 30 ml of deionized water, and rehydrated for approximately 4 h in a laboratory, under low-light conditions (Boyer et al. 2008). Upon rehydration, the turgid weight was determined, and the leaf segment immediately wrapped in aluminium foil, snap frozen and stored in a −80°C freezer until measurement of $\pi_{100}$. The $\pi_{100}$ measurements were conducted using a freezing point osmometer (Model 210, Fiske®, Advanced Instruments Inc., Norwood, MA, USA) on sap expressed from thawed leaf samples. OA was determined by the difference in $\pi_{100}$ in rehydrated leaves of WS plants relative to the WW plants.

**Pressure–volume (PV) curves:** Pressure–volume curve analysis was conducted to further explore the relationship between $\pi_{100}$ and $\Psi_{TLP\_PV}$ under WW and WS conditions. A comparison of psychrometric and

Table 1: Australian cultivars selected for this study and their physiological traits related to drought tolerance

| Genotype | Year of release | Predominant trait of interest | Reference |
|---|---|---|---|
| 'Drysdale' | 2001 | High water-use efficiency and drought tolerance | Seednet (2005) |
| 'Gladius' | 2006 | Drought tolerant | Wheeler (2007) |
| 'Hartog' | 1982 | Putative high osmotic adjustment capacity; low transpiration efficiency. High drought tolerance | Rebetzke et al. (2002), Nio et al. (2011) |
| 'Janz' | 1989 | Low transpiration rates; no change in bulk leaf elastic modulus under water deficit | Unpublished data |
| 'Kukri' | 1991 | Low transpiration response to increasing VPD; putative low osmotic adjustment capacity; low stomatal conductance under water deficit. Low drought and heat tolerance | Izanloo et al. (2008), Schoppach and Sadok (2012) |
| 'Mace' | 2008 | New variety, very popular in WA. Putative drought tolerance | AGT (2010) |
| 'Sunco' | 1986 | Putative low osmotic adjustment capacity | Morgan (2001); Nio et al. (2011) |
| 'Westonia' | 1997 | Bulk leaf elastic modulus decreases under water deficit; maintains lower $\Psi_{leaf}$ than 'Janz' under well-watered conditions | Unpublished data |

pressure chamber techniques used to measure leaf water potential and osmotic potential is given in Turner (1981). PV measurements were conducted 13 days after the initiation of the drought treatment for 'Kukri' WS plants and 19 days for 'Hartog' WS plants, when symptoms of permanent wilting were observed; measurements for WW plants were conducted two days later. The night prior to PV measurements, flag leaves of the main stem were removed and the excised end immediately placed in 30 ml of deionized water. Leaf samples were rehydrated overnight in the laboratory under low-light conditions. The bench-drying method was used for the PV determination (Sack et al. 2011), where leaves were progressively dried and $\Psi_{leaf}$ and leaf mass were measured at intervals until $\Psi_{leaf}$ values became more negative than $-2$ MPa. PV curves were plotted using the PV analysis spreadsheet tool of Sack and Pasquet-Kok (2011). $\Psi_{TLP\_PV}$ was determined from the inflection point on the curves.

**Field sampling for osmotic potential at full hydration ($\pi_{100}$):** Leaves were sampled from a field trial consisting of a range of historical wheat cultivars planted by the breeding company InterGrain Pty. Ltd. under rainfed conditions. The trial was located in York, Western Australia, which has an average annual rainfall of 400 mm (Bureau of Meteorology 2015). Thirty-eight wheat cultivars were sampled based on their year of release, which spanned seven decades from the 1950s to 2010s. Flag leaves were taken from four plants per cultivar plot at the ear emergence stage of development (Zadoks growth stage GS50–59, (Zadoks et al. 1974)), rehydrated in moist paper towel for approximately 4 h (Ryser et al. 2008) while being transported from the field back to the laboratory, snap frozen in liquid nitrogen and stored in a $-80°C$ freezer. $\pi_{100}$ was measured with the freezing point osmometer, as described above.

**Statistical analysis:** Data analysis was conducted using the software package R version 3.03 (R Core Team 2014) or GraphPad Prism® version 6.04 (GraphPad Software Inc., La Jolla, CA, USA). All data were tested for normality using Shapiro–Wilk normality test and Bartlett's test of homogeneity of variances. Quadratic equations (in the form of $y = a + bx + cx^2$) were best fitted to the PAW data with the constant term 'a' constrained to 100%. The replicates test for lack of fit was used to test that the quadratic model was appropriate ($P > 0.05$) for each cultivar. The extra sum-of-squares $F$-test was used to determine whether the best-fit regression parameters differed between cultivars, followed by Tukey's multiple-comparisons test to identify contrasting cultivars. Cultivar and treatment effects for plant water relation parameters were tested using two-way ANOVAs, followed by Tukey's HSD *post hoc* test to identify differences between cultivars. The relationship between PV and osmometer-determined $\pi_{100}$ and $\Psi_{TLP\_PV}$ was assessed using regression analysis and Pearson's product–moment correlation. Genotypic variation and the significance of the year of release in $\pi_{100}$ in field-grown cultivars were tested using a two-way ANOVA and Tukey's HSD *post hoc* test was used to determine the most significantly contrasted cultivars.

## Results

### Visual assessment of turgor loss point ($\Psi_{TLP\_visual}$), $\pi_{100}$ plasticity and plant growth

The rate of depletion of PAW depended on cultivar ($P < 0.0001$; Fig. 1 and Table S1). 'Westonia', 'Sunco', 'Hartog' and 'Drysdale' exhausted PAW in $14.9 \pm 0.25$ days, compared with 'Gladius', 'Janz', 'Kukri' and 'Mace' that used PAW in $19.4 \pm 0.25$ days. Gravimetric soil moisture content at the visually determined turgor loss point was not significantly different between cultivars ($P = 0.1987$) and was on average $7.23 \pm 0.07\%$.

Sampling times for turgor loss point were dependent on the rate of depletion of PAW and visual assessment of permanent wilting for individual replicates of each cultivar. $\Psi_{TLP\_visual}$ was



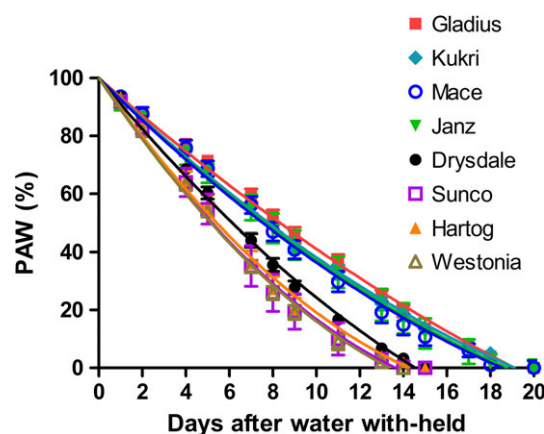Fig. 1: Depletion in plant-available water (PAW) over time in eight wheat cultivars after watering was withheld. Data are means $\pm$ SEM ($n = 3$–4). Coefficients for each curve and the goodness of the fits are shown in Table S1
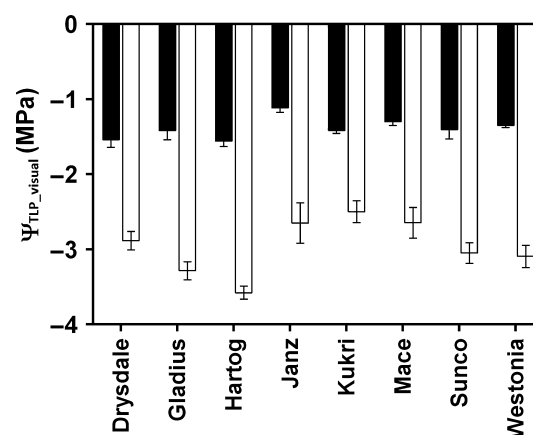


Fig. 2: Leaf water potential at the visually determined turgor loss point ($\Psi_{TLP\_visual}$) in well-watered (closed bars) and water-stressed (open bars) wheat cultivars. The values are mean $\pm$ SEM ($n = 4$)

significantly different between cultivars ($P < 0.01$) and was reduced by water stress ($P < 0.01$) (Fig. 2). Cultivars 'Hartog' and 'Kukri' showed the most significant contrast in $\Psi_{TLP\_visual}$ ($P < 0.01$), ranging from $-3.6 \pm 0.05$ MPa for 'Hartog' and $-2.5 \pm 0.08$ MPa for 'Kukri' (Fig. 2).

There was a significant cultivar and treatment interaction for $\pi_{100}$ ($P = 0.027$) because the drought treatment had varying effect on OA and plasticity in $\pi_{100}$ (Fig. 3). Under WW conditions, $\pi_{100}$ ranged from $-1.47 \pm 0.07$ ('Drysdale') to $-1.15 \pm 0.03$ MPa ('Kukri') and under WS conditions, from $-1.81 \pm 0.02$ ('Hartog') to $-1.38 \pm 0.01$ MPa ('Kukri') (Fig. 3). Cultivars 'Drysdale' and 'Sunco' had the smallest OA, with a 0.15 and 0.16 MPa shift in $\pi_{100}$, respectively, while 'Gladius' and 'Hartog' had the greatest OA with a shift of 0.43 and 0.53 MPa, respectively (Fig. 3).

Shoot biomass was significantly affected by water stress ($P < 0.01$; Fig. 4) and differed between cultivars ($P < 0.01$; Fig. 4). 'Mace', 'Drysdale' and 'Kukri' had greater than 30% reduced biomass under drought conditions (Fig. 4). 'Westonia' and 'Gladius' had a 15% reduction, while 'Hartog', 'Sunco' and 'Janz' maintained biomass with less than a 10% decrease (Fig. 4).
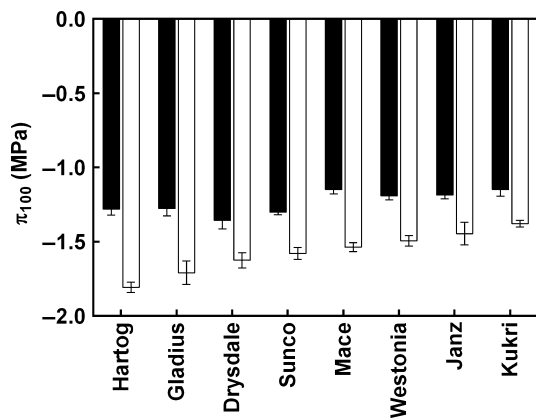
Fig. 3: Osmotic potential at full turgor ($\pi_{100}$) of eight wheat cultivars under well-watered (closed bars) and water-stressed (open bars) conditions measured with an osmometer at the visually determined turgor loss point. Values represent mean $\pm$ SEM (n = 4)

## PV analysis

Eight leaves could be processed concurrently for the PV determination, which took 6–7 h using the bench-dry method. PV estimates for 'Hartog' and 'Kukri' showed contrasts in $\pi_{100}$ and $\Psi_{TLP\_PV}$ between the cultivars (Table 2). $\Psi_{TLP\_PV}$ of 'Hartog' was more negative than 'Kukri' (P = 0.01) and was significantly decreased by water stress in both cultivars (P = 0.04). There was a significant cultivar and treatment interaction for both $\varepsilon$ and $\pi_{100}$ (P < 0.01 and P = 0.04, respectively) (Table 2). In response to WS, 'Hartog' $\varepsilon$ increased and $\pi_{100}$ decreased, whereas $\varepsilon$ and $\pi_{100}$ of 'Kukri' changed in the opposite direction to 'Hartog' (Table 2). There was no difference between the cultivars for $a_f$ (P = 0.56), but WS increased $a_f$ by an average of 47% (P < 0.01) (Table 2). Cultivar and treatment had no significant effect on RWC$_{TLP}$ (Table 2).

## Predicting turgor loss point

PV-determined $\pi_{100}$ was significantly correlated with $\Psi_{TLP\_PV}$ (P = 0.02) for 'Hartog' and 'Kukri' under WW (r = 0.74) and WS (r = 0.72) conditions, with PV $\pi_{100}$ explaining 55% and 52% of the variance in $\Psi_{TLP\_PV}$, respectively (Fig. 5a). The relationship between cultivar means of osmometer-determined $\pi_{100}$ and $\Psi_{TLP\_visual}$ was also significant (P < 0.01) under WW (r = 0.91) and WS conditions (r = 0.74). The variance in $\Psi_{TLP\_visual}$ was explained more by variation in $\pi_{100}$ under WS (82%) compared with WW conditions (41%) (Fig. 5b).

## Genetic variation in $\pi_{100}$ under field conditions

There was substantial variation in osmometer-determined $\pi_{100}$ (P < 0.01) for the 38 cultivars grown in the field, ranging from −1.95 to −0.94 MPa (Fig. 6). The two most significantly contrasted cultivars, 'Cunderin' and 'Clearfield', were released between 1995 and 2000. Throughout 1950–2012, $\pi_{100}$ showed no distinct trend of improvement or decline as a genetic trait, with the year of release having no significant effect (P = 0.56; Fig. 6).

## Discussion

The $\pi_{100}$ determined by osmometer and PV methods correlated with $\Psi_{TLP}$, suggesting that $\pi_{100}$ could potentially be used to predict the turgor loss point of wheat. The osmometer-determined method also provides several hundred-fold higher throughput in estimating $\Psi_{TLP}$ compared with PV analysis, making it a much more viable option for use in breeding programmes screening for drought tolerance.

In a meta-analysis comprising woody, herbaceous, evergreen and deciduous species, across an array of biomes ranging from semi-desert to tropical rainforests, Bartlett et al. (2012a) showed that $\pi_{100}$ was a good predictor of $\Psi_{TLP}$, which was subsequently
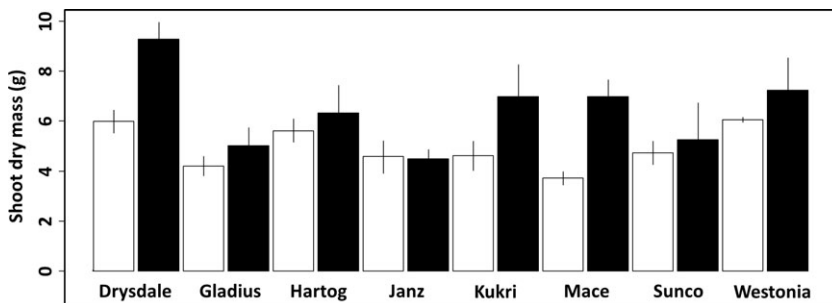


Fig. 4: Mean shoot dry mass of eight wheat cultivars under well-watered (closed bars) and drought (open bars) conditions. Values are mean $\pm$ SEM (n = 4)

Table 2: Pressure–volume parameters for wheat cultivars 'Hartog' and 'Kukri' under well-watered (WW) and water-stressed (WS) conditions

| Genotype | Treatment | $\Psi_{TLP\_PV}$ (MPa) | $\pi_{100}$ (MPa) | $\varepsilon$ (MPa) | RWC$_{TLP}$ (%) | $a_f$ (%) |
|---|---|---|---|---|---|---|
| 'Hartog' | WW | −1.51 ± 0.02 | −1.19 ± 0.02 | 7.56 ± 1.16 | 83.0 ± 1.9 | 20.2 ± 5.3 |
| | WS | −1.76 ± 0.02 | −1.32 ± 0.04 | 10.48 ± 0.70 | 87.1 ± 0.7 | 44.8 ± 4.8 |
| 'Kukri' | WW | −1.38 ± 0.03 | −1.17 ± 0.03 | 11.28 ± 0.33 | 89.1 ± 0.5 | 27.5 ± 4.4 |
| | WS | −1.47 ± 0.05 | −1.00 ± 0.04 | 6.11 ± 0.06 | 83.0 ± 1.6 | 46.8 ± 2.1 |
| Statistics | Interaction | 0.28 | 0.04* | 0.01** | 0.06 | 0.74 |
| | Genotype | 0.01** | 0.02* | 0.8 | 0.69 | 0.56 |
| | Treatment | 0.04* | 0.54 | 0.2 | 0.49 | 0.01** |

Parameters include turgor loss point ($\Psi_{TLP\_PV}$), osmotic potential at full turgor ($\pi_{100}$), bulk leaf elastic modulus ($\varepsilon$), relative water content at turgor loss point (RWC$_{TLP}$) and apoplastic water fraction ($a_f$). Values are mean $\pm$ SEM (n = 4). * and ** indicate significance at P < 0.05 and P < 0.01, respectively.
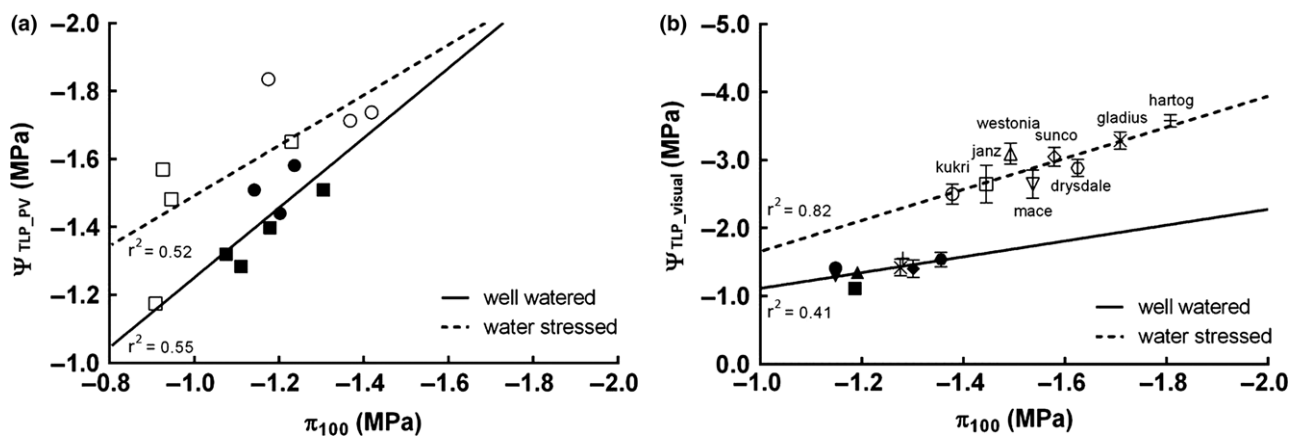
Fig. 5: Relationship between pressure volume determined turgor loss point ($\Psi_{TLP\_PV}$) and osmotic potential at full hydration ($\pi_{100}$) for two wheat cultivars, 'Hartog' (circles) and 'Kukri' (squares) (a), and relationship between the visually determined turgor loss point ($\Psi_{TLP\_visual}$) and osmometer-determined $\pi_{100}$ for eight wheat cultivars (b). Data represent individual plants in (a) and cultivar means ± SEM (n = 4) in (b), under well-watered (solid symbols, solid line) and water-stressed (open symbols, dashed line) conditions
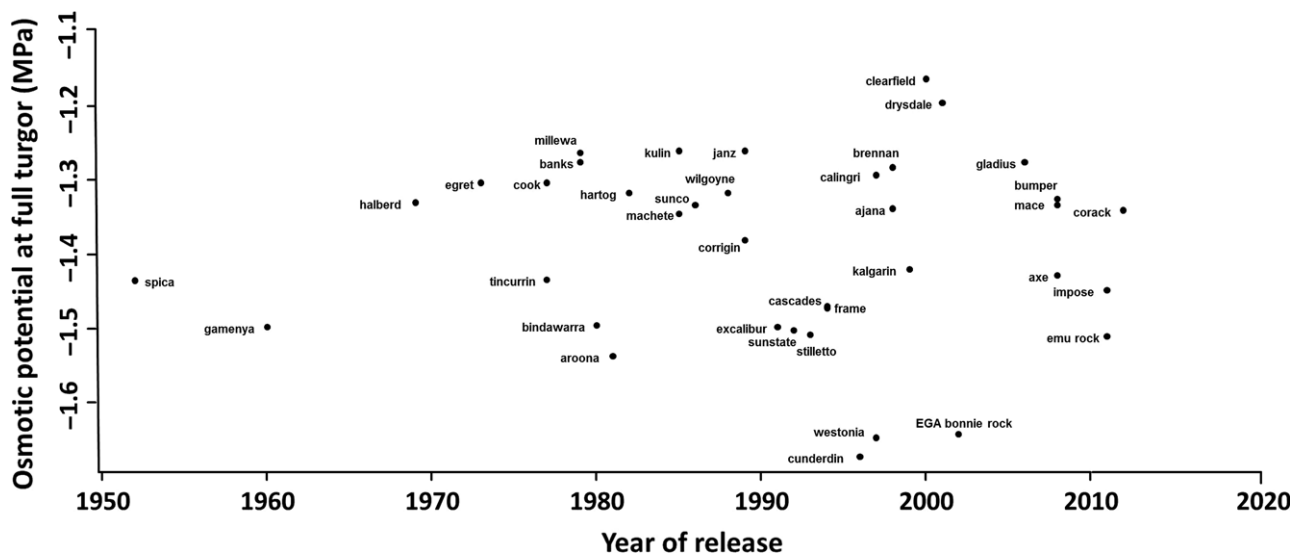


Fig. 6: Osmotic potential at full hydration ($\pi_{100}$) for 38 wheat cultivars in relation to their year of release, grown under rainfed field conditions in York, Western Australia. $\pi_{100}$ was measured using the osmometer method. Values represent means of four plants sampled within each cultivar plot. Text labels indicate wheat cultivars.

confirmed in a study of 30 species growing under two different rainfall regimes (Bartlett et al. 2012b). To our knowledge, such a comparison has not been previously conducted under controlled drought conditions or between cultivars of the same species. In our study, $\pi_{100}$ and $\Psi_{TLP}$ correlated well in both WW and WS plants and $\pi_{100}$ explained a large proportion of the variation in $\Psi_{TLP}$ for the cultivars tested. However, water stress induced plasticity in $\pi_{100}$ and hence, $\Psi_{TLP}$, depending on capacity for OA of individual cultivars. This suggests that knowledge of OA is needed to predict postdrought $\Psi_{TLP}$ from $\pi_{100}$ under WW conditions, or that $\pi_{100}$ needs to be measured under WS conditions.

$\Psi_{TLP\_visual}$ was much more negative than $\Psi_{TLP\_PV}$, which resulted in different regressions between $\pi_{100}$ and $\Psi_{TLP}$. It is possible that $\Psi_{TLP\_visual}$ was sampled later than the real turgor loss point due to the difficulty in identifying the point visually. A method to identify zero turgor *in situ* more accurately is needed, such as the non-invasive leaf patch clamp pressure probes (Bramley et al. 2013). In addition, osmometer $\pi_{100}$ was

more negative than PV $\pi_{100}$ and the osmometer method showed greater OA in 'Hartog' than was estimated by PV analysis. The cause of this disparity is not known, but a comparison with greater number of cultivars with contrasting OA capacities measured by both methods may provide some indication. Despite these discrepancies, the direction of the changes in $\Psi_{TLP}$, $\pi_{100}$ and OA in response to WS was the same by both methods for both of the cultivars compared. This shows an evident link between $\Psi_{TLP}$ and $\pi_{100}$ and suggests that a method for prediction is possible. Furthermore, the magnitude of disparity in these traits among cultivars suggests that selection for $\pi_{100}$ is warranted, thus providing support that postdrought $\Psi_{TLP}$ in wheat could potentially be predicted with a more rapid and simplified osmometer method.

As expected, 'Hartog' had a more negative $\Psi_{TLP\_PV}$ than 'Kukri' as it is considered more drought tolerant (Izanloo et al. 2008, Nio et al. 2011, Ryan 2013). Drought conditions affected PV parameters in a manner that was consistent with the theory. In 'Hartog', the small decrease in $\pi_{100}$ and increased $a_f$ led to a

significant decrease in $\Psi_{TLP\_PV}$, despite an increase in $\varepsilon$. By contrast, in 'Kukri', a simultaneous decrease in $\varepsilon$ and a significant increase in $a_f$ had less effect on lowering $\Psi_{TLP\_PV}$. Although an in-depth discussion on the underlying physiology of these individual traits is beyond the scope of this article with respect to breeding, it demonstrates that an integrative trait like $\Psi_{TLP}$ will be more useful in selection programmes for drought tolerance rather than specific individual traits.

Traits that are useful for selection in breeding programmes must show significant variation among an extensive range of germplasm (Lafitte 2002). This study demonstrated that there is genetic variation in $\pi_{100}$ under controlled environment and rainfed field conditions. Moreover, there was wider variation in $\pi_{100}$ in the field under rainfed conditions and this variation was independent of year of release or pedigree. Identification of genetic variation in $\pi_{100}$ is the first step towards the possibility of using this trait as a selection criterion for plant breeders. The evident pattern in the changes of $\Psi_{TLP}$ and $\pi_{100}$ due to water stress provides confidence that $\pi_{100}$ could potentially be used to predict $\Psi_{TLP}$. Therefore, having the ability to rapidly measure $\pi_{100}$ through osmometry represents an efficient technique to identify drought tolerance, thus enforcing the potential this trait has as a selection criterion in breeding programmes.

Lower $\Psi_{TLP}$ has long been associated with drought tolerance, biogeographic distribution and ability of plant species to adapt to low-rainfall environments (Lenz et al. 2006 and references therein). Although the main aim of this study was to demonstrate the proof of concept that $\Psi_{TLP}$ could be predicted rapidly from measurements of $\pi_{100}$ with the osmometer, shoot biomass and depletion of PAW were measured in the pot study to provide an indication of some physiological traits typically related to drought tolerance. Under glasshouse conditions, shoot biomass production was related to $\pi_{100}$. 'Gladius' and 'Hartog' displayed the greatest shifts in $\pi_{100}$ under WS conditions and maintained 85–90% of shoot biomass relative to WW conditions. On the other hand, 'Kukri' showed the smallest shift in $\pi_{100}$ and greatest reduction in biomass. However, there were some inconsistencies. 'Drysdale' showed the third largest shift in $\pi_{100}$, used PAW at a similar rate as 'Hartog', but shoot biomass production decreased by 35% and 'Janz' displayed the second smallest shift in $\pi_{100}$, dried the soil more gradually, but maintained 97% of shoot biomass relative to WW conditions. These results demonstrate the complex interaction between physiological traits and responses to drought. All of the cultivars in this study, except 'Kukri', are considered drought tolerant (see references in Table 1) and were bred in different environments. For example, 'Drysdale' was developed for high transpiration efficiency through selection based on carbon isotope discrimination (Condon et al. 2004), but is known to close its stomata in response to soil drying while leaf water potential also declines (Saradadevi et al. 2014, 2015), so carbon assimilation and hence growth would be reduced, supporting the observation in this study. 'Janz' is a widely adapted cultivar, which may be related to its conservative behaviour as observed here in terms of shoot biomass under WW conditions. 'Westonia' is an older cultivar that performed well in south-west Western Australia, possibly because its higher rate of water use enabled it to effectively capture the intermittent rainfall during the winter growing season in the south-west Mediterranean-type climate.

Because $\Psi_{TLP}$ is an integrative trait it may prove more useful as a selection tool for drought tolerance given that in contrast with individual traits such as OA, $\varepsilon$, RWC and $\Psi_{leaf}$, it encapsulates all the plant water relations parameters and plasticity in $\Psi_{TLP}$ could be used for targeted development of new cultivars for specific environments. In addition, using the osmometer to measure $\pi_{100}$ provides a high-throughput method in predicting $\Psi_{TLP}$ for wheat genotypes. Further study is required to investigate whether the variation in $\pi_{100}$ and hence $\Psi_{TLP}$ is linked to maintenance of growth and yield under drought conditions in the field and on a wider range of germplasm. OA often correlates with grain yield in a range of crop species exposed to water stress (Lilley et al. 1996, Lafitte 2002), and as OA causes a shift in $\pi_{100}$, it is likely that $\pi_{100}$ would also be related to yield maintenance under drought in wheat.

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## Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Table S1.** Linear and quadratic coefficients and goodness of fit for regressions fit to depletion of plant-available water over time after watering was withheld from eight wheat cultivars (shown in Fig. 1).