Two potato (Solanum tuberosum) varieties differ in drought tolerance due to differences in root growth at depth

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Abstract. To test the hypothesis that root growth at depth is a key trait explaining some genotypic differences in drought tolerance in potato (Solanum tuberosum L.), two varieties (Horizon and Maris Piper) differing in drought tolerance were subjected to different irrigation regimes in pots in a glasshouse and in the field under a polytunnel. In the glasshouse, both cultivars showed similar gas exchange, leaf water potential, leaf xylem ABA concentration and shoot biomass independently of whether plants were grown under well watered or water deficit conditions. Under well watered conditions, root growth was three-fold higher in Horizon compared with Maris Piper, 3 weeks after emergence. Water deficit reduced this difference. In the polytunnel, applying 60% or less irrigation volume compared with full irrigation significantly decreased tuber yield in Maris Piper but not in Horizon. This was coincident with the higher root density of Horizon in deep soil layers (>40 cm), where water content was stable. The results suggest that early vigorous root proliferation may be a useful selection trait for maintaining yield of potato under restricted irrigation or rainfall, because it rapidly secures access to water stored in deep soil layers. Although selecting for vigorous root growth may assist phenotyping screening for drought tolerance, these varieties may require particular environmental or cultural conditions to express root vigour, such as sufficiently deep soils or sufficient water shortly after emergence.

Additional keywords: abscisic acid, deficit irrigation, genotypic selection, partial rootzone drying, variety.

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Introduction

Potato (Solanum tuberosum L.) is considered a drought-sensitive species, especially during the tuber initiation and bulking stages (Onder \textit{et al.} 2005) and thus many crops receive supplemental irrigation to stabilise yield (Shalhevet \textit{et al.} 1983). Water-saving irrigation techniques like partial rootzone drying (PRD) and controlled deficit irrigation can significantly reduce the amount of water applied without reducing tuber yield (Shahnazari \textit{et al.} 2007; Saeed \textit{et al.} 2008; Jensen \textit{et al.} 2010). PRD is an emerging irrigation technique based on exploiting the long-distance hormonal control of plant water loss. Theoretically, part of the root system is allowed to dry the soil, stimulating root hormone biosynthesis and hormone transport to the shoot (especially the phytohormone ABA) to effect partial stomatal closure, while the rest of the root system receives sufficient irrigation to maintain root water uptake to sustain growth and yield (Dodd 2009). Irrigation scheduling of PRD has applied a fraction or total crop evapotranspiration to one of the sides of the crop row (Dodd 2009; Sepaskhah and Ahmadi 2010). As the physiological effects of PRD on crop water loss tend to diminish as water uptake from roots in drying soil decreases (Dry \textit{et al.} 2000), drying and irrigated parts of the rootzone are usually alternated, usually at a fixed (arbitrary) frequency. Some studies using split-pots suggest that hormonal signalling is maximised when the dry side is maintained at a certain soil water content, allowing appreciable root water uptake from that side (Dodd \textit{et al.} 2008; Liu \textit{et al.} 2008). However, to our knowledge, there are no published studies on automated scheduling of PRD based on soil moisture sensors to optimise its application.

Plant breeding can also help to maintain crop yield under low water availability by selecting drought-resistant varieties (Cabello \textit{et al.} 2012). Selecting for drought-tolerant traits may include the ability to maintain turgor and stomatal conductance (g\textsubscript{s}) under water deficits (Jeffries 1993) and drought avoidance traits like enhanced root vigour and rooting depth. Potato has high potential for genetic selection of drought tolerance traits, mainly because of the existence of Andean wild races adapted to dry environments (Anithakumari \textit{et al.} 2012) and there is increasing interest in finding the most suitable methods to select for drought tolerance (Monneveux \textit{et al.} 2013).

Large root systems have been recognised as a key trait for drought resistance in many crops (Palta \textit{et al.} 2011). Rooting depth in potato can be appreciable (around 80–90 cm) and highly variable between varieties (Stalham and Allen 2001; Wishart...
et al. 2013). Although some studies report that varietal differences in root growth are linked to drought tolerance (Lahlou and Ledent 2005), making root growth a candidate trait for phenotypic selection, there is a need to understand the complex interactions between root growth and environment before using it in extensive breeding programs. Moreover, these varietal differences could also be associated with other physiological traits conferring drought tolerance or avoidance such as increased intrinsic water use efficiency (IWUE) (Galmés et al. 2011), enhanced stomatal control of water loss (Jefferies and MacKerron 1997) or maintenance of plant water potential during water stress (Levy et al. 2013). Establishing the relative importance of root growth and other physiological traits is essential to validate it as a phenotyping trait for selecting drought-tolerant genotypes.

Variate differences in drought tolerance may have an impact on the physiological effects of water-saving irrigation techniques. Specifically, since differences in root growth determine the extent and density distribution of the rootzone, they will also alter the proportion of roots growing in wet and dry soil under a given soil moisture distribution. During PRD, exposing more roots to drying soil resulted in greater inhibition of leaf growth and higher foliar ABA accumulation (Martin-fluence access to soil water, it is difficult to disentangle varietal differences in root vigour from other traits that may influence drought tolerance. For this reason, we performed a pot and a field experiment in two potato varieties with reported differences in sensitivity to water deficit (British Potato Council 2011). In the pot experiment, we assessed morphological (biomass allocation) and physiological traits (leaf gas exchange, ABA concentration in xylem sap ([X-ABA]), leaf water potential (Ψleaf) traits in a soil volume that was large enough to express early root growth differences with minimal restriction but where those differences presumably cannot influence access to soil water. In a field experiment under a polytunnel (to prevent rainfall compromising the experimental design) where root systems were unconstrained, soil moisture profile, root density and yield were measured under different water-saving irrigation treatments automatically triggered based on soil moisture thresholds. It was hypothesised that root growth was the main trait that differed between the drought-tolerant and drought-sensitive varieties.

Materials and methods

Plant material

For both the pot and field experiments, seed tubers of two varieties with contrasting drought resistance were purchased from Higgins Agriculture Ltd (Doncaster, UK). *Solanum tuberosum* L. cv. Maris Piper was bred in United Kingdom (UK) around 1960 and is the most popular cultivar in the UK. It is a high yielding main crop variety, with tall haulms and small leaflets. *S. tuberosum* L. cv. Horizon was more recently bred in the UK from Russet Burbank (origin: United States) × Sante (origin: Netherlands) parentage. It is considered to possess excellent drought resistance in field trials according to the British Potato Council variety database (British Potato Council 2011) and seed tubers are exported to Mediterranean countries such as Egypt. It is also a high-yielding main crop variety, with medium sized haulms and large leaflets.

**Pot experiment**

Twenty-four seed tubers of the two varieties were planted in 5-L pots (one tuber per pot) filled with topsoil taken from the field plot (see next section) and sieved (5 mm mesh size) to eliminate stones and organic debris. Tuber FW did not statistically differ between varieties ($F = 0.08; P = 0.77$; average and s.e.: 45.6 ± 1.7 g). Pots were randomly placed in an unheated glasshouse, watered to field capacity and weighed. Temperature ranged between a maximum of 22–29°C and a minimum of 16–19°C. To guarantee a 14-h photoperiod at a minimum of 600 μmol m<sup>−2</sup> s<sup>−1</sup>, metal halide lamps were activated when natural light was below this value during the photoperiod. Maximum PPFD on sunny days was up to 1000 μmol m<sup>−2</sup> s<sup>−1</sup>. Due to differential emergence success rates between varieties, 16 plants of Maris Piper and 22 of Horizon were obtained. Saturated soil weight (SSW) was recorded and soil volume estimated (as a truncated cone after measuring height, upper and basal diameters of the soil plug) to calculate pot weight thresholds and irrigation volumes for the different treatments throughout the experiment. To establish weight thresholds, soil volumetric water content ($θ_v$) was measured at saturation ($Θ_{sat}$) with a soil moisture probe (Thetaprobe, Delta-T Devices Ltd, Burwell, UK) to estimate water weight at saturation ($W_{WS} = Θ_{sat} \times V$) and thus soil dry weight in the pot ($SDW = SSW − WWS$). Therefore a certain $θ_v$, threshold corresponded to a soil weight value (SW) according to $SW = (θ_v \times V) + SDW$.

Pots were watered to SSW when estimated $θ_v$ fell below 0.35 cm<sup>3</sup> cm<sup>−3</sup>. On 7 March 2012, 7 days after emergence (DAE), half of the pots from each variety were randomly assigned to one of the following two irrigation treatments. Treatments were imposed for almost four weeks (until 33 DAE). Under full irrigation (FI), plants were watered to SSW when $θ_v$ was above 0.35 cm<sup>3</sup> cm<sup>−3</sup>. Under deficit irrigation (DI), plants were watered to $θ_v = 0.35$ cm<sup>3</sup> cm<sup>−3</sup> when $θ_v$ was below 0.20 cm<sup>3</sup> cm<sup>−3</sup>.

Pots were weighed every other day and watered according to the water content thresholds described above. Plant FW was negligible compared with soil weight (<3% at the end of the experiment). Total irrigation volume was recorded for each plant.

Leaf gas exchange, $Ψ_{leaf}$ [X-ABA] and biomass were measured at 21 DAE (2 weeks after treatment imposition) and 33 DAE. Since measurements were destructive, only half the plants were maintained until 33 DAE. Leaf gas exchange was measured with an infrared gas analyser (CIRAS-2, PP Systems, Hitchin, UK) on a fully expanded sunlit leaf from the upper third of the canopy. Measurements were taken between 1100
and 1300 hours under a constant PPFD of $1000 \mu\text{mol m}^{-2}\text{s}^{-1}$, provided by a light-emitting diode light unit, and 390 parts per million of CO$_2$ atmospheric concentration. Net photosynthesis rate ($A$) and $g_s$ were recorded. IWUE was calculated as $A / g_s$.

Simultaneously with gas exchange measurement, an adjacent leaf was excised and inserted in a pressure chamber to determine water potential. Pressure was then increased by 0.5 MPa over the balancing pressure to collect xylem sap. Sap was sampled with a micropipette (30–100 μL was obtained) and placed in an Eppendorf vial. Samples were immediately frozen in liquid nitrogen and stored at $-18^\circ\text{C}$ for [X-ABA] determination by a radioimmunoassay (Quarrie et al. 1988). The xylem sap of $S.\ tuberosum$ does not present nonspecific interference in the assay (Liu et al. 2005).

Leaf area was measured with a LI-3100 leaf area meter (LI-COR, Lincoln, NE, USA), including the leaf used for water potential determination and sap extraction. Roots were extracted from the pots, tubers and stolons were removed, and the roots were washed. Tubers and stolons were neglected in the analyses due to their small contribution (<5%) to the total weight. DW of leaves, stems and roots were oven-dried at 70°C for 48 h to obtain DW.

Field experiment

Seed tubers (around 50 g in FW) of both varieties were planted on 27 May 2011 under a 20 × 16 m transparent plastic roof suspended 3 m above the ground to exclude rainfall without disturbing the air flow around the crop canopy. The plot was located at the experimental farm of Myerscough College, in Bilsborrow Preston, UK (Lee Farm, 53°51'11"N 2°45'47"W). The soil is a sandy loam with a high organic matter content (13%) with a clay horizon at variable depth between 60 and 100 cm. Prior to planting, soil was disinfected with 76 g m$^{-2}$ of dazomet (Basamid, BASF Company, Ludwigshafen, Germany). Ammonium sulfate (70 kg ha$^{-1}$ N) was applied following soil analysis to determine the required fertiliser via Planet V3 software (ADAS, Wolverhampton, UK). Seed tubers were shallowly planted in rows and covered with a ridge of soil 0.2 m high. Rows were spaced 0.75 m apart and seed tubers were spaced 0.3 cm apart within the row.

The experimental area was divided in four blocks (8 m × 6 m) with eight rows per block. The experiment was arranged in a split-plot design, with variety (two levels) and irrigation treatment (four levels) as factors. Within each block, two contiguous rows formed a whole-plot (8 m × 1.5 m) randomly assigned to one of the four irrigation treatments. Each of the two rows (subplots) within each whole plot was randomly assigned to one of the two varieties (Fig. 1).

Each irrigation treatment was controlled via separate valves using data loggers and dielectric soil moisture sensors, (GP1 and SM200 respectively, Delta-T Devices). The GP1 contained a relay switch that prevented the irrigation timer from activating if the sensors detected sufficient soil moisture. The irrigation
timer could irrigate for 15 min twice a day. Every irrigation treatment had four SM200 sensors connected to the GP1: two could activate the relay and the other two only recorded soil moisture. All sensors were placed in the Maris Piper rows of one of the irrigation whole-plots. Sensors were placed 4 m apart on both sides of the row. Sensors were inserted vertically between the middle and the base of the ridge at ~10 cm deep (approximately at ground level). The data loggers were programmed to deactivate and activate the irrigation timer when either of the two controlling sensors reached a fixed upper and lower threshold, respectively (described below). The sensors measured soil permittivity (ε) hourly, and this was converted to soil volumetric content (θv). Soil refractive index (ε′) and θv are related through a positive linear relationship, with parameters depending on the characteristics of the soil. The parameters for topsoil in the plot were obtained by drying soil samples from saturation to completely dry soil in 3-L pots in the laboratory and comparing the ε′ readings from the sensors and actual θv measured by weighing water losses. The resultant equation was ε′ = 1.0 + 8.4θv – 0.3, (r2 = 0.97).

Water was delivered to each planted row by two parallel trickle tapes placed halfway up either side of the ridge. Each trickle tape could be closed manually by a tap. Emitters were spaced 20 cm apart and the delivery rate was 2.5 L m⁻¹ h⁻¹. Irrigation treatments were imposed during the tuber bulking stage, from 6 July (21 DAE and 9 days after tuber initiation) to the end of the experiment on 31 August (77 DAE). During that period, the daily temperature (± s.e.) was 15.2 (±0.2)°C, 19.3 (±0.3)°C and 11.1 (±0.3)°C for the mean, maximum and minimum temperatures respectively.

Soil water content thresholds, soil moisture probe and tap arrangements in the different irrigation treatments were as follows: Under FI, θv was between 0.35 and 0.45 cm³ cm⁻³, with one controlling probe per side and position within the row. Both taps were permanently open. Under partial rootzone drying with fixed sides (PRD-F), θv thresholds in the wet side were as in FI. Initially, irrigation was planned to keep θv in the dry side between 0.20 and 0.25 based on physiological responses observed in potted plants growing in topsoil. However, soil drying was very slow and lowest threshold was never reached (Fig. 2). Therefore no irrigation was applied to this side. Both controlling probes were placed in the wet side of the row. The tap on the trickle tape of the dry side remained closed at all times during the application period. Under partial rootzone drying with alternating sides (PRD-A), θv thresholds in the wet side were as in FI. Initially, it was planned to swap dry and wet sides when θv in the dry side was 0.20 cm³ cm⁻³. As in PRD-F, this threshold was never reached because of the slow rate of soil drying. Instead, alternation was carried out manually when the average moisture of the two sensors in the dry side seemed to stabilise, which occurred at ~0.3 cm³ cm⁻³. Controlling probes were placed in the initial wet side and changed to the opposite side of the row after each alternation. Alternation was carried out by turning on the tap on the initially dry side and turning off the previously wet side. Under whole rootzone drying (WRD), the thresholds were initially set as in the dry side of PRD-F. Because of the same problem described above, no irrigation was applied during the whole period.

The amount of water delivered for each treatment was measured by flow meters (Model MS, Arad Ltd, Yokneam, Israel) installed just downstream of each valve. The total amount was divided by the total area irrigated in each treatment (48 m²; 4 x 8 m long x 1.5 m wide).

Soil refractive index (ε′) was periodically measured during the irrigation treatment period in the first 100 cm of the soil profile (10, 20, 30, 40, 60 and 100 cm from the surface) via access tubes placed in the middle of the row (Fig. 1) with a soil moisture probe (PR2/6, Delta T Devices). Four tubes per block could be installed, two of them assigned to each of the varieties (Fig. 1). Therefore block could not be considered in the experimental design for this variable and a completely randomised design was used instead. Two replicates of each variety x irrigation treatment were randomly assigned within the plot (eight replicates per variety and four per irrigation treatment). An access tube was installed in each of the monitored locations just after planting the seed tubers. Each tube was tightly fitted inside a hole made by an auger. Due to differences in organic matter content and texture along the profile, and the variability of the depth of different soil horizons within the plot, the calibration of ε′ against actual θv was not accurate enough to compare different depths across the plot. Therefore, the plot was irrigated to saturation at the end of the experiment and the percentage of actual ε′, compared with saturated ε′ (%ε′/ε) was calculated for each measurement, tube and depth as a proxy of θv. Soil moisture profile measurements were taken at the middle of the irrigation treatment period (26 July; 41 DAE) and at the end of the experiment (31 August).
At the end of the experiment, soil cores were taken from the first 80 cm of the soil profile in the vicinity of the 16 access tubes (8 cores per variety). Each core was composed of four layers 20 cm long and 9 cm in diameter, dug out sequentially with an auger. In each portion, roots were carefully extracted by washing the soil over a fine sieve. Roots were oven-dried at 70°C for 48 h and weighed. Root dry weight density (RDWD) was calculated dividing root DW by the volume of each core (1272 cm³).

Haulms and tubers were harvested at the end of the experiment (31 August) in two separate 0.75 m² areas for each subplot (1 m × 0.75 m centred in the row). Three plants were harvested in each segment. Haulms were dried at 70°C for 48 h and weighed. Tubers were counted to calculate unitary tuber weight. Aboveground DW and tuber FW per unit area (kg m⁻²) were calculated for each subplot by adding the weights of the two segments (in kg) divided by the sampled area (1.5 m²). The incidence of common scab (Streptomyces scabies) was assessed in the harvested tubers. Tubers were considered to be affected when more than 12.5% of the surface was covered by scab. Less than 10% of tubers were affected, with no differences between varieties.

Statistical analyses

For the field experiment, tuber yield, unitary weight, tuber number and aboveground biomass were analysed by split-plot ANOVA. Irrigation was considered the whole-plot factor and variety the subplot factor. RDWD and %/E in different layers of the soil profile were analysed as a randomised factorial repeated-measures ANOVA, with irrigation treatment and variety as between-subject factors, depth as a within-subject factor and no block factor included. For %/E, the analysis was repeated for each measurement date. A large inequality of variances in root density among layers was detected because this variable was much greater in the upper two layers. Therefore the same separate repeated-measures ANOVA described above was performed for the first (0–40 cm) and second (40–80) half of the soil profile, with the two measuring depths in each half as the within-subject factor. When variety × irrigation treatment was statistically significant, a similar analysis compared the effect of variety in each irrigation treatment. Each pair of values corresponded to one of the blocks.

For the pot experiment, soil water content on the measurement date, leaf gas exchange, Ψ_leaf and [X-ABA] were analysed by three-way ANOVAs, with irrigation, variety and date as factors. Fresh tuber weight before planting was analysed by a single-factor (variety) ANOVA.

Results

Pot experiment

Irrigation volume did not differ between varieties (data not shown) but was 55% higher in FI than in DI pots. On the measurement days, Ψ, was higher in FI (0.43 ± 0.01 cm³ cm⁻³) than in DI (0.24 ± 0.01 cm³ cm⁻³, F = 233.5; P < 0.001). Variety did not affect Ψ, (F = 2.3; P = 0.14).

Although gs was higher in FI plants (Table 1; Fig. 3a, b), there were no statistically significant effects of variety, date, irrigation × variety and irrigation × date on gs (Table 1). Similarly, A was higher in FI plants (Fig. 3c, d). Since A increased on the second date but only in the DI treatment, date and irrigation × date were statistically significant. No differences in A were observed between irrigation treatments on the second date. IWUE (= A/gs⁻¹) was greater in DI plants (Fig. 3e, f), but decreased from the first to the second date. Variety did not influence IWUE.

Leaf water potential (Ψ_leaf) was slightly (~0.1 MPa) but significantly lower in DI plants (Table 1, Fig. 4a, b) but did not vary with variety or date. [X-ABA] was higher in Horizon and in DI, with no effect of date or water × variety interaction (Table 1, Fig. 4c, d).

Shoot biomass was higher in FI plants and increased during the experiment (Fig. 5a, b), but no interaction between date and irrigation or variety was observed (Table 2). Root biomass was higher in Horizon and in the FI treatment. Since the greater root biomass of Horizon was only observed in the FI treatment, a variety × irrigation interaction was observed (Fig. 5c, d). Root biomass was greater on the second date, but the differences were not statistically significant, reflecting limited root growth between the first and the second date. Despite the interactive effects on root mass, total biomass was not statistically affected by variety or variety × irrigation effect (F = 1.20, P = 0.28 and F = 2.59, P = 0.12 respectively). Shoots had much more biomass than roots, so clear differences in root biomass contributed little to variability of total biomass. The shoot to root ratio was significantly lower in Horizon (Table 2, Fig. 5e, f).

However, it increased largely in DI plants on the second date due to a strong reduction in root growth but not in shoot growth (Fig. 5a–f).

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**Field experiment**

During the treatment period, irrigation volume in the FI treatment was 169 mm, whereas the PRD-A, PRD-F and WRD treatments received 149 mm, 101 mm and 0 mm respectively. The higher irrigation volumes of PRD-A compared with the PRD-F treatment (despite the same irrigation settings) were due to delayed rehydration of previously dried soil in the PRD-A treatment (Fig. 2), resulting in greater irrigation volume. Mean tuber yield was not affected by irrigation ($F=0.25$, $P=0.86$), but variety and the irrigation × variety interaction were statistically significant ($F=11.03$, $P=0.006$ and $F=6.61$, $P=0.007$ respectively). Maris Piper yield was significantly lower in WRD and PRD-F but not in PRD-A and FI (Fig. 6a). Tubers were lighter in Maris Piper in the WRD treatment (53.6 ± 4.4 g in Maris Piper and 80.7 ± 6.4 g in Horizon), with no changes in tuber number per plot (95.0 ± 4.7 and 96.0 ± 15.3 for Horizon and Maris Piper respectively). In PRD-F, Maris Piper tubers were lighter (68.1 ± 6.3 and 77.8 ± 6.0 g for Maris Piper and Horizon respectively) and fewer (86.3 ± 7.2 and 101.0 ± 3.4 g for Maris Piper and Horizon respectively). Aboveground DW was significantly higher in Horizon ($F=7.32$, $P=0.02$) but did not vary by irrigation ($F=0.37$, $P=0.57$).

**Fig. 3.** (a, b) Stomatal conductance to water vapour ($g_s$), (c, d) net photosynthesis ($A$) and (e, f) instantaneous intrinsic water use efficiency (IWUE = $A g_s$) for both potato varieties in the pot experiment (Horizon: black; Maris Piper: grey) under different irrigation treatments (DI, deficit irrigation; FI, full irrigation) and two measurement dates: (a, c, e) intermediate measurement at first harvest 2 weeks after imposing irrigation; (b, d, f) final measurement at second harvest after 4 weeks ($n=4–6$, mean and s.e.). Different letters denote statistical significant differences between each variety × irrigation for each measurement date.
Irrigation × variety was almost statistically significant ($F = 3.13, P = 0.06$). The observed trend was similar to tuber yield, with haulm DW decreasing in the drier treatments but only in Maris Piper (Fig. 6b). RDWD did not change with irrigation ($F = 1.14, P = 0.40$ and $F = 0.61, P = 0.61$ for the 0–40 cm and 40–80 cm layers respectively) and no variety × irrigation interaction was observed ($F = 1.24, P = 0.36$ and $F = 1.07, P = 0.42$ for the 0–40 cm and 40–80 cm layers respectively). However, Horizon had greater RDWD than Maris Piper in the lower (40–80 cm) part of the soil profile ($F = 15.75, P = 0.005$ for variety effect), but no differences were observed in the upper (0–40 cm) part ($F = 0.97, P = 0.36$) (Fig. 7).

The degree of saturation ($\%\ell / e$) was lower with depth in all treatments and on both measurement dates ($F = 43.15, P < 0.001$; $F = 37.20, P < 0.001$ for depth factor on 26 July and 31 August respectively). The index was above 90% for all the treatments at 60 cm and 100 cm in depth (Fig. 8). The depth × irrigation treatment interaction was almost significant on 26 July ($F = 1.84, P = 0.06$) and significant on 31 August ($F = 3.08, P = 0.002$). In FI, ($\%\ell / e$) was above 70% throughout the whole profile, but it was lower for the other treatments for the layers above 40 cm. At the end of the experiment (31 August), soil moisture was higher in PRD-A and FI than in WRD above 40 cm (Fig. 8b). Overall, the irrigation effect was only significant on August 31st ($F = 8.09, P = 0.008$).

**Discussion**

In the field experiment with automatically scheduled irrigation, irrigation volumes differed between treatments. Thus comparing the efficiency of different irrigation techniques independent of the amount of water applied is not possible. Nonetheless, it was clear that the volume applied in PRD-A (80% of full irrigation), which kept soil moisture always above 0.3 cm$^3$ cm$^{-3}$, was enough to maintain yield of the two varieties, even though soil water content in the upper soil layers was not optimum (Fig. 2). Similar yield maintenance during PRD-A irrigation, despite decreased irrigation volumes, has been reported previously (Shahnazari et al. 2007; Xie et al. 2012) although the physiological changes underpinning this agronomic response and whether they are root- or shoot-mediated are not always clear.

Irrigation volumes (and distributions) had different effects in each variety. Although the yield in the drought-tolerant Horizon was independent of irrigation volume, Maris Piper production decreased in the two treatments that applied less water (PRD-F and WRD) (Fig. 6). Decreased yield even under moderate restriction of water (66% of water requirement) has
been observed previously in potato (Onder et al. 2005), especially during tuber bulking stage (Ierna and Mauromicale 2012). This lower yield was not associated with a higher incidence of common scab or any other disease. No effect of irrigation treatments or irrigation × variety interaction on root distribution in the soil profile or interaction was detected. The lack of influence of PRD on vertical root distribution agrees with previous experiments in potato (Ahmadi et al. 2011). Therefore, it seems that different varietal responses of root DW to different irrigation treatments did not explain the varietal yield responses observed.

Sustained tuber yield in Horizon even after watering ceased can only be explained by sufficient water availability in deep soil layers (<40–60 cm) throughout the experiment (Fig. 8), which is not uncommonly observed in potato crops (see Gaze et al. 2002; Satchithanantham et al. 2014). Even under irrigation, in certain soils, water ascending by capillarity from the groundwater table can account for up to 92% of the water consumption in potato, as estimated by a hydrological model (Satchithanantham et al. 2014). Therefore, genotypic differences in drought tolerance (Fig. 6) were associated with observed differences in root density in deep soil layers (Fig. 7). Some studies report that

![Figure 5](image-url)
Deep roots can extract a considerable fraction of the total water used. Under drought conditions, deep roots (80–100 cm) in the variety 'Cara' accounted for less than 5% of total root length but more than 50% of water uptake (Stalham and Allen 2004). Moreover, root-to-shoot ABA signalling, which limits water use, was attenuated when there were pronounced vertical gradients in soil moisture (Puértolas et al. 2013). Accessing water at depth could buffer the physiological effects of upper soil drying so that changes in bulk root water potential are minimised. Therefore, differences in root density in the deep layers during tuber bulking, even as small as those reported here, could profoundly affect plant water uptake and ultimately carbon gain, favouring varieties with slightly higher root density in the deepest layers.

Superior performance of genotypes under drought conditions can be also related to other physiological traits conferring drought tolerance, such as increased leaf-level water use efficiency linked to increased [X-ABA] (Thompson et al. 2007) or conservative water use (Khan et al. 2007). However, studying these traits under field conditions can be confusing, since varietal differences in water uptake caused by differences in rooting at depth can alter stomatal behaviour (Jefferies and MacKerron 1997). Possible genetic variation in some of these traits was assessed by growing plants in large pots in the greenhouse, but the two varieties did not differ in shoot responses to water deficits (Table 2). A and gs were the same under well watered conditions and decreased similarly under DI in both varieties (Fig. 3) and were associated with similar reductions in Ψleaf (Fig. 4), suggesting that water consumption and the efficiency of stomatal control of water loss did not differ between varieties. This agrees with the lack of genotypic differences in soil water depletion in the field experiment.

<p>| Table 2. Three-way ANOVA of the morphological variables measured in the glasshouse experiment |</p>
<table>
<thead>
<tr>
<th>d.f.</th>
<th>LA</th>
<th>SLA</th>
<th>SDW</th>
<th>RDW</th>
<th>SDW : RDW</th>
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<tbody>
<tr>
<td></td>
<td>F</td>
<td>P</td>
<td>F</td>
<td>P</td>
<td>F</td>
</tr>
<tr>
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<td>0.75</td>
<td>5.75</td>
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<tr>
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<td>0.04</td>
<td>0.01</td>
<td>0.95</td>
</tr>
<tr>
<td>D</td>
<td>1</td>
<td>8.99</td>
<td>0.005</td>
<td>9.95</td>
<td>0.004</td>
</tr>
<tr>
<td>V × W</td>
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<td>1.82</td>
<td>0.19</td>
<td>0.00</td>
<td>0.98</td>
</tr>
<tr>
<td>V × D</td>
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<td>6.55</td>
<td>0.02</td>
<td>1.73</td>
<td>0.20</td>
</tr>
<tr>
<td>W × D</td>
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<td>1.01</td>
<td>0.32</td>
<td>0.73</td>
<td>0.40</td>
</tr>
</tbody>
</table>

**Fig. 6.** (a) Tuber yield and (b) aboveground DW for the different potato variety × irrigation treatment combinations (n = 4, mean and s.e.) at the end of the field experiment. An asterisk indicates statistically significant differences between varieties within an irrigation treatment (P < 0.05). FI, full irrigation; PRD-F, fixed partial rootzone drying; PRD-A, alternating partial rootzone drying; WRD, whole rootzone drying.

**Fig. 7.** Root dry weight density along the soil profile for the two potato varieties (n = 8, mean and s.e.) at the end of the field experiment. An asterisk indicates statistically significant differences between varieties within an irrigation treatment (P < 0.05).
Some studies report that conservative water use when soil water is available is more important than root growth for drought tolerance because it delays the onset of drought (Zaman-Allah et al. 2011). Our pot experiment suggests that differences in water use did not explain the better response of Horizon to water shortage in the field. However, it must be highlighted that some studies observed genotypic differences in $g_s$ that led to higher soil moisture deficits and greater yield limitation in potato (Jefferyes 1995).

Some studies reported that the drying-induced increase in ABA concentration is lower in drought-resistant varieties (Wang and Huang 2003), allowing stomatal opening and continued photosynthesis with mild soil drying. In contrast, [X-ABA] was higher in the drought-tolerant variety (Horizon; Fig. 4c, d). However, this was not associated with lower $g_s$ and did not affect varietal responses to drought, suggesting possible genotypic differences in stomatal sensitivity to ABA (Blum and Sinmena 1995).

It has been argued that genotypic differences in root proliferation are positively correlated with shoot vigour in some crops like wheat (Triticum aestivum L.) (Palta et al. 2011). This could facilitate phenotyping screening to select for drought tolerance. However, our pot experiment shows no difference in shoot biomass despite large differences in root growth (Fig. 5), in agreement with previous glasshouse screening of 28 potato genotypes, where root length density could be poorly correlated with shoot weight (Wishart et al. 2013). As early potato growth relies on carbon and nutrients stored in tubers (Bohl et al. 2001), genetically controlled plasticity of biomass partitioning might be much larger than in other crops, which could increase the potential use of this variable in selecting for drought tolerance compared with other crops with a more limited range of root to shoot ratios.

The root growth of Horizon slowed in the second 2 weeks of the pot experiment, probably due to insufficient pot size. Generally, a root biomass density above 1 g L$^{-1}$ limits plant growth in pots (Poorter et al. 2012). Well watered Horizon plants reached that threshold after the first 2 weeks, whereas the rest of the plants remained below 0.5 g L$^{-1}$ at the end of the experiment. Regardless, it seems clear that differences in root vigour can be detected within 2–3 weeks of emergence (Wishart et al. 2013), which could considerably reduce screening time.

It must be highlighted that varietal differences in root growth were drastically diminished under water stress in the pot experiment, unlike in the field. These apparently contradictory results can be explained by differences in the timing of water stress occurrence and the limitations imposed by rootzone restriction in the pot experiment. In the field, all the plants were irrigated optimally during the first three weeks after emergence, but the upper layer soil moisture did not reach the set threshold level (0.2 cm$^{-2} \text{cm}^{-3}$) until two weeks later. With no restriction to root extension, Horizon secured access to water, thus maintaining yield during subsequent deficit irrigation. In potted well watered plants, varietal differences in root growth rate presumably occurred during this early stage. In the pot experiment, with limited soil volumes, water deficits were applied after the first two weeks and soil moisture rapidly reached threshold levels in the entire rootzone, impairing root growth and diminishing varietal differences.

It has been suggested that drought tolerance traits associated with a certain genotype may have opposite effects in different drought scenarios (Tardieu 2012). Drought-tolerant potato varieties relying on superior early root vigour like Horizon should be able to express their advantage during the early stages of the crop. Therefore optimal irrigation (or rainfall) is required during that period until the roots colonise the deeper soil layers where soil moisture is more stable throughout the whole growing season. Although care is needed to extrapolate the results from glasshouse to field conditions, it seems that water stress occurring shortly after emergence, or shallow soils, would potentially negate the superior drought tolerance of varieties with vigorous root growth. Under those circumstances, other traits enhancing conservative water use (such as effective stomatal control of water loss, increased IWUE or enhanced capacity for maintaining water potential under water stress) could be more efficient in diminishing yield penalties than profuse rooting (Zaman-Allah et al. 2011).

Finding straightforward phenotyping methods for drought tolerance selection in important crops such as potato is a major challenge to improve yield in water-limited environments and reduce agricultural water consumption via irrigation. Our results
highlight that in some circumstances, usually complex traits, such as drought tolerance, can be correlated with simple physiological features like early root growth. However, the complexity of environmental constraints (and plant responses to them) means that genotype selection should carefully consider climatic characteristics and soil features where crops will be growing.

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References


