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DROUGHT STRESS

Drought Tolerance Screening Under Controlled Conditions Predicts Ranking of Water-Limited Yield of Field-Grown Soybean Genotypes

E. M. Pardo[1,*], G. R. Vellicce[1,*], L. Aguirrezabal[2], G. Pereyra Irujo[2], C. M. L. Rocha[1], M. G. García[1], S. Prieto Angueira[3], B. Welin[1], J. Sanchez[1], F. Ledesma[1] & A. P. Castagnaro[1]

1 Estación Experimental Agroindustrial Obispo Colombres (EEAOC) – Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Instituto de Tecnología Agroindustrial del Noroeste Argentino (ITANOA), Las Talitas, Tucumán, Argentina

2 Laboratorio de Fisiología Vegetal, Unidad Integrada Balcarce, Facultad de Ciencias Agrarias, Universidad Nacional de Mar del Plata – Instituto Nacional de Tecnología Agropecuaria, Balcarce, Argentina

3 EEA Santiago del Estero, Instituto Nacional de Tecnología Agropecuaria, Santiago del Estero, Argentina

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Correspondence
A. P. Castagnaro
Estación Experimental Agroindustrial Obispo Colombres (EEAOC)- Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Instituto de Tecnología Agroindustrial del Noroeste Argentino (ITANOA). Av. William Cross 3150, C.P. T4101XAC, Las Talitas, Tucumán, Argentina
Tel.: +54-381-4521000 (ext. 162)
Fax: +54-381-4521008
Email: atilio@eeaoc.org.ar

*Both authors contributed equally to this work.

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Abstract

Drought is a major limitation to crop yields worldwide. Screening for soybean yield under water deficit is often a bottleneck in breeding programmes. We assessed the validity of a standardized drought tolerance screening method to predict water-limited field performance of soybean in NW Argentina. First, to determine the phenological period when yield of glasshouse-grown plants was more sensitive to water deficit, we applied treatments during 21 days in V_7, R_3 or R_5 stages, being the period from R_5 to R_6 the most critical for yield. Afterwards, two glasshouse experiments were carried out to quantify the tolerance of either eight or four genotypes, respectively, by applying a controlled water deficit of constant intensity during the critical period. Finally, yield data obtained in field trials in Argentina across several locations and seasons classified according to rainfall were analysed. Drought Susceptibility Index was calculated for each experiment and for field data, and rankings of tolerance were similar in all cases. This standardized method, which can be automated for high-throughput phenotyping, could represent a useful tool in breeding programmes for identifying soybean cultivars with improved performance under drought conditions.

Introduction

Drought is one of the most important environmental stresses in agriculture worldwide. It is expected that water limitation to crop yields will increase in many regions due to the effects of climate change on rainfall and evaporative demand (IPCC 2007). Many efforts have therefore been made to improve crop productivity under water-limiting conditions. The ability to produce high seed yield in drought-affected environments is taken as the ultimate indicator of drought tolerance (Tardieu 2005, Du et al. 2009).

Genetic improvement for drought adaptation has been largely addressed through the conventional approach of selecting for stable, high-yielding cultivars over varied locations and years (Babu et al. 2003, Hall et al. 2013). The high genotype-by-environment interaction usually observed for drought tolerance (Chapman 2008), combined with the low repeatability of water deficit under field conditions, can lead to inaccurate rankings of drought tolerance (Wery et al. 1997), and therefore, many field trials across time and space are usually required to assess the tolerance of a given genotype. Precise phenotyping is therefore currently seen as a major bottleneck for the improvement

of drought tolerance (Dolferus et al. 2011, Hall et al. 2013).

Soybean [*Glycine max* (L.) Merr.] is the most widely grown oil crop in the world (FAO 2013) and, similar to others crops, water deficit is the most important factor limiting its yield. As for other species, genetic variability for drought tolerance exists in soybean (Frederick et al. 2001). Identifying drought-tolerant genotypes by means of improved phenotyping methods under controlled conditions could be advantageous for soybean breeding programmes. Gravimetrically controlling soil water content is the most widespread method for reliably imposing a reproducible drought treatment to potted plants under controlled conditions, allowing to manage the timing, intensity and duration of the stress, therefore reducing the influence of environmental conditions and water-use rate of individual plants. This method has been successfully used in model and crop species, such as *Arabidopsis thaliana* (Bacsó et al. 2008), sunflower (Pereyra-Irujo et al. 2007), maize (Chapuis et al. 2012) and soybean (Earl 2003). Correlating results obtained using controlled environment phenotyping with field performance remains one of the most important challenges in breeding for drought-tolerant crops (Passioura 2012). One of the few examples in which this issue has been addressed is the work of Chapuis et al. (2012), who showed that tolerance values of the response of leaf growth to water deficit of maize obtained in a phenotyping platform were consistent with those of resilience of grain number to drought in the field. Their results indicate that the capacity to maintain leaf growth under water deficit was the most important trait leading to higher grain yield under water stress in different field environments. These results suggest that drought tolerance rankings of glasshouse-grown potted plants could be extrapolated to field-grown plants provided that measurements focus on those traits that are critical. Although a method has been automated for soybean phenotyping (Pereyra-Irujo et al. 2012), correlation between the response to drought of soybean grain yield under controlled and field conditions using a standardized, reproducible, phenotyping method remains to be tested.

Determining the period when yield is most sensitive to drought stress could aid in designing the treatment which maximizes the possibilities of obtaining a high correspondence between controlled and field conditions. This most-sensitive period is usually found during reproductive stages as yield is mostly driven by grain number in several crops species including soybean (Kantolic and Slafer 2005, 2007), although the response of leaf development (which determines radiation interception and biomass accumulation) to drought during earlier vegetative stages could also affect yield.

The objective of this work was to evaluate the validity of a standardized drought tolerance screening method using glasshouse-grown potted plants to predict water-limited field performance in soybean. To achieve this, we (i) identified the phenological period when yield is most sensitive to drought, (ii) determined the drought tolerance ranking of several soybean genotypes exposed to water deficit during that period and (iii) compared this ranking with that obtained in field trials. To the best of our knowledge, such study has not been previously performed.

Materials and Methods

Glasshouse experiments

Plant material

In Experiment 1, soybean commercial genotype NA8000 was used to determine the phenological period when yield was most sensitive to water deficit. In Experiment 2, eight genotypes were selected on the base of their genetic background: six commercial genotypes (NA8000, Munasqa, TJ2049, BR16, Conquista and NA7001), one Plant Introduction (PI416937); and one elite genotype (EE_124), considered as water deficit tolerant (Devani M, personal communication). Based on results found in Experiment 2, four genotypes were tested in Experiment 3: NA8000, Munasqa, TJ2049 and PI416937.

Growth conditions and water availability treatments

Glasshouse experiments were conducted during three consecutive growth seasons at the Estación Experimental Agroindustrial Obispo Colombres (EEAOC), Las Talitas, Tucumán, Argentina (S26°50′, W65°12′). Plants were grown in pots, in an environmentally semi-controlled glasshouse. The pots (diameter: 20 cm, height: 50 cm) contained 6.2 kg of sandy loam soil–sand mixture (3 : 1). Four seeds per pot were sown, seedlings were thinned when the first trifoliate leaf emerged, keeping one seedling per pot. To minimize soil water evaporation, the top soil was covered with a 2-cm layer of perlite. Pots were moved and rearranged weekly to minimize uncontrolled differences in environmental conditions in the glasshouse. Air and soil temperatures, air relative humidity and incident photosynthetically active radiation (PAR) were measured every 15 min and averaged and recorded every 1 h, with data loggers (Cavadevices.com, Buenos Aires, Argentina). Thermal time was calculated as the daily integral of the difference between temperature and base temperature of 8.0 °C (Jones et al. 1991).

The estimation of soil water content in each pot was performed as described by Pereyra-Irujo et al. (2012). Briefly, the weight of each empty pot and the dry substrate was determined at the beginning of the experiment.

Additionally, the estimated fresh weight (FW) of the plant was entered regularly (usually on a weekly basis). These data were then used for calculating the soil water content of each pot and the amount of water that had to be added every day (Experiments 1 and 3) or every two days (Experiment 2) to reach the desired soil water content. A relationship between soil water content and soil water potential was determined (Richards 1965). All pots were well-watered to a soil water content of 22 % corresponding to a soil water potential (ψw) of −0.05 MPa until the imposition of water deficit treatments in all experiments. The targeted soil water potential corresponding to water deficit treatments was reached within 2–3 days in all glasshouse experiments.

Irrigation was performed with a 2 g l^{-1} nutrient solution (Red HAKAPHOS®, COMPO Argentina SRL, San Isidro, Argentina) which contained nitrogen to avoid possibly confounding effects from biological nitrogen fixation or from its response to drought. The composition of the nutrient solution was 18 %N, 7.9 %P, 15 %K, 0.6 %Mg, 0.8 %S, 0.05 %Mn, 0.019 %Zn, 0.01 %B, 0.05 %Fe, 0.019 %Cu and 0.01 %Mo. Seeds were not inoculated with *Bradyrhizobium japonicum*.

Experiment 1 was sown at 12 January 2010 to determine the phenological period where yield was most sensitive to water deficit. A similar and constant water deficit level was imposed to plants of soybean genotype NA8000 on different phenological stages, which were determined visually according to the scale defined by Fehr et al. (1971). Three groups of five pots each were subjected to treatments of water deficit (D1, D2 and D3) consisting of maintaining soil water content at 14 % ($\psi_w = -0.65$ MPa) during 21 days. Treatment D1 was applied at a vegetative stage (V_7) and ended when plants where at the R_2 stage (Fig. 1). The other treatments were applied at reproductive stages: treatment D2 was applied at R_3 and ended at $R_{5.5}$; treatment D3 was applied at R_5 and ended when plants reached R_6 (Fig. 1). A group of five pots remained well-watered (Control treatment) during the whole experiment. At the end of the water deficit period, pots were re-watered to Control treatment levels and remained well-watered until physiological maturity.

Experiments 2 and 3 were performed to determine and confirm the ranking of tolerance to water deficit among different soybean genotypes, grown in pots in a glasshouse. A water deficit similar to that used in Experiment 1 was applied at the time and duration corresponding to the period of maximum sensitivity (from R_5 to R_6). Sowing was performed late in the season (January 12 for MG VIII and VII while MG V and VI January 14) to reduce differences in phenological stages among genotypes. The R_5 stage was registered on 09 March and 12 March for TJ2049 and NA8000, the two genotypes with more contrasting cycle length. Thirty plants per genotype were grown. Water deficit was applied to 15 plants (WD treatment), while 15 plants were well-watered during the whole experiment (Control treatment). For each treatment, five plants were harvested (one plant each 5–7 days) and weighed for FW determination to adjust the estimation of soil water content for pots of different genotypes.

Measurements

In all glasshouse experiments, plants were harvested at physiological maturity. Seeds were separated manually into non-aborted and aborted. Grain samples were oven-dried at 60 °C for 48 h. In the non-aborted seed subsample, seed yield per plant, seed number and 100-seed weight were determined.

Field trials

Yield data belonging to genotypes which showed contrasting responses in Experiments 2 and 3 were obtained from a database of a regional trial network of commercial soybean cultivars. Available data were only those from genotypes Munasqa, NA8000 and TJ2049. This trial network is conducted by the Soybean Breeding Program of the Estación Experimental Agroindustrial Obispo Colombres (EEAOC). Macro plots were located at different locations in northwestern Argentina (Devani et al. 2012). The goal of this trial network was to evaluate adaptation and yield of commercial soybean cultivars in large-scale plots placed at about 14 locations across NWA (from 63°30′ until 66°00′ South Latitude, and from 22°30′ until 28°30′ West longitude). In each experiment, cultivars of late maturity groups

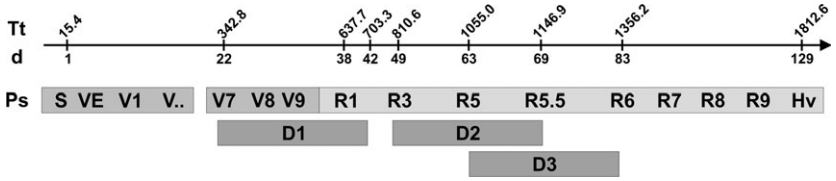

Fig. 1 Time and duration of water deficit treatments applied during different phenological periods according to Fehr et al. (1971). D1: treatment started at stage V_7 and finished at stage R_1; D2: treatment started in stage R_3 and finished at stage $R_{5.5}$; and D3: treatment started at stage R_5 and finished at stage R_6. Ps: phenological stages. V_7: plants with seven nodes; R_3: pod appearance; R_5: pod-filling stage; $R_{5.5}$: pod-filling stage with >75 % of final weight; R_6: full pods and green leaves; d: days; Tt: thermal time.

(MG) (VII and VIII) were planted apart from those of early MG (IV, V and VI). Each plot consisted of 1000 m^2 with rows spaced 0.52 m apart. The plant density was between 18 and 22 plants m^{-1}. Recommended agronomical practices were applied, which include inoculation with *Bradyrhizobium japonicum* before sowing. Yield data were corrected by grain moisture (13.5 %).

Data analysis

Data obtained in glasshouse experiments were subjected to analysis of variance using Infostat (Di Rienzo et al. 2008). Each analysis was set with a significance level of P = 0.05. When statistical differences in more than one experiment were detected, only the highest *P* value was considered. Tukey's HSD test was applied to compare yield and yield components for each genotype under different water status. Data from Experiment 2 and Experiment 3 were analysed by a two-way analysis of variance (ANOVA) to determine the effect of the genotype (genetic variability) and of water deficit (phenotypic plasticity) on yield and its components and to determine the presence of interactions between these two factors (genetic variability for phenotypic plasticity).

For glasshouse and field experiments, a Drought Susceptibility Index (DSI) was calculated for each genotype according to Du et al. (2009) considering yield obtained under water deficit (WD) and well-watered (WW) conditions. For the calculation of the DSI under field conditions, yield data of genotypes Munasqa, NA8000 and TJ2049 grown in 58 trials were considered. Trials were separated into three groups according to rainfall during a period beginning 3 months before harvest and ending a month before harvest. This is based on a rule of thumb frequently used by local growers to classify years as 'wet', 'intermediate' or 'dry'. For this region, 'dry' years are those with <175 mm rainfall during this critical 2-month period, and 'wet' years are those with at least twice as much rainfall (Devani et al. 2012). According to this rule, 13 trials were classified as 'dry' and 16 trials as 'wet' ('intermediate' trials were not considered in the analysis).

Results

Growing conditions

The range of values found in glasshouse and field experiments for different environmental variables is shown in Table 1. Environmental conditions in glasshouse experiments were very similar between years and were characterized by higher minimum temperatures (and slightly higher mean temperatures), higher relative humidity and lower incident radiation than field experiments. Variability in environmental conditions between locations and years was mainly due to differences in rainfall, relative humidity and incident solar radiation.

Table 1 Minimum and maximum whole-experiment average values of daily mean and minimum temperature, mean relative humidity and mean incident solar radiation in glasshouse and field experiments

	Glasshouse experiments		Field experiments	
	Minimum	Maximum	Minimum	Maximum
Mean temperature (°C)	22.2	23.4	19.4	22.9
Minimum temperature (°C)	17.1	18.0	14.0	16.5
Relative humidity (%)	86	88	48	71
Incident solar radiation (W m^{-2})	126	132	182	312
Rainfall (mm)			58	679

Glasshouse experiments

Experiment 1 was performed to determine the phenological period where yield was most sensitive to water deficit by applying a similar deficit level on different stages of a soybean genotype. Yield per plant and seed number per plant significantly decreased only in treatment D3, in which plants were subjected to a water deficit between R_5 and R_6 stages (Table 2). Differences with the Control treatment were not significant for treatments D1 and D2, in which plants were subjected to a water deficit between V_7 and R_2, or R_3 and $R_{5.5}$, respectively. The weight of 100 seeds (P100) was unaffected by water deficit applied at any stage. These results indicated that applying a water deficit between R_5 to R_6 period is appropriate for phenotyping soybean yield tolerance to water deficit. This information was further used for screening genotypes in Experiments 2 and 3.

In Experiment 2, eight genotypes were subjected to a water deficit during the period of maximum sensitivity determined in Experiment 1 to determine the ranking of

Table 2 Effect of water deficit treatments applied at different stages (see Methods) on yield per plant and its components of soybean genotype NA8000 in Experiment 1

Treatment	Number of seeds/plant	Yield per plant	100-seed weight
D3	45.2 A	8.56 A	15.92 A
D1	74.8 B	12.7 B	16.95 A
D2	80.8 B	13.65 B	17.13 A
C	90.0 B	14.29 B	19.02 A

Different letters indicate statistical differences (Tukey's test, P = 0.05).

tolerance among them. Yield and its components showed statistical differences among genotypes ($P \leq 0.01$) and water treatments ($P \leq 0.01$). The interactions between genotypes and water treatments were also significant ($P \leq 0.045$), meaning that at least some genotypes behave in a different way in response to water deficit.

Seed yield per plant (Fig. 2a) under well-watered conditions was higher for NA8000, BR16 and NA7001 and lower for PI416937 and TJ2049. Conquista, Munasqa and EE_124 showed intermediate yield values which were not significantly different from the other genotypes. This ranking was modified when plants were subjected to water deficits (Fig. 2c). Under this condition, Munasqa and NA8000 showed the greatest seed yield, while TJ2049 showed the lowest value. Yields under WD and WW were not correlated ($P = 0.29$).

In well-watered plants, seed number per plant showed a similar behaviour among genotypes to that described for seed yield per plant. This correspondence was altered when comparing both traits in plants subjected to water deficit. Weight of 100 seeds showed in some cases a different behaviour to those described for per plant seed yield and number (data not shown).

In the Experiment 3, four genotypes selected taking account the results of Experiment 2 were subjected to water deficit treatment. Results obtained were similar to Experiment 2. NA8000 and Munasqa showed the greatest seed yield again, while TJ2049 and PI416937 showed a lowest value under well-watered condition (Fig. 2b). When water deficit was applied, this ranking was maintained and genotypes NA8000 and Munasqa showed the greatest seed yield (Fig. 2d).

Field trials

The three genotypes tested showed similar yields when yield data from the complete field trial database was considered (Fig. 3a). Despite these similar average and median values of yield data among the studied genotypes across environments (Fig. 3a), the tenth percentile for TJ2049 was lower indicating a lower yield in some environments. Consistently, different responses were detected among genotypes to environmental conditions when yield data were separated into groups according to rainfall during a period beginning 3 months before harvest and ending a month before harvest (rainfall ≥350 and ≤175 mm, respectively, Fig. 3b,c, respectively). The three genotypes showed similar average, median and variability of yield when data corresponding to environments without water deficit (rainfall ≥350 mm) were considered. When yield data corresponding to field trials subjected to water deficit were considered (rainfall ≤175 mm), all genotypes showed a decrease in yield compared to environments without water deficit (compare Fig. 3b,c) but TJ 2049 presented even lower yield (Fig. 3c).

Correlation between glasshouse and field rankings

Drought Susceptibility Index (DSI) values (Du et al. 2009) were calculated using data obtained in the glasshouse

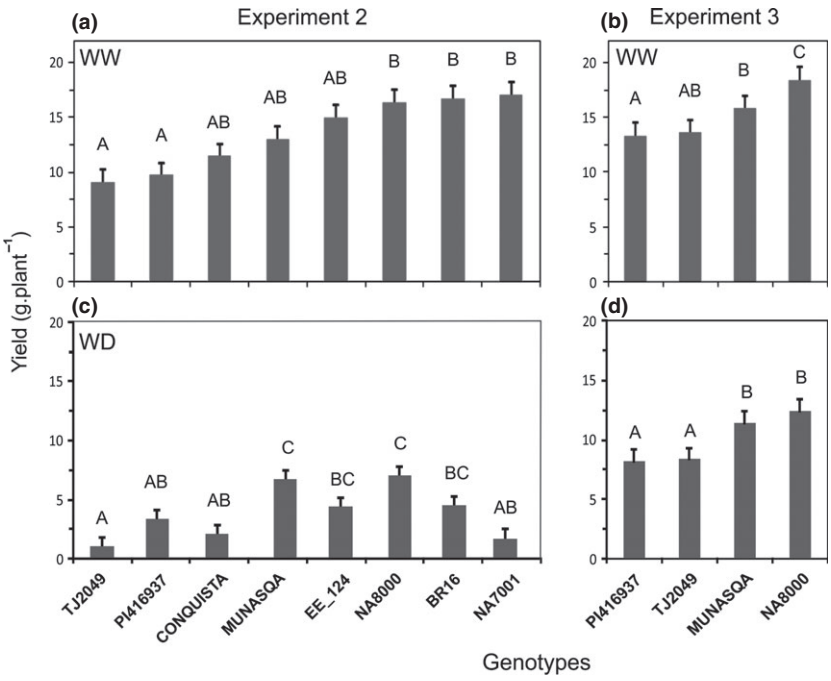

Fig. 2 Seed yield per plant for different genotypes subjected to two water regimes (WW = −0.01 MPa, top panels and WD = −0.65 MPa, bottom panels) in two different experiments (Experiment 2, well watered (a) and water deficit (c); Experiment 3 well watered (b) and water deficit (d)) bars with different letters are significantly different ($P < 0.05$). Bars represent mean values; error bars represent standard deviation of mean values.

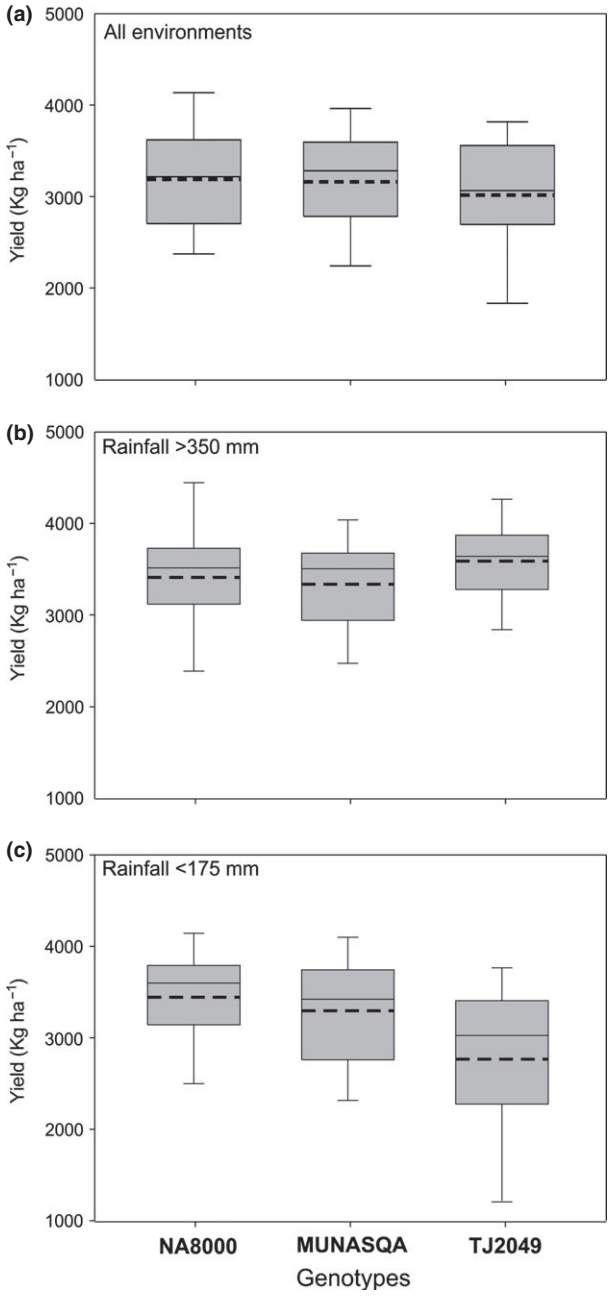

Fig. 3 Boxplot for the values of yield data of the soybean genotypes Munasqa, NA8000 and TJ2049 obtained from 58 environments from a field trial network where macro plots were located at different locations in the north-western Argentina (see Methods). (a) Yield data from all environments, (b) yield data corresponding to field trials without water deficit (rainfall ≥350 mm) and (c) yield data corresponding to field trials subjected to water deficit (rainfall ≤175 mm). The boundary of the box closest to zero indicates the twenty-fifth percentile, a line within the box marks the median, the dotted line indicates the average value, and the boundary of the box farthest from zero indicates the seventy-fifth percentile. Whiskers above and below the box indicate the ninetieth and tenth percentiles.

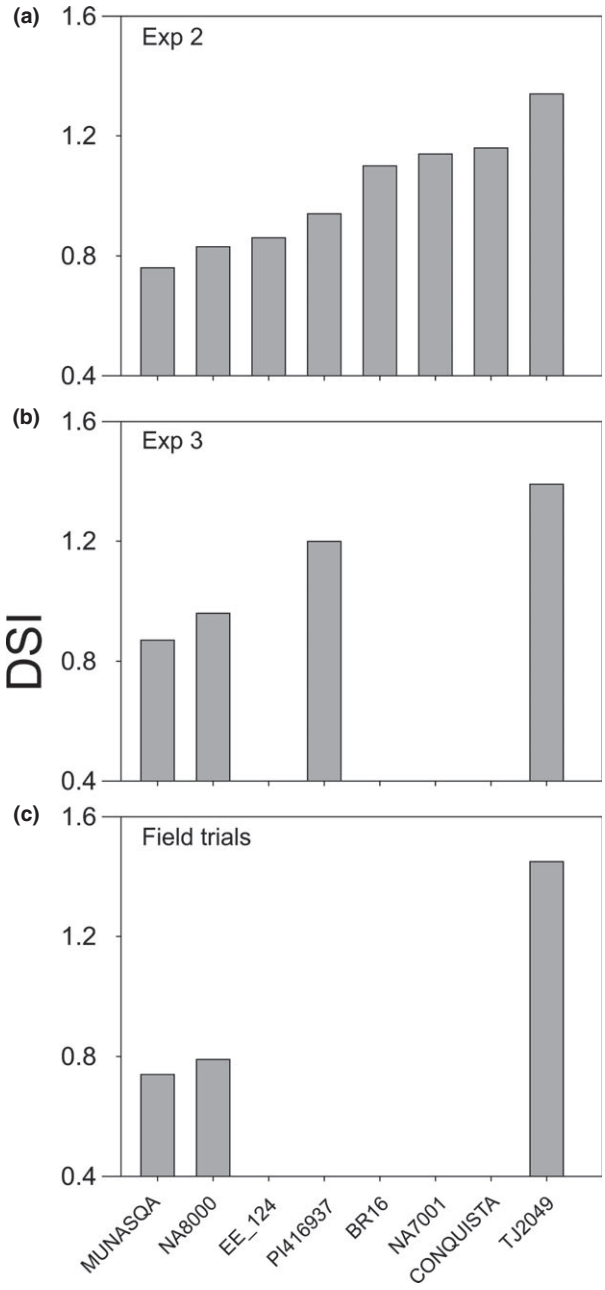

Fig. 4 Values of Drought Susceptibility Index for different soybean genotypes calculated according to Du et al. (2009) for seed yield per plant (g plant^{-1}) of potted plants grown in the glasshouse in Experiment 2(a), Experiment 3(b) and for yield per unit soil surface data (kg ha^{-1}) obtained from a Field Trials Network Database (c).

(Experiments 2 and 3) and in field trials and was used to establish a ranking for yield tolerance among genotypes (Fig. 4). In both experiments and in field trials, Munasqa was the more tolerant genotype, followed by NA8000, while TJ2049 presented the highest susceptibility to drought throughout analysis (Fig. 4).

Discussion

In this work, we used a standardized protocol to determine the ranking of drought tolerance of several soybean genotypes grown in pots in the glasshouse, exposed to drought during the phenological period when yield is most sensitive to drought. This ranking was conserved when yield from several field trials was analysed.

Particular phases during plant development are more relevant for the determination of crop yield. Several authors have explored critical periods for the determination of yield and its components in several species (Fischer 1975, Kiniry and Ritchie 1985, Savin and Slafer 1991, Jiang and Egli 1993, Kantolic and Slafer 2007, Arisnabarreta and Miralles 2008). The knowledge of many critical periods has been helpful for understanding the mechanisms underlying plant responses to environmental factors. In soybean, seed number per plant (the component driving yield of cereals and oilseed species) is linearly related to biomass accumulation during a critical period comprised between R_3 and R_6 (Vega et al. 2001b). The critical period for yield for plants subjected to water deficit identified in this work was comprised of the critical period identified by others for plants subjected to other environmental stresses (Vega et al. 2001a, Kantolic and Slafer 2007).

Controlled environments offer the stability to search for attractive phenotypes or genotypes under a well-defined scenario. Moreover, if the method can be automated, high-throughput phenotyping could be achieved by means of phenotyping platforms. However, as controlled conditions usually differ greatly from those in the field, Passioura (2012) argues that breeders are unlikely to take much notice of research in these environments unless the worth of specific traits has been demonstrated in the field. In this work, we have demonstrated that it is possible to extrapolate results obtained in potted plants grown in the glasshouse to predict yield tolerance rankings in the field. Two reasons probably explain the reproducibility in the field of glasshouse results of yield tolerance to water deficit found in this work by applying the method used by Pereyra-Irujo et al. (2012) to impose and maintain water deficit in potted plants. First, this method is standardized and allows the imposition of similar drought treatments to different genotypes within and across experiments. Second, the time and duration of water deficit was applied to potted plants grown in the glasshouse during the critical period when yield was most sensitive to drought, as determined in Experiment 1. In agreement with our results, Chapuis et al. (2012) found similar rankings for water deficit of maize measured in a phenotyping platform and in the field considering in the last case the soil water status during a critical period. Du et al. (2009) correlated data from field and glasshouse in a soybean mapping population, although common QTLs for yield under all water conditions were not found in this work. In the glasshouse, they irrigated pots of a same water treatment with a same water rate independently of the genotype (the water deficit treatment was irrigated when 50 % of RILs visually showed signs of wilting). Imposing and maintaining water deficit by such method often results in a low repeatability and accuracy of the rankings among genotypes as the intensity of the water deficit depends largely on each genotype leaf area and genotypes with larger leaf area at the moment of imposition of water deficit deplete soil water first (Granier et al. 2006). On the other hand, QTLs for water-saving traits measured in pot experiments through a precise phenotyping protocol co-mapped with a yield-based drought tolerance QTL in pearl millet (Kholová et al. 2012).

Daily weighing and watering of each pot is recommended for adequately controlling soil water content using the protocol applied in this work to impose and maintain the desired soil water potential. One drawback of this method is that it requires intensive labour if it is carried out manually. Devices for automating this task have been developed, such as a conveyor belt carrying pots to a weighing and watering station (Andrew and Cowper 1973) or a continuous weighing and watering system for each pot (Hunter 1981). As cited in the Introduction section, in recent years, high-throughput devices have been developed, which can handle hundreds or even thousands of pots automatically (Furbank and Tester 2011, Pieruschka and Poorter 2012), which enable this method to be used for phenotyping in breeding programmes. One of these platforms has been recently developed specifically for soybean (Pereyra-Irujo et al. 2012).

The ability to produce high seed yield in drought-affected environments is taken as the ultimate indicator of drought tolerance (Tardieu 2005, Du et al. 2009). The low repeatability of water deficit under field conditions would nevertheless lead to confounding rankings of drought tolerance (Wery et al. 1997). Side-by-side rainfed and irrigated trials used by Du et al. (2009) allow a better environmental control for assessing water deficit tolerance in the field than variety trials conducted under rainfed conditions over extended periods of time. Some successful experiences for improving water deficit tolerance were achieved in maize through an adequate characterization or control of the environments where plots were phenotyped (Bänziger et al. 2006). In this work, the challenge was to obtain information about drought tolerance from a field trial network, dealing with other sources of yield variation associated with years and seasons (including available water stocked in the soil before the beginning of the critical period). As rankings were similar between field and glasshouse and databases of field trials are largely available around the world, it could be suggested that characterizing environments using a rule

of thumb such as the one used in the present work could represent a low-cost and less time-consuming procedure than quantifying water tolerance using side-by-side water treatments in the field. Simple, locally established empirical relationships have been successfully used to characterize soil water availability in different environments as well as its impact on yield of different crops, including soybean (Calviño and Sadras 1999). Water availability of different environments in a trial network could be better characterized with some additional simple environmental measurements (Chapuis et al. 2012). Furthermore, complex models that estimate soil water balance could be used to better characterize water availability during the critical period established in this work for different genotypes in field trials (Messina et al. 2009).

Long cycle duration tends to improve yield under favourable conditions by increasing the amount of intercepted light, but decrease it under severe terminal drought because it causes depletion in soil water before the end of the crop cycle (Tardieu 2005). Such a behaviour was not found in this study; genotype TJ2049 showed a similar yield than Munasqa and NA8000 under field conditions when rainfall did not limit yields, despite its lower cycle duration. Moreover, yields under good water availability during the critical period were not modified when only results obtained in late sowing dates were considered in the analysis (data not shown). Collectively, these results indicate that drought tolerance and yield potential are independent traits in these genotypes, as yields under well-watered and stressed conditions were not correlated. Genotype TJ2049, which showed a low tolerance to water deficit, and NA8000 and Munasqa, which were identified as tolerant genotypes, could be used as parentals of segregating populations aimed at identifying the genes or genetic regions linked to tolerance of yield to water deficit in soybean.

Drought tolerance can involve several mechanisms. The tolerance of biological nitrogen fixation (BNF) to drought has been proposed as an important component of drought tolerance in soybean (Sall and Sinclair 1991, Serraj et al. 1997, Sinclair et al. 2008). In our glasshouse study, plants were not inoculated and fertilized with N, which indicates that differences in drought tolerance among genotypes were not due to differences in BNF under water deficit conditions (nodules were not observed on the root system at harvest in any of the glasshouse experiments). In the field, inoculation with *B. japonicum,* together with the natural variation in soil N content, would lead us to think that significant BNF could have occurred in at least part of the field trials. Finding a consistent drought tolerance ranking among genotypes in diverse conditions makes it unlikely that observed differences in drought tolerance could have been due mainly to differences in BNF. Nevertheless, drought tolerance can involve several mechanisms that probably co-occur. Recently, two articles showed that slow-wilting genotypes (another mechanism possibly underlying drought tolerance in soybean, Fletcher et al. 2007, Pathan et al. 2014) also exhibit drought-tolerant BNF (Bellaloui et al. 2013, Devi and Sinclair 2013). Moreover, Fenta et al. (2012) suggest that the response of BNF to drought could be a consequence of the plant's ability to maintain photosynthesis under stress. The mechanisms underlying differences in water deficit tolerance among genotypes described in this work still need to be studied.

The three genotypes studied in all experiments (Munasqa, NA8000 and TJ2049) presented similar values of DSI (Fig 3) regardless of (i) the condition under which plants were grown (glasshouse or field), (ii) the basis used to express yield (yield per plant or yield per unit area) or (iii) the approach used to assess drought tolerance (controlled water deficit in pots or field trial data analysis). Furthermore, genotype rankings were similar between both glasshouse experiments. These results collectively indicate that the standardized method to impose stable and repeatable soil water deficits to potted glasshouse-grown soybean plants used previously by Pereyra-Irujo et al. (2012) represents a useful tool to obtain reproducible rankings of yield tolerance to water deficit and that these rankings predict those obtained in the field.

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