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GlyPh: a low-cost platform for phenotyping plant growth and water use

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Abstract. Breeding drought-tolerant crop varieties with higher water use efficiency could help maintain food supply to a growing population and save valuable water resources. Fast and accurate phenotyping is currently a bottleneck in the process towards attaining this goal, as available plant phenotyping platforms have an excessive cost for many research institutes or breeding companies. Here we describe a simple and low-cost, automatic platform for high-throughput measurement of plant water use and growth and present its utilisation to assess the drought tolerance of two soybean genotypes. The platform allows the evaluation of up to 120 plants growing in individual pots. A cart moving in only one direction carries the measuring and watering devices. Watering and measurement routines allow the simulation of multiple water regimes for each plant individually and indicate the timing of measurement of soil water content and image capture for growth estimation. Water use, growth and water use efficiency were measured in two experiments with different water scenarios. Differences in water use efficiency between genotypes were detected only in some treatments, emphasising the importance of phenotyping platforms to evaluate a genotype's phenotype under a broad range of conditions in order to capture valuable differences, minimising the chance of artefacts and increasing precision of measurements.

Additional keywords: abiotic stress, drought tolerance, dry matter accumulation, plant phenomics, transpiration, *Glycine* spp.

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Introduction

Water deficit is one of the major limitations to crop yields worldwide, particularly in developing countries (Huang *et al*. 2002; Rosegrant and Cline 2003). Furthermore, altered precipitation patterns as a consequence of climate change are likely to increase the frequency of droughts in agricultural areas worldwide (Gornall *et al*. 2010). In the future, agriculture may have a lower priority for water use because of competition with other uses (e.g. human consumption, Tardieu 2005) As a consequence, drought-tolerant crop varieties with higher water use efficiency could help maintain food supply to a growing population and help save valuable water resources.

One difficulty in obtaining drought-tolerant varieties is the unequivocal evaluation of plant responses to water deficits (Pereyra-Irujo *et al*. 2007). Breeding for drought tolerance requires measuring structural and functional characters –the phenotype– of plants exposed to controlled soil water regimes in order to identify favourable genotypes. Fast and accurate phenotyping is currently seen as a major bottleneck for the improvement of drought tolerance (Richards *et al*. 2010).

Plant phenotyping platforms are devices with the ability to automatically generate and collect data on the phenotype of plants, therefore simplifying tasks which would otherwise be conducted manually. Some phenotyping platforms are specialised for certain types of plants (e.g. Phenopsis (Granier *et al*. 2006), the LemnaTec ScanalyzerHTS (LemnaTec GmbH, Würselen, Germany) and GROWSCREEN (Walter *et al*. 2006) for small plants like *Arabidopsis thaliana* (L. Heynh.), the TraitMill (Reuzeau *et al*. 2006) for rice, Phenodyn (Sadok *et al*. 2007) for cereals and grasses) and others, such as the LemnaTec Scanalyzer (LemnaTec GmbH) are flexible enough to grow and measure different types of plants. Other platforms have been designed for measuring specific plant organs (e.g. roots (Iyer-Pascuzzi *et al*. 2010; Nagel *et al*. 2012; Wells *et al*. 2012) or tillers (Yang *et al*. 2011)). Some platforms have the ability to not only collect data, but also to automatically modify the plant's environment so as to measure adaptive traits. Examples of this type of platform are Phenopsis (able to phenotype the response of plants to water stress) and the LemnaTec Scanalyzer, which can measure the response to water stress and salinity (e.g. Harris *et al*. 2010). Automatic

phenotyping should aim at not only increasing the number of species or varieties, but also effectively quantifying the phenotypes of these different genotypes under a range of environmental conditions (Nicotra and Davidson 2010). Therefore, it is this type of platforms that is most likely to contribute to enhancing the breeding process of drought-tolerant varieties.

The development of high-throughput genotyping technologies has lowered the cost-per-genotype to levels that enabled an explosion of genetic studies in many fields (Ragoussis 2009). Phenotyping technologies still have high initial costs and as a consequence, phenotyping platforms are not becoming widely accessible to potential users, i.e. researchers and breeders (Kolukisaoglu and Thurow 2010). Evidence of this limitation is clear in the fact that most platforms are currently located in large phenotyping facilities, usually one or few in each country (e.g. http://www.plantphenomics.com/partners/, http://www.plant-phenotyping-network.eu/eppn/installations, accessed 29 June 2012). The tendency in plant phenotyping technology development has been mostly towards increasing resolution and the number of variables measured, through sensors of increasing complexity (e.g. Fiorani *et al*. 2012). Few efforts have been made in order to develop low-cost phenotyping options, one example being the imaging system developed by Tsaftaris and Noutsos (2009) using wireless-connected consumer digital cameras. Lowering the cost of these platforms could, therefore, lead to a rapid expansion of breeding projects targeting complex traits.

Soybean is the most widely grown oil crop in the world (FAO 2012), including in many developing countries; and similar to many other crops, water deficit is the most important factor limiting soybean yields. None of the existing platforms have been adapted for phenotyping soybean under water deficit conditions. Our objective was to develop a low-cost, automatic platform for high-throughput measurement of plant water use and growth. In this paper we will present a detailed description of the GlyPh (*Glycine max* phenotyping) platform. An example of its possible utilisation is also presented: an assessment of drought tolerance in two soybean (*Glycine max* (L.) Merr.) genotypes, aimed at better understanding the behaviour of different soybean varieties when exposed to water deficit. Experiments were also used to demonstrate the capabilities of the platform for (i) simulating different water scenarios, (ii) estimating the plant growth and water use efficiency by using data obtained through image analyses of automatically taken pictures, (iii) discarding any artefactual results (e.g. the effect of vibration on plant growth and transpiration) obtained when using an automatic device for high throughput measurements.

Materials and methods

Platform description

The GlyPh platform allows the evaluation of up to 120 plants growing in individual pots. Watering and measurement routines are entered as worksheet files. Watering routines allow the simulation of multiple water regimes for each plant individually. Measurement routines indicate the timing of measurement of soil water content (or transpiration rate, through consecutive water content measurements) and capture of images for growth estimation. The software controls the movement of the sensors through the 120 plants, performing the specified routines, with a frequency of up to 1 h and saves the data to a database.

A general view of the platform, located in an environmentally controlled greenhouse, is shown in Fig. 1*a*, *b*.

The platform structure is made of four 10 m-long, bridge-like structures supporting 120 pots (four rows of 30 pots). Cylindrical 2.7 L (10 cm diameter, 35 cm high) pots are located on plastic trays, at fixed positions. A moving cart, located under this structure, carries the measuring and watering devices. This cart moves in only one direction, stopping at each pot position. All devices are connected to a personal computer, which is also located in the cart. The computer's peripherals are located outside the platform's structure, in a user control desk.

Soil water content and water use is measured gravimetrically with four balances (Ohaus Trooper TR6RS, Ohaus Corporation, Pine Brook, NJ, USA), one for each row of pots. At each pot position, the balances are lifted so that the pots can be weighed.

Watering is provided by four peristaltic pumps (Verderflex OEM M1000, Verderflex, Castleford, UK) connected to a reservoir. These pumps supply a precise amount of water or nutrient solution, as indicated by the software. Specially designed acetate funnels are located in every pot, which allow some distance between the watering hose and the pots, thus preventing damage to the plants and also decreasing soil evaporation (Fig. 1*c*).

Eight (two per row) three-megapixel digital cameras (model EUCC-997, Eurocase, Miami, FL, USA) take images of the plants from different angles. These can be located so as to take top- and side-view images of the plants, in order to measure traits such as height, width and projected leaf area (Hartmann *et al*. 2011). Alternatively, they can be located both at the top of the structure, a few cm apart (as shown in Fig. 1*a*), in order to take stereoscopic images of the plants which can be used to measure leaf angle and other structural parameters (Biskup *et al*. 2007). It is also possible to add different types of cameras or sensors (e.g. infrared temperature sensors or cameras, time-of-flight cameras).

The platform is controlled by simple software that uses Microsoft Excel files with daily or hourly routines as input together with Microsoft Access database files as output. Additionally, the platform can be operated manually.

Each pot has a unique code and is assigned both a position in the platform (1–120) and a routine (1–120), which is equivalent to an experimental treatment. It is possible, for example, to set up the same experimental treatment to every plant or 120 different treatments. Routines contain the date and time for the actions that will be conducted by the platform (i.e. weighing, watering, imaging) and the soil water content that has to be maintained. It is possible to set up hourly weighing routines for measuring water use or hourly imaging routines to visualise wilting, or to plan long-term water stress and recovery regimes.

For each pot, the weight of the empty pot, the tray (and other accessories) and the (dry) substrate is entered into the database at the beginning of the experiment. Additionally, the estimated FW of the plant must be entered regularly (usually on a weekly basis) by the user. These data is then used for calculating the

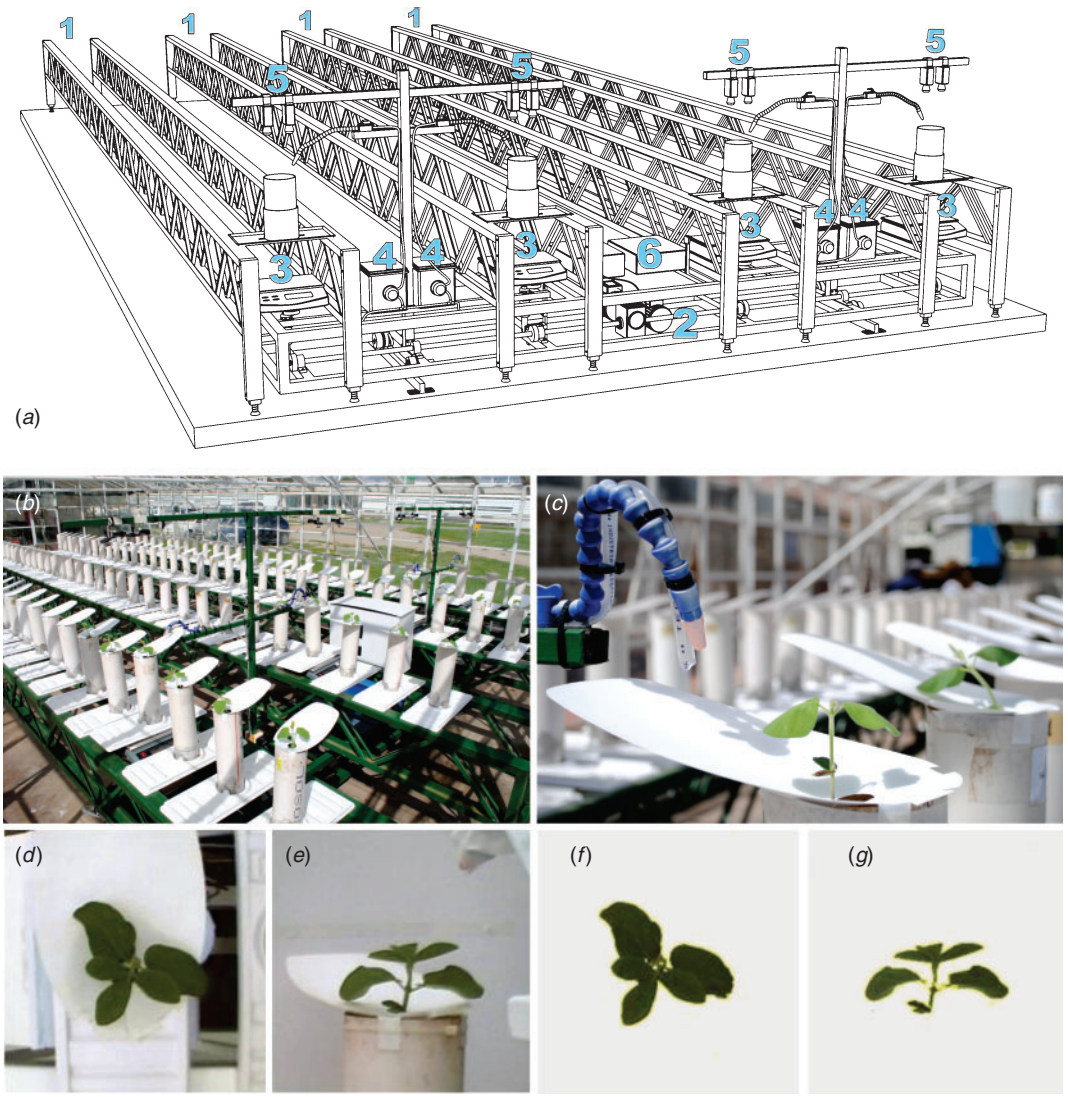

Fig. 1. (*a*) Schematic illustration of the GlyPh phenotyping platform, showing the structures that support the pots (1), the cart (2), balances (3), pumps (4), cameras (5) and the computer (6). (*b*) Photograph of the phenotyping platform located in a greenhouse. (*c*) Detail of a funnel and one of the watering hoses. (*d*, *e*) Top- and side-view images of soybean plants taken automatically by the platform. (*f*, *g*) Processed images for growth measurement. Note: the green structure and blue hoses shown in (*b*, *c*) were later painted white to aid in image segmentation, as seen in (*d*, *e*).

soil water content of each pot and the amount of water that has to be added to reach the soil water content indicated in the routine.

Images are taken automatically when indicated by the routine and saved under a name indicating the pot code, date and time (Fig. 1*d*, *e*). Image analysis for estimating leaf area is performed using an algorithm that has been tested with soybean plants (Fig. 1*f*, *g*; Benalcázar *et al*. 2011).

Assessment of drought tolerance in two soybean genotypes

The drought tolerance of two soybean (*Glycine max* (L.) Merr.)) genotypes (cv. A3550, Nidera Semillas, Argentina and cv. DM3100, Don Mario Semillas, Chacabuco, Argentina) was analysed. Both genotypes are indeterminate cultivars of maturity group III and under on-farm conditions DM3100 has been observed to perform better than A3550 in years with low rainfall (Atilio Castagnaro, pers. comm.). Cultivar A3550 also shows more elongated internodes than DM3100, which could aid in testing the validity of image-based growth measurements for different plant architectures.

Two experiments were conducted. In the first experiment with six different water scenarios, we evaluated the possibility of detecting genotypic differences in growth and water use efficiency in plants under mild and severe stresses and also at different stages of development. In this experiment, the stress levels were obtained by stopping irrigation of pots until the desired soil water content was obtained. In the second experiment, such genotypic differences were tested under a water deficit treatment imposed by slowly drying the soil, which is a scenario similar to a drought in the field.

In Experiment 1, 120 plants of both genotypes were sown on 18 May 2011, in the previously described pots filled with soil (typic Argiudoll, horizon A). Plants were grown in a growth chamber (16 h photoperiod, 520 µmol m^{-2} s^{-1} PAR, 25/23C temperature day/night, 1.5/1.6 vapour pressure deficit (VPD) day/night) until 48 days after sowing (DAS) and then transferred to the phenotyping platform (mean values: 15 h photoperiod, 250 µmol m^{-2} s^{-1} PAR, 17/11°C temperature day/night, 1.0/0.5 kPa VPD day/night).

Automatic watering was provided daily, according to the programmed routines. Six treatments were imposed to the plants: four mild (0.18 g water g $soil^{-1}$, –0.44 MPa), short-term water deficit treatments (D1, D2, D3 and D4) starting at different moments (48, 54, 58 and 63 DAS), a severe (0.16 g water g $soil^{-1}$, –0.94 MPa), long-term water deficit treatment (D5) and a well-watered (0.26 g water g $soil^{-1}$, –0.02 MPa) control (WW).

Plants were harvested 48 (before treatments), 58 (D1), 63 (D2), 68 (D3) and 73 DAS (D4, D5 and WW) and shoot dry mass was measured. The mean developmental stage of the plants ranged from V5 at the first sampling date to R1 at the final harvest (Fehr *et al*. 1971). After harvesting, pots with no plants were kept in the platform to measure direct soil evaporation. Leaf area was estimated non-destructively when transferred to the platform, by measuring the width and breadth of all terminal leaflets with a ruler (Wiersma and Bailey 1975) and destructively (Li-3100 leaf area meter, Li-Cor, Lincoln, NE, USA) in harvested plants.

In Experiment 2, 116 plants of the same two genotypes were sown on 11 April 2012, as previously described for Experiment 1. Plants were grown in a growth chamber (16 h photoperiod, 525 µmol m^{-2} s^{-1} PAR, 25/21°C temperature day/night, 1.0/1.2 kPa VPD day/night) until 15 DAS and then transferred to the phenotyping platform (mean values: 16 h photoperiod, 365 µmol m^{-2} s^{-1} PAR, 26/17°C temperature day/night, 1.9/0.7 kPa VPD day/night). Four pots were placed in the platform, one per row, each carrying a green, 8 cm-diameter sphere, in order to automatically calibrate the relationship between pixels and real spatial units for each camera.

Automatic watering was provided daily, at 0800 hours, according to the programmed routines. Two treatments were imposed to the plants: a water deficit treatment (D), in which soil water content was decreased in a controlled fashion from 0.29 to 0.20 g water g $soil^{-1}$ (–0.02 to –0.66 MPa) during 21 days starting at 22 DAS and a well-watered (0.29 g water g $soil^{-1}$, –0.02 MPa) control (WW).

Forty-eight plants were kept until the end of the experiment (43 DAS), in order to evaluate water use efficiency and transpiration rate. The rest of the plants were harvested at different times during the experiment (15, 21, 28 and 36 DAS) in order to calibrate the estimation of shoot area and dry mass through image analysis. The mean developmental stage of the plants ranged between V2 at the first sampling date to V5 at the final harvest (Fehr *et al*. 1971). After harvesting, pots with no plants were kept in the platform to measure direct soil evaporation. Harvested plants were scanned and total shoot area measured using ImageJ (Hartmann *et al*. 2011), then dried to constant weight to determine shoot dry mass.

Transpiration rate was measured by weighing the pots hourly and then dividing the slope of weight *v.* time by the shoot area. This was conducted between 0900 and 1600 hours during 13 consecutive days starting at 30 DAS, on 28 of the 48 pots that were kept until the end of the experiment. The remaining 20 pots were weighed only once a day for watering during the first 10 days and they were not used for measurements of transpiration rate during such period. During the last 3 days before the final harvest, transpiration measurements were made on all plants, in order to evaluate the possible effects of increased vibration on the plants. Transpiration rates were analysed dividing the data into daytime (1000–1600 hours) and night-time (1600–0800 hours) transpiration.

Top- and side-view images were obtained for each plant at 0700 hours, every 2–3 days in Experiment 2. Images were segmented and the number of pixels corresponding to the plant were counted and converted to cm^2 using the corresponding calibration factor for each camera and then summed to obtain the projected shoot area. Projected shoot area of harvested plants were correlated with actual shoot area. Shoot dry mass was estimated as a function of shoot area and plant age, as in work by Golzarian *et al*. (2011). The obtained equations were used to estimate these values for all the plants throughout the experiment.

In Experiment 1, water use efficiency (WUE) was calculated for each plant as the ratio between dry mass accumulation (final – initial dry mass) and plant water use (total water use – soil evaporation). Initial dry mass values were estimated for each plant as a function of leaf area and plant age, as previously described. In Experiment 2, WUE was calculated for each plant as the slope of the relationship between estimated dry mass (from image data) and plant water use (total water use – soil evaporation) during the whole experiment and after plant water use differed significantly ($P < 0.05$) between control and water stressed treatments.

Results

Platform performance

Imaging routines took ~31 min to complete, weighing routines 26 min and irrigation routines 42 min. A complete routine (weighing, irrigation and imaging) took 53 min. This allowed programming hourly weighing routines for the measurement of transpiration, in addition to daily imaging and irrigation.

Daily oscillations of soil water content for each treatment are shown in Fig. 2*a* for Experiment 1 and Fig. 2*b* for Experiment 2.

Growth measurement through image analysis

A power function ($y = 1.451x^{0.914}$) described adequately the relationship between actual shoot area and projected shoot area obtained through image analysis, for both genotypes and both treatments. A good fit was observed between measured and estimated shoot area values from Experiment 2, as shown in Fig. 3*a*.

Shoot DW was estimated as a function of shoot area and plant age, as in work by Golzarian *et al*. (2011). The calibrated function was: Shoot DW (g) = 0.1 + 0.00184 × area (cm^2) + 0.0000926 × area (cm^2) × age (days). This equation allowed a satisfactory estimation of shoot DW from both experiments ($R^2 = 0.99$, data not shown). A good precision in the estimation of shoot DW could be also obtained using

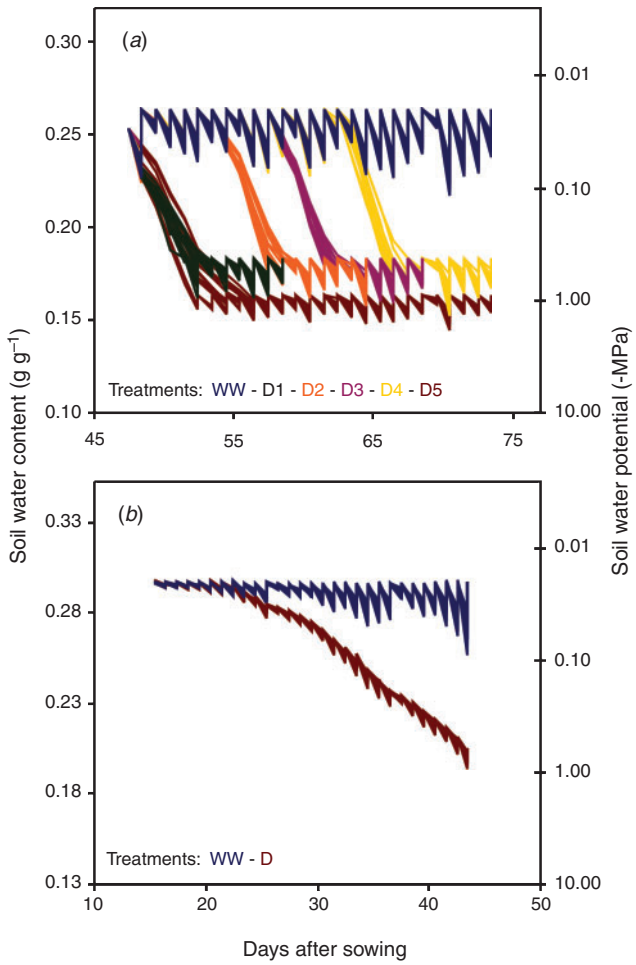

Fig. 2. Time courses of soil water content and soil water potential of individual pots in the six treatments of Experiment 1 (*a*) and the two treatments of Experiment 2 (*b*).

estimated shoot area values from Experiment 2 (instead of measured shoot area), as shown in Fig. 3*b*.

Drought tolerance assessment

In Experiment 1, WUE was higher for genotype DM3100 than A3550, being this difference statistically significant only in treatments D4 and D5 (Fig. 4). Figure 5 shows the shoot dry mass data for individual plants, obtained through image analysis during the whole Experiment 2, in relation to water use data recorded by the platform, showing the genotypic differences in WUE in the control (Fig. 5*a*) and water deficit (Fig. 5*b*) treatments.

Transpiration rates during daytime hours in the water deficit treatment, relative to those in the controls, were similar between genotypes at high soil water contents and then decreased more rapidly in genotype DM3100 than in genotype A3550 (Fig. 6*a*). Night-time transpiration rates decreased with soil water content, also more rapidly in DM3100 (Fig. 6*b*).

None of the measured parameters – transpiration rate, shoot area and WUE – differed significantly between plants which were weighed hourly (84 movements) and plants that were weighed once a day (10 movements) during the first 10 days of transpiration measurements, either in control or stressed plants.

Discussion

The objective of this work was to develop a low-cost, automatic platform for high-throughput measurement of plant water use and growth. To our knowledge, the GlyPh platform is the first low-cost system that allows plant cultivation, treatment imposition and measurement of plant water use and growth. Its design, with few moving parts and generally standard components implies not only a low construction cost, but also simplicity and flexibility.

The GlyPh platform was designed specifically for soybean and constitutes the first automatic phenotyping platform for this crop. However, the simple structure allows for easy

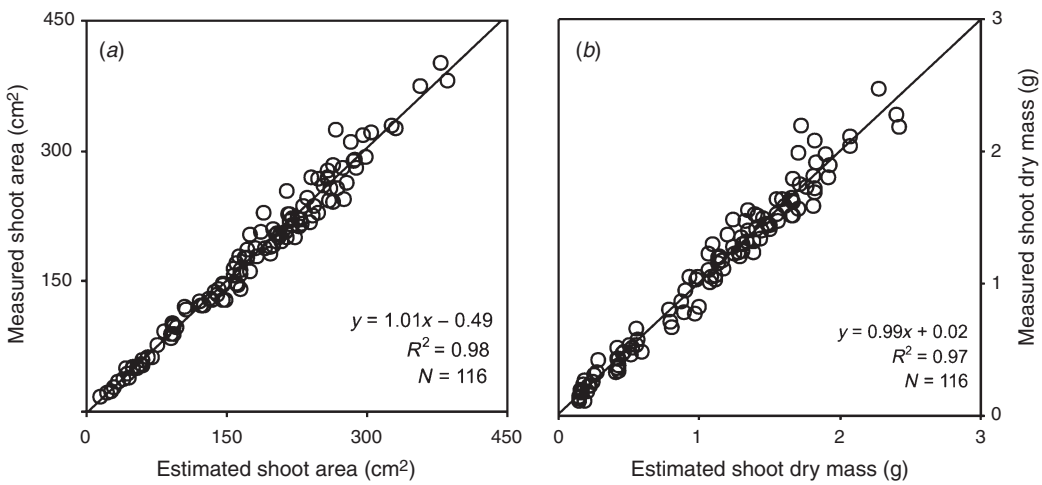

Fig. 3. (*a*) Relationship between measured soybean shoot area and shoot area estimated as a function of projected leaf area from top- and side-view images of the plants. (*b*) Relationship between measured shoot dry mass and shoot dry mass estimated from plant age and shoot area obtained from image analysis. Data from Experiment 2.

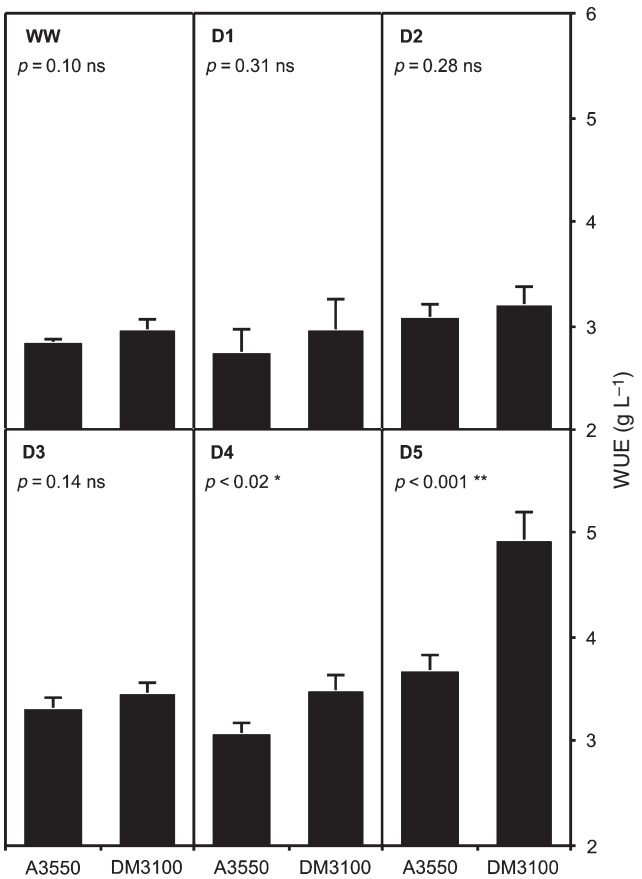

Fig. 4. Mean water use efficiency (+s.e.) for genotypes A3550 and DM3100 in Experiment 1, for each of the six treatments. Significance values are from Student's *t*-test comparison between genotype means.

adaptation to other plant species. In the current configuration of the platform, pots within a row are spaced at 30 cm, but the distance can be modified to accommodate for smaller or bigger plants by changing the stop marks. Balances and pump hoses can be replaced in order to have more capacity or more precision. This can be done in all rows or only in part of them, thus for instance allowing phenotyping of crop plants in some rows (e.g. with 6 Kg capacity and 1 g precision) and *A. thaliana* or *Medicago truncatula* plants in other rows (e.g. with 500 g capacity and 0.1 g precision).

Another limitation of certain platforms is the flexibility in terms of the number of plants that can be analysed. The current size (four rows of 30 plants) of the GlyPh platform is only one of the many possible formats. There are two ways in which the size and therefore throughput, of this platform can be modified. The most costly way is by changing the number of rows (and therefore balances, pumps and cameras). The easiest way of increasing the capacity of this platform is by increasing the length of the rows, therefore, accommodating more pots per row. The maximum length will depend on the weight that can be supported by the structure without significant bending (the weight of the pots and the structure itself). In this case, the platform was set up for soybean plants, which require large containers; smaller plants would allow for a longer structure and less space between pots. Platforms that use conveyor belt systems, such as the Scanalyzer, allow for a very straightforward expansion in their capacity. However, a drawback of conveyor belts is the need for sophisticated calculations for managing the logistics of pot movement and the time lost in plant routeing (~40% of the time of a scan, Helmert and Lasinger 2010). In platforms such as Phenodyn, in which there is one balance and one set of sensors per pot, the cost of expansion is much greater.

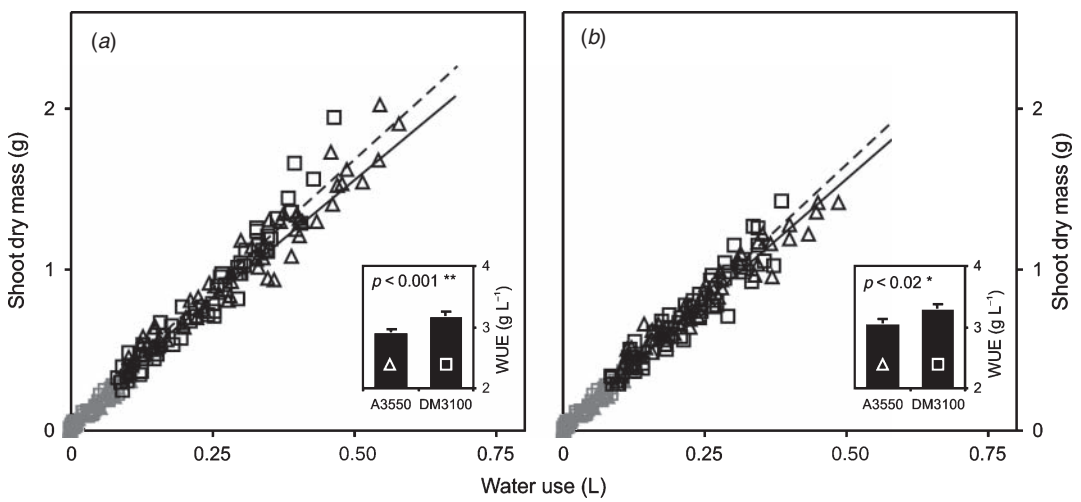

Fig. 5. Relationship between shoot dry mass and water use in the control (*a*) and the water deficit (*b*) treatments, for genotypes A3550 (triangles) and DM3100 (squares) in Experiment 2. Lines represent the water use efficiency for genotypes A3550 (solid line) and DM3100 (dashed line). Black symbols indicate the data used for calculating water use efficiency, starting on the day in which water use began to differ significantly between treatments. Insets: mean water use efficiency (+s.e.) for both genotypes in the control (*a*) and water deficit (*b*) treatments. Significance values are from Student's *t*-test comparison between genotype means.

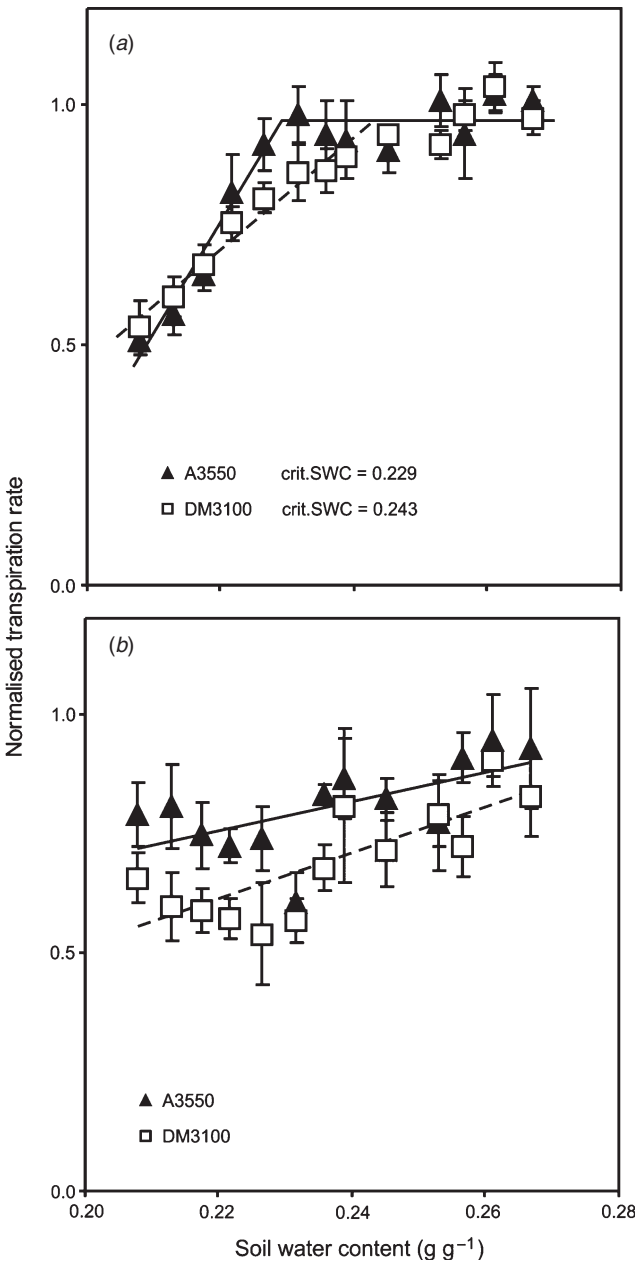

Fig. 6. Transpiration rate in the water deficit treatment, normalised to mean transpiration in the controls, as a function of soil water content, for genotypes A3550 (closed triangles) and DM3100 (open squares), during (*a*) daytime hours (1000–1600 hours) and (*b*) night-time hours (1600–0800 hours). A linear-plateau model was fit to daytime transpiration data and a linear model to night-time transpiration, for each genotype.

One of the main differences between available platforms is the movement of the pots. The extreme cases are the Scanalyzer platforms, in which pots are moved around the greenhouse for each action to be conducted (weighing, watering, measuring) and Phenodyn, in which pots are completely static (although automatic watering is not provided). The platform presented in this work and Phenopsis are intermediate cases, where plants remain in the same place, but are lifted temporarily only for weighing. One of the possible disadvantages of pot movement is vibration, which could lead to changes in plant architecture (Niklas 1998) or even changes in the response to water deficit, due to soil particle movement around the roots (Faiz and Weatherley 1982). None of these effects was detected in Experiment 2, where a set of pots were lifted for weighing about eight times more than a set of control pots for 10 days during soil drying. To the best of our knowledge, this lack of effect of pot movement on plant performance has not been previously tested for any automatic phenotyping platform. Another possible disadvantage of pot movement could be the need to contain some plants with a wire cage (e.g. barley (Hartmann *et al*. 2011) or rice (Crowe *et al*. 2012) plants in a Scanalyzer platform). In the case of our platform, such a structure would have been needed in order to avoid damage from the moving watering hose; this issue was solved with a specially designed, simple funnel which allows some distance between the hose and the plant (Fig. 1*c*).

Estimation of shoot area from image analysis yielded a good correlation, for two genotypes which differ in plant architecture and irrespective of soil water content. Biomass estimation from image data was achieved with a precision similar to that obtained by Golzarian *et al*. (2011) for wheat and barley ($R^2 = 0.97$ vs 0.98), despite the use of only only one side image instead of two and the large differences in plant architecture between cereals and soybean. However, these results are limited to plants within the explored range of leaf area and biomass (up to 400 cm^2 and 2.5 g, Fig. 3). Preliminary tests with significantly bigger plants showed a tendency to underestimate shoot area due to increased leaf occlusion. A change in the relationship between shoot area and biomass would also be expected after the transition between vegetative and reproductive phases. These results do demonstrate the possibility of using this method for high-throughput biomass estimation during vegetative growth in broadleaf species.

One key aspect in image-based phenotyping is the control of the lighting environment for image acquisition. This issue is easily solved in other platforms that are entirely located inside a growth chamber, or in which plants are transported to a closed cabinet for imaging. In our platform (located in a greenhouse), the main limitation is direct sunlight incident on the leaves, which could hinder segmentation during image analysis. In this case, images were taken early in the morning when incident light is mainly diffuse. Flashlamps can be used to overcome this limitation, as used by Polder *et al*. (2009) for imaging plants in a greenhouse.

The work presented here has been focussed mainly in the development of the phenotyping hardware and the minimum software necessary for its operation. An important part of phenotyping technology resides in image analysis (e.g. Furbank and Tester 2011), but image analysis software is not a component that contributes largely to the total cost of phenotyping systems. In our case, an image analysis pipeline was developed specifically designed for soybean plants (Benalcázar *et al*. 2011), but the systems allows the use of other image analysis tools. In addition to commercial packages or custom-made solutions, there are also open-access options such as HTPheno, an image analysis pipeline for plant

phenotyping based on the open-source ImageJ program (Hartmann *et al*. 2011).

Drought tolerance assessment

The phenotyping platform allowed the automatic imposition of multiple, diverse water scenarios to two soybean genotypes, a task that would have been extremely laborious to conduct manually. The precision in soil water content management, as shown in Fig. 2, minimises the chance of artefacts, which can arise when phenotyping for drought tolerance is conducted by just withholding watering (Pereyra-Irujo *et al*. 2007). As demonstrated in Fig. 2, GlyPh could be use to simulate a controlled dry-down water deficit. This drying scenario is similar to a drought in the field and also prevents imposing a more severe stress to larger plants from the beginning of stress imposition to the moment when the desired soil water content is reached.

Genotypic difference in WUE was evident only in some of the explored water deficit scenarios. This points out the need to evaluate a genotype's phenotype under a broad range of conditions in order to capture valuable differences, therefore, emphasising the importance of phenotyping platforms being able to modify environmental conditions besides trait measurement. This is especially true in the case of drought tolerance, because a phenotypic trait which confers tolerance in one scenario might confer susceptibility in another (Tardieu 2005).

Hufstetler *et al*. (2007) found the minimum leaf conductance to be negatively correlated with WUE in soybean. The lower night-time transpiration rates observed in DM3100 could, therefore, be a possible indicator of a low minimum conductance in this genotype. Blum (2005) argued that higher WUE is frequently achieved through mechanisms that reduce not only water use, but also growth and yield. In this case, genotype DM3100 achieved a higher WUE under drought through reduced transpiration rate, but also higher dry mass accumulation relative to A3550 (data not shown). This is a notable trait for yield maintenance under drought, since seed number per plant (the main yield component of cereals and oil-seed species) is linearly related to biomass accumulation during a critical period in soybean (Vega *et al.* 2001). This result also supports the observation that under on-farm conditions DM3100 performed better than A3550 in years with low rainfall (Atilio Castagnaro, pers. comm.). Moreover, these results emphasise the capability of our platform not only for evaluating cultivars for water deficit tolerance but also for improving the understanding of the behaviour of different soybean varieties when exposed to water deficit.

WUE increased under water deficit, as shown previously by Liu *et al*. (2005) and the relative order of WUE values for the two genotypes was conserved in all experiments and treatments. These results support the suggestion made by Earl (2002) that WUE in soybean is a constitutive trait.

Conclusions

An automatic platform has been developed to allow the assessment of water use, growth and WUE of soybean genotypes under multiple soil water scenarios. It is simpler and has a significantly lower construction cost than previously existing platforms and is, therefore, suitable for low-budget research groups or seed companies, as well as for use in the developing world.

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