Deeper and wider root growth as phenotypic markers for avoidance of moisture deficit in young faba bean (*Vicia faba* L.) plants

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Background. Soil moisture deficiency causes yield reduction and instability in faba bean (*Vicia faba* L.) production. The extent of sensitivity to drought stress varies across accessions originating from diverse moisture regimes of the world. Hence, we conducted successive greenhouse experiments in pots and rhizotrons to identify genotypic sources and root phenotypic markers for drought tolerance.

Methods. A set of 89 accessions from wet and dry growing regions of the world were screened in a greenhouse experiment grown in a perlite-sand medium under well watered conditions. Stomatal conductance, canopy temperature, chlorophyll concentration, root and shoot dry weights were recorded during the 5th week of growth. Eight accessions representing the range of responses were selected for further investigation. Starting 5 days after germination, they were subjected to a root phenotyping experiment using the automated phenotyping platform GROWSCREEN-Rhizo. The rhizotrons were filled with peat-soil under well watered and water limited conditions. Root architectural traits were recorded 5, 12, and 19 days after the treatment (DAT) began.

Results. In the germplasm survey, accessions from dry regions showed significantly higher values of chlorophyll concentration, shoot and root dry weights than those from wet regions. Root and shoot dry weight as well as seed weight, chlorophyll concentration and root dry weight were positively correlated with each other. Accession DS70622 combined higher values of root and shoot dry weight than the rest. The experiment in GROWSCREEN-Rhizo showed large differences in root response to water deficit. There was genotype by environment interaction in taproot and second order lateral root lengths at 12 and 19 DAT, and the taproot length was reduced up to 57% by drought. The longest and deepest root system under both treatment conditions, were recorded by DS70622 and DS11320, and total root length of DS70622 was 3 times longer than that of WS99501, the shortest rooted accession. The maximum horizontal distribution of a root system and root surface coverage were positively correlated with taproot and total root lengths and root system depth. DS70622 and WS99501 combined maximum and minimum values of these traits, respectively. Thus, DS70622 and DS11320, from dry regions, showed drought-avoidance characteristics whereas WS99501 and Mèlodie/2, from wet regions, showed the opposite.

Discussion. The combination of the germplasm survey and use of GROWSCREEN-Rhizo allowed exploring of adaptive traits and detection of root phenotypic markers for potential drought tolerance. The greater root system depth and root surface coverage in tolerant accessions agreed with previous research in other grain legumes. Hence, DS70622 and DS11320 can be new sources of root traits that can be tested for their effect on drought response.

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INTRODUCTION

Faba bean (*Vicia faba* L.) is an agronomically important crop for sustainable cropping system (de Visser, Schreuder & Stoddard, 2014) and has value for both food and feed (Crépon, 2010). Drought poses a great challenge to the sustainable production of the crop (Khan et al., 2010). Most faba bean genotypes are sensitive to soil moisture loss and heat stress (Loss, Siddique & Martin, 1996), showing leaf wilting symptoms even at moderate soil water potential (McDonald & Paulsen, 1997). Yield losses and instability are the main problems of the crop in drought-affected areas (Khan et al., 2010). Nevertheless, faba bean shows drought adaptation potential in the field (Reid, 1990) and diversity exists in abiotic stress tolerance (Khazaei et al., 2013; Belachew & Stoddard, 2017). Line ILB938 has demonstrated drought tolerance in different experiments (Link et al., 1999; Khan et al., 2007). Khazaei et al. (2013) studied the leaf morphophysiological traits of two sets of 201 faba bean accessions collected from dry and wet regions of the world, chosen according to the Focused Identification of Germplasm Strategy

(FIGS). The results indicated the potential of FIGS in the search for target traits for drought stress adaptation, but its focus on leaf traits, left root traits open for later study.

High-throughput screening and phenotyping of plants grown in pots allows controlled uniform moisture stress, a situation difficult to achieve under field condition (Tuberosa, 2012). Screening of faba bean in well watered conditions provided initial information about leaf traits related to drought adaptation (Khazaei et al., 2013). Chlorophyll content is a key trait determining the source capacity in affecting cumulative photosynthesis (Tuberosa, 2012) and it is positively correlated with dry root biomass and used to discriminate accessions of peanut for drought stress (Songsri et al., 2009). Stomatal conductance and canopy temperature depression (CTD) are two methods to screen cool-season legumes for drought stress (Stoddard et al., 2006). Large stomatal response, which is the expression of sensitivity to soil moisture deficiency, is regarded as useful for long-term drought (Munns et al., 2010) and considered as a consistent indicator of growth rate response to stress. CTD, the difference in temperature between the canopy surface and the surrounding air, incorporates the effects of multiple biochemical and morphophysiological features acting at the root, stomata and the plant canopy (Tuberosa, 2012). Accessions exhibiting cooler canopy temperature under drought stress avoid excessive dehydration through the use of more of the available moisture in the soil. Hence, CTD indicates plant water status in monitoring plant responses to water stresses (Tarek et al., 2014) and it is reported as the most responsive trait in faba bean accessions (Khan et al., 2007; Khazaei, 2014).

Together with shoot traits, identifying root phenotypic markers will help to understand the mechanisms by which they affect tolerance to drought. Root studies in legumes are relatively few and much less is known about roots than about shoots. When plants were grown in tall cylinders containing 1:1 Vertisol:Sand mixture (w/w), trait diversity for drought tolerance in chickpea (*Cicer arietinum* L.) was readily detected, including deeper rooting and greater biomass proportion in roots (Kashiwagi et al., 2006). Shovelomics and automated image phenotyping methods revealed genotypic variation in cowpea (*Vigna unguiculata* (L.) Walp.) root architecture, such as number of lateral roots and volume of soil enclosed by roots (Burridge et al., 2017). In a controlled environment, GROWSCREEN-Rhizo, a novel automated phenotyping robot, has enabled relatively high-throughput and non-invasive root phenotyping through characterization of root geometry (Nagel et al., 2012; Gioia et al., 2015; Avramova et al., 2016). Real time automated imaging, and quantification of the functional and structural parts of the crop using image analysis software are the methods used (Nagel et al., 2009; Rascher et al., 2011; Nagel et al., 2012). Therefore, this research investigated root depth and width as potential means to tolerate moisture stress.

MATERIAL AND METHODS

Germplasm Survey

This experiment was conducted at the University of Helsinki's Viikki Campus greenhouse facility in a randomized complete block design (RCBD) with 4 replications. The blocks were sown at 7 day intervals (owing to space limitation) and allowed to grow for 34 days.

The original set of 201 wet-adapted and 201 dry-adapted accessions (Khazaei et al., 2013) was reduced to 88 based on differences in canopy temperature depression measured in the glasshouse (Khazaei et al., 2013), country of origin and availability of seeds. Ten other accessions (7 from Ethiopia and 3 from Europe) were selected from the previous screening experiment for acid-soil and aluminum toxicity tolerance (Belachew & Stoddard, 2017). ILB938/2 and Mélodie/2 were chosen as checks as they have been well studied previously (Link et al., 1999; Khan et al., 2007; Khazaei et al., 2013). Poor germination of 11 accessions further reduced this set of 100 to 89 (Table 1). Since seed quantities were limited, seed size was evaluated as one-tenth of 10-seed weight.

Pots of 3 L capacity, 20 cm deep and 15 cm diameter with 4 drainage holes of 2 cm diameter, were used. Potting was done by covering the bottom of the pot with a thin membrane sheet and then alternating layers of sand and perlite one after the other as follows: 0.2 liter sand at the bottom + 2.6 liter perlite at the middle + 0.2 liter sand at the top. Two seeds per pot were sown and after 5 days, the weaker seedling was removed, leaving the stronger seedling to grow. Nutrient solution was applied at 200 ml automatically every other day from sowing to harvesting for 34 days to keep the medium at field capacity. Photoperiod was set at 14 h light and 10 h dark, and the temperature maintained at 22 °C during the day and 16 °C in the night. Pest control was conducted using biological control methods. Nutrient solution was 1 g/L of Superex Peat (Kekkilä Oy, Vantaa, Finland) supplemented with 2 mmol/L $CaCl_2$, as previously described (Belachew & Stoddard, 2017).

Stomatal conductance was measured using a Leaf Porometer (Decagon Devices, Inc, Pullman, WA, U.S.A.) once per plant at 30 days after sowing (DAS). Canopy temperature was measured using a FLUKE Model 574 Precision Infrared Thermometer (Fluke Corporation, Everett, WA, U.S.A.) at 31 DAS, chlorophyll content was measured as leaf SPAD values from two leaves per plant and the average of the two was recorded using SPAD-502 (Minolta Camera Co, Ltd, Japan) at 32 DAS. Measurements were taken between 11:00 and 13:00 GMT. Harvesting was done 34 DAS. Shoots were removed above the collar region and roots were carefully removed from the perlite and placed in a drying oven at 70 °C for 48 h. Root and shoot dry weight were measured to the nearest 0.01 g and root to shoot dry weight ratio was calculated by dividing the root weight by the corresponding shoot weight. Root mass fraction was calculated as root dry mass divided by total plant dry biomass.

Root Phenotyping Experiment

The experiment was conducted at Jülich Plant Phenotyping Center (JPPC) (www.jppc.de), Forschungszentrum Jülich GmbH, Germany from 23 January to 20 February 2017.

Eight accessions were chosen (Table 1) from the germplasm survey according to their performance in stomatal conductance, canopy temperature, chlorophyll concentration, root and shoot dry weights and root mass fraction values. In selection, accessions showing higher values of stomatal conductance and canopy temperature were considered as drought susceptible, and those showing higher chlorophyll concentration, root dry weight and root to shoot dry weight ratio, root mass fraction, and lower values of stomatal conductance and canopy temperature were considered as drought tolerant.

The experiment was conducted in the automated root and shoot phenotyping platfrom GROWSCREEN-Rhizo using rhizotrons with a size of 90x70x5 cm (Nagel et al., 2012). The growth medium used was GRAB-ERDE, a dark peat-based substrate (Plantaflor Humus Verkaufs-GmbH, Germany). A total of 2400 L peat was first machine broken and then passed through a 0.8 cm sieve. The initial moisture content of the peat-soil was 66.3% measured using Electronic Moisture Analyzer (version 1.1, 03/2013, KERN and Sohn GmbH, Germany). Of this, 1600 L was air dried to 40% moisture content, when it had a water potential of 0.006 MPa according to the water retention curve analysis conducted by the Institute of Plant Nutrition and Soil Science, University of Kiel, Germany.

Nutrient content and other physical and chemical properties of the growth medium were analyzed by LUFA NRW Laboratory, Germany. Dry matter content was 35%, wet bulk density 450 g/L, dry bulk density 158 g/L, pH 5.8, EC 733 µS/cm, KCl in H_2O 1.76 g/L, KCl in $CaSO_4$ 0.45 g/L, total nitrogen 27 mg/L in $CaCl_2$/DPTA-Extract (CAT, where DPTA is diethylenetriamine-pentaacetic acid), NH_4^+-N 4 mg/L in CAT, NO_3^--N 23 mg/L in CAT, P_2O_5 22 mg/L in CAT, K_2O 178 mg/L in CAT, Mg 125 mg/L in CAT, and Mn 11 mg/L in CAT.

Water-limited treatment boxes were filled with air dried peat-soil, whereas well watered treatment boxes were filled without drying. Each rhizotron contained approximately 21 L of growth medium. Filling was done in 3 steps of 7 L peat-soil each followed by regular pressing, to make the compaction of the medium as uniform as possible among boxes. The boxes were then fixed in the robotic system in the greenhouse and tilted at 43° from vertical.

Research design

The experiment was arranged in a split-plot design, with 4 replicate blocks, 2 treatments (well watered and water limited) as the main plots and 8 accessions as subplots.

Planting and treatment management

The experiment was conducted for 28 days, from seed soaking to plant harvesting, during the vegetative stage of plant growth. Seeds of uniform size were selected from all 8 accessions, washed three times, surface sterilized with 1% NaClO (sodium hypochlorite) (w/v) for 5 min and rinsed 3 times with running tap water. The seeds were soaked in tap water for 24 h, transferred to three layers of moist filter paper in 14 cm diameter Petri dishes (14 seeds/dish) as described in Belachew and Stoddard (2017), and incubated for 96 h at 22°C in the dark. The seedlings showing uniform root growth were selected and transferred into the rhizotrons. Initially, for establishment, each seedling in well watered treatment received 200 ml water in the automatic irrigation system and those in water limited treatment received 50 ml of water to their roots manually. Following this, the well watered plants were given 100 ml of water every 12 h until the end of the treatment period. In the water-limited treatment, plants received the second 50 ml of water 4 days after transplanting and thereafter received no more water. The average peat-soil temperature, air humidity and air temperature were 22.6°C, 58% and 20.9°C. Plants were grown in 15 h light and 9 h dark conditions.

Data collected

Root images were automatically taken every day except on Saturday and Sunday from 30 January to 20 February 2017. Images taken 5 days after treatment (DAT), 12 DAT, and 19 DAT were analyzed using the PaintRHIZO software package and dimensions were converted to SI units using 55.53 pixel = 1 cm. The following special root distribution and individual root traits were computed (Nagel et al. 2009 & 2012): length (cm) of taproot and of first and second order lateral roots, total root length (cm), root system depth (cm), which represents the maximum vertical distribution of the root system, root system width (cm), which represents maximum horizontal distribution of the root system, and convex hull area (cm^2) which is a measure for the surface area along the transparent plate of the rhizotrons covered by a root system. To evaluate how much of the whole root system was visible at the transparent plate of the rhizotrons, we measured total root length destructively. Accession DS70622 was chosen because it had the largest root system in both irrigation treatments. The roots were carefully removed from the potting medium 19 days after the treatment, washed, and preserved in ethanol solution until analysis. One week later, each root system was thoroughly washed, cut into manageable lengths and spread in water on the WinRhizo scanner.

Data analysis

Root images obtained with GROWSCREEN-Rhizo and manual root scanner EPSON A3 Transparency Unit (Model EU-88, Japan) were analyzed using PaintRHIZO and WinRHIZO, respectively, following the methods developed by Mühlich et al. (2008) and Nagel et al. (2009 & 2012). Quantitative data were subjected to analysis of variance using SPSS version 22.0 (IBM Inc., Chicago, IL, USA) software package. Treatment means were separated by Duncan Alpha (5%). Student's t-test was conducted using Independent Samples Test to test the significance of the difference between group means of the dry-adapted and wet-adapted accessions.

RESULTS

Germplasm Survey

There were significant differences between accessions in stomatal conductance, chlorophyll concentration, root and shoot dry weight and root mass fraction values ($P<0.001$), root to shoot dry weight ratio ($P<0.01$) and canopy temperature ($P<0.05$). Stomatal conductance ranged 6-fold, shoot dry weight 12-fold, root dry weight 7-fold, root mass fraction 2-fold, seed size 18-fold and canopy temperature by 3.1°C (Table 2). Accessions originating from dry growing regions of the world showed significantly higher chlorophyll concentration ($P<0.001$), shoot ($P<0.05$) and root

($P<0.01$) dry weights than those from wet regions. The Ethiopian accessions showed low stomatal conductance and high canopy temperature (Table 2 and Table S1).

Chlorophyll concentrationwas correlated negatively with stomatal conductance and positively with root dry weight (Table 3). Root and shoot dry weight were positively correlated with each other and with seed weight. Root mass fraction was negatively correlated with seed weight.

The five accessions with the greatest root and shoot weights were from the dry set and the five with the lowest were from the wet set (Fig. 1). The two highest dry weight values were recorded by accession DS70622 (shoot 3.5 g and root 1.6 g) and accession DS74573 (shoot 3.4 g and root 1.4 g) (Fig. 1). Based on the criteria mentioned earlier, 8 accessions (Table 4) were chosen for the root phenotyping experiment.

Root Phenotyping

The water-limited treatment was sufficiently strong to reduce the lengths of all three classes of root (taproot, lateral and second order lateral roots) at all three time points (5, 12 and 19 days after treatment started (DAT)) below the values found in the well watered treatment (Table S2). The main effect of accession on all three root lengths was also significant at all time points. The treatment x accession effect was significant for taproot and second order lateral root lengths at 12 and 19 DAT, but not for lateral root lengths, in which the standard error was large. Lateral roots made the largest contribution to total root length at 19 DAT (Fig. 2).

At 19 DAT, accession DS70622 had the longest lateral roots in both treatments, the longest second order lateral roots in the well watered treatment, the greatest total root length in both treatments, and the smallest difference in taproot and lateral root growth between treatments (Fig. 2). DS11320 had the longest tap root, the second-longest laterals and the second-longest total root length in the well watered treatment. EH06006-6 had the second-longest taproots in the well watered treatment. Mélodie/2 had the shortest taproot, lateral and second order lateral roots in the well watered treatment, whereas in the water-limited treatment, DS74573 had the shortest tap root, and WS99501 had the shortest laterals, second order laterals and total (Fig. 2).

In nearly 50% of the accessions, second order lateral roots were not visible at 5 DAT (Table S2). In the water-limited condition, only a quarter of the accessions showed second order lateral roots at 12 DAT, but at 19 DAT, all of the test materials had these roots.

At 19 DAT, total root length and root system depth were positively correlated ($r=0.86$, $n=8$, $P<0.01$), as were taproot length and total root length ($r=0.82$, $n=8$, $P<0.05$).

At the end of the treatment period, the total root length of DS70622 was 3 times longer than those of Mélodie/2 and WS99501. Accessions DS11320 and DS70622 showed the two deepest root system consistently at all 3 time points and WS99501 had the shallowest root system (Table 5 & Table S3).

On average, the total root length and root system depth recorded under well watered condition was twice that of water-limited condition. Droughted roots continued to grow throughout the experiment, but more slowly than in well watered conditions, such that the total root length of the droughted treatment was 50%, 41% and 27% of non-droughted at 5, 12, and 19 DAT, respectively (Fig. 2 & Table S3). Similarly, root system depth was reduced by 40%, 46%, and 50%, respectively, at these three time points (Table 5 & Table S3).

Comparison of total root length records obtained from PaintRHIZO and WinRHIZO image analysis software using root system of accession of DS70622 as a sample indicated that roots in rhizotrons were 25.5% visible in the well watered condition and 39.3% in the water-limited condition, the average of the two giving 32.4% visibility (Fig. 3).

Root system width differed between treatments, but not significantly between accessions. The mean root system width of plants grown in well watered condition was 46 cm, in contrast to 28 cm in the water-limited treatment

Convex hull area showed large differences between treatments and between accessions (Fig. 3), but the interaction was not significant (Table 5). Treatment differences in convex hull area increased across the 3 time points (Table 5 & Table S3). Plants grown in the well watered condition showed about 3 times more root area coverage (convex hull area) than plants grown in the water-limited condition. Maximum convex hull area was shown in accession DS70622, closely followed by EH06006-6 and DS11320, while WS99501 and Mélodie/2 had the two minimum values (Table 5). Root system width and convex hull area were positively correlated ($r=0.97$, $n=8$, $P<0.01$), and both traits were positively correlated with taproot length, total root length, and root system depth ($r=0.82$ to 0.89, $n=8$, $P<0.01$, $P<0.05$).

DISCUSSION

The germplasm survey showed that there was wide variation in morphological root traits of faba bean, that they were correlated with shoot traits but that there were important outliers from that correlation. In the root phenotyping experiment, the water deficit was sufficiently harsh that it affected the length and width of all root systems, but there were large differences among accessions. Accession DS70622 had a larger root system than the benchmark drought-tolerant accession, ILB938/2, so it may be a potential source of genes for drought avoidance by improved access to soil water. These results are discussed below.

Accessions from dry regions of the world showed higher chlorophyll concentration, and root and shoot dry weight than those from wet regions in the survey. The correlation between growth of different plant parts is expected, and it leads to relatively consistent root:shoot ratio or root mass fraction (RMF). The outliers from the correlation are interesting as sources of potential breeding traits. In the present survey, root mass fraction ranged relatively widely, from 0.24 to 0.50. In a set of 211 chickpea accessions, RMF ranged from 0.38 to 0.53 at 35 days after sowing (Kashiwagi et al., 2005). The overall higher value of chickpea RMF may relate to its acknowledged greater drought tolerance. The outliers above regression line (Fig. 1) in the current set of faba bean were mostly in accessions from the “wet set”, indicating that there may be useful sources of drought tolerance among this material. Our recalculations of RMF values from literature show higher values in each paper from drought-tolerant lines than from drought-susceptible ones: 0.57 to 0.66 in 133 recombinant inbred lines of lentil (*Lens culinaris* Medik) (Idrissi et al., 2015), 0.44 to 0.47 in 40 genotypes of lentil (Sarker, Erskine & Singh, 2005), and 0.20 to 0.25 in cowpea (*Vigna unguiculata*) genotypes (Matsui & Singh, 2003).

The substantial reduction in root length early in the phenotyping experiment emphasizes the importance of establishing faba beans with adequate moisture, particularly in agricultural regions subject to water deficit (Loss, Siddique & Martin, 1996). The reduction in root length was highly variable among accessions, being as high as 77% in DS74573 and as low as 30% in DS70622 (Fig. 2) at 19 DAT. This variation was shown to be significant in the genotype by environment interaction beginning from 12 DAT. The taproot of DS70622 in the water-limited condition was nearly 6x and 3x longer than those of WS99501 and Mèlodie/2, respectively. Similarly, drought-tolerant cultivars of common bean showed deeper roots than the sensitive ones (Sponchiado et al., 1989). Deep-rooted pulses can benefit from stored water in times of drought more readily than shallow-rooted ones (French & White, 2005).

Root phenotyping research technology provides new opportunities for assessing the effect of stress on different classes of root. Drought limited the length of laterals and second order lateral roots beginning from the onset of the treatment period. In sorghum (*Sorghum bicolor* Moench), the production of seminal root laterals was hindered by drought at the onset of the treatment and nodal roots produced few laterals only after some time (Pardales & Kono, 1990). Chickpea produced longer laterals when sown with sufficient moisture than when droughted (Krishnamurthy, Johansen & Ito, 1994). Mélodie/2 and WS99501 showed the greatest detrimental effect of drought already at 5 DAT and continued in that way for the rest of the experiment (Fig. 2 & Table S2). Even DS70622, the most prolifically rooting accession, did not show second order lateral roots in the water-limited condition until at least 12 DAT. Though the formation was first noted late, at 19 DAT, this accession was found to have the second longest second order lateral root next to DS74573.

There were positive correlations between root area coverage (root system width and convex hull area) and root depth (tap root and total root lengths, and root system depth) measurements, indicating that faba beans expand their root system in depth and breadth in a more or less balanced way. Drought-tolerant chickpea genotypes showed adaptive root distribution, with a higher root length density at deeper soil layers during a severe drought year (Kashiwagi et al., 2006), whereas roots of this species remained near the surface in moist conditions (Benjamin & Nielsen, 2006). This plasticity is especially important for the crop to avoid both terminal drought in chickpea (Kashiwagi et al., 2006; Gaur, Krishnamurthy & Kashiwagi, 2008) as well as transient drought. Peanut genotypes with a large root system showed high water use efficiency under drought condition (Songsri et al., 2009). Prolific and deep root systems have been shown in drought-avoiding accessions of chickpea (Kashiwagi et al., 2005), cowpea (Matsui & Singh, 2003), field pea (*Pisum sativum* L.) and soybean (*Glycine max* L. Merr.) (Benjamin & Nielsen, 2006). Hence, accessions with a larger root system probably avoid drought through increased access to water in the soil by increased tap root length as well as overall root system depth and width.

In the germplasm survey, the benchmark accessions Mélodie/2 and ILB938/2 showed low stomatal conductance, high chlorophyll concentration, and low shoot and root dry weight as compared to the rest. This was in agreement with the findings of Khazaei et al. (2013) in which Mélodie/2 and ILB 938/2 were reported to express efficient use of water and water use efficiency, respectively. In the root phenotyping experiment, however, the two accessions performed well below other accessions such as DS70622 and DS11320. This contradiction might be due to the initiation of the treatment at a much earlier stage of growth, which is in agreement with the finding that the root distribution of peanut genotypes at 37 and 67 days after sowing did not adequately predict the effects of drought, and best prediction being obtained at 97 days after sowing (Songsri et al., 2008). There are many ways in which plants respond to water deficit (Pereira & Chaves, 1993). Those from dry areas may show tolerance by increased root system depth and cavitation resistance (Hacke, Sperry & Pittermann, 2000), root growth at the expense of above-ground parts (Husain et al., 1990; Reid, 1990), osmotic regulation and solute buildup, and expression of water channel proteins (aquaporins) (Lian et al., 2004; Galmés et al., 2007). Crop plants that tolerate drought through the biosynthesis of abscisic acid (ABA) may also show reduced water use and low biomass production because of low leaf growth, low stomatal conductance (Galmés et al., 2007) and hence low photosynthesis even during the wet growing conditions (Tardieu, 2003). ILB938/2 follows this model. Other plant internal changes can regulate the opening of stomata as well (Galmés et al., 2007).

CONCLUSIONS

The GROWSCREEN-Rhizo phenotyping platform allowed detection of useful differences in root responses to water deficit. In both the survey and the rhizotron experiments, the shoot and root traits varied widely among accessions, and these traits were positively correlated among each other. In both cases, higher values of morphophysiological shoot and root measurements were recorded from accessions originating from the drier growing regions of the world, confirming the significance of FIGS to identify drought-adaptive traits.

The growth of the root system of faba bean in depth and width followed a balanced pattern, a strategy of wider and deeper soil exploration for water. Accessions DS70622 and DS11320 showed outstanding results in almost all of root traits measured under both treatment conditions in the phenotyping experiment. Thus, these two accessions can be new sources of root traits for future breeding of drought tolerant cultivars.

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Table 1 (on next page)

List of experimental materials by country of origin and source.

GU is University of Göttingen; HARC is Holeta Agricultural Research Center, Ethiopia; ICARDA is International Center for Agricultural Research in the Dry Areas; INRA is French National Institute for Agricultural Research; Prefixes DS and WS indicate material originally allocated to the dry set and wet set (Khazaei et al., 2013). Accessions with asterisk were used in the subsequent root phenotyping experiment.

S.N.	Accessions	Country of origin	Source	S.N.	Accessions	Country of origin	Source	S.N.	Accessions	Country of origin	Source
1	Aurora	Sweden	Svalöf Weibull	31	DS137675	Tajikistan	ICARDA	61	WS115134	Nepal	ICARDA
2	Babylon	Netherlands	Nickerson Limagrain	32	DS13918	Sudan	ICARDA	62	WS115177	Nepal	ICARDA
3	DOSHA	Ethiopia	HARC	33	DS70622*	Syria	ICARDA	63	WS115182	Nepal	ICARDA
4	DS11202*	Jordan	ICARDA	34	DS72271	Morocco	ICARDA	64	WS115186	Nepal	ICARDA
5	DS11207	Syria	ICARDA	35	DS72309	Syria	ICARDA	65	WS115352	Nepal	ICARDA
6	DS112096	Morocco	ICARDA	36	DS72310	Syria	ICARDA	66	WS115430	Nepal	ICARDA
7	DS11210	Syria	ICARDA	37	DS72366	Syria	ICARDA	67	WS11688	Afghanistan	ICARDA
8	DS11236	Iraq	ICARDA	38	DS72387	Syria	ICARDA	68	WS117830	China	ICARDA
9	DS11281	Afghanistan	ICARDA	39	DS72396	Syria	ICARDA	69	WS117841	China	ICARDA
10	DS11286	Iran	ICARDA	40	DS72455	Syria	ICARDA	70	WS117849	China	ICARDA
11	DS11294	Spain	ICARDA	41	DS72493	Syria	ICARDA	71	WS117853	China	ICARDA
12	DS11317	Macedonia	ICARDA	42	DS72523	Syria	ICARDA	72	WS117855	China	ICARDA
13	DS11320*	Macedonia	ICARDA	43	DS74370	Oman	ICARDA	73	WS117857	China	ICARDA
14	DS11437	Turkey	ICARDA	44	DS74554	Algeria	ICARDA	74	WS117864	China	ICARDA
15	DS11480	Lebanon	ICARDA	45	DS74573*	Russia	ICARDA	75	WS117868	China	ICARDA
16	DS11561	Algeria	ICARDA	46	DS99515	Kyrgyzstan	ICARDA	76	WS12315	Sweden	ICARDA
17	DS11591	Tunisia	ICARDA	47	EH 06006-6*	Ethiopia	HARC	77	WS124242	China	ICARDA
18	DS11689	Afghanistan	ICARDA	48	Gebelcho	Ethiopia	HARC	78	WS13039	Ethiopia	ICARDA
19	DS11701	Afghanistan	ICARDA	49	GLA 1103	Austria	Gleisdorf	79	WS130600	Russia	ICARDA
20	DS11788	Afghanistan	ICARDA	50	ILB938/2*	Ecuador	ICARDA/GU	80	WS130731	Azerbaijan	ICARDA
21	DS11909	Ethiopia	ICARDA	51	Kassa	Ethiopia	HARC	81	WS13107	Greece	ICARDA
22	DS12257	Syria	ICARDA	52	Mélodie/2*	France	INRA/GU	82	WS13185	Turkey	ICARDA
23	DS124062	Kazakhstan	ICARDA	53	Messay	Ethiopia	HARC	83	WS132238	China	ICARDA
24	DS124138	China	ICARDA	54	NC 58	Ethiopia	HARC	84	WS132258	China	ICARDA
25	DS124353	Greece	ICARDA	55	Tesfa	Ethiopia	HARC	85	WS132266	China	ICARDA
26	DS13042	Italy	ICARDA	56	WS11309	Poland	ICARDA	86	WS132274	China	ICARDA
27	DS131708	Tajikistan	ICARDA	57	WS11313	Ethiopia	ICARDA	87	WS99379	Portugal	ICARDA
28	DS13463	Cyprus	ICARDA	58	WS11344	Russia	ICARDA	88	WS99465	China	ICARDA
29	DS13473	Cyprus	ICARDA	59	WS114476	Bangladesh	ICARDA	89	WS99501*	China	ICARDA
30	DS13481	Cyprus	ICARDA	60	WS114576	Bangladesh	ICARDA				

1

Table 2(on next page)

Mean values of shoot and root measurements of 45 faba bean accessions from dry zone, 37 from wet zone and 7 from Ethiopia. Seed weight data were unreplicated.

*, **, *** p<0.05, 0.01, 0.001, respectively.

Data	Stomatal Conductance (mmol $H_2O/m^2/s$)	Canopy temperature (°C)	Chlorophyll concentration (SPAD value)	Shoot dry weight (g)	Root dry weight (g)	Root to shoot dry weight ratio	Root mass fraction	Seed weight (g)
Minimum	109	20.7	24.1	0.27	0.21	0.31	0.24	0.12
Mean	316	22.2	33.1	1.82	0.80	0.47	0.32	0.92
Maximum	752	24.8	41.0	3.49	1.59	0.94	0.50	2.11
SE	65	0.7	1.6	0.27	0.12	0.13	0.04	
LSD (5%)	182	2.0	4.4	0.75	0.35	0.37	0.10	
P-value	***	*	***	***	***	**	***	
Mean dry set	321	22.1	34.5	1.99	0.89	0.46	0.33	1.15
Mean wet set	318	22.1	31.2	1.60	0.71	0.48	0.32	0.64
Mean Ethiopian	263	22.9	33.4	1.95	0.75	0.40	0.29	0.92

Table 3 (on next page)

Pearson correlations of shoot and root data of 89 faba bean accessions.

*, ** $P < 0.05$, 0.01 (2-tailed), respectively.

	Stomatal conductance	Canopy temperature	Chlorophyll concentration	Shoot dry weight	Root dry weight	Root to shoot dry weight ratio	Root mass fraction
Canopy temperature	-0.14						
Chlorophyll concentration	-0.23*	-0.04					
Shoot dry weight	-0.11	0.05	0.12				
Root dry weight	-0.01	-0.05	0.23*	0.89**			
Root to shoot dry weight ratio	0.08	-0.05	0.08	-0.60**	-0.24*		
Root mass fraction	0.07	-0.03	0.09	-0.56**	-0.21*	0.90**	
Seed weight	-0.78**	0.001	0.36**	0.61**	0.58**	-0.34**	-0.23*

Table 4(on next page)

Faba bean accessions chosen root phenotyping experiment and the bases of selection in the screening experiment.

Chosen accessions	Selection criteria
DS11202	High canopy temperature, low chlorophyll concentration, low root mass fraction and root to shoot dry weight ratio
DS11320	Low canopy temperature, high root and shoot dry weights
DS70622	Low canopy temperature, high root and shoot dry weights
DS74573	High shoot and root dry weight
EH 06006-6	High canopy temperature, low chlorophyll concentration, low root mass fraction and low root to shoot dry weight ratio
ILB 938/2	Benchmark from previous research
Melodie/2	Benchmark from previous research
WS99501	High stomatal conductance, high canopy temperature, low root weight, low root to shoot ratio and low root mass fraction

Table 5 (on next page)

Mean root system depth and convex hull area of 8 faba bean accessions at 19 DAT, n=4.

*** $p < 0.001$, ns (not significant), SE is standard error. DAT is days after treatment given.

Accessions	Root system depth (cm)		Convex hull area (cm^2)	
	Well watered	Water limited	Well watered	Water limited
DS11202	74	29	2061	410
DS11320	78	46	2491	927
DS70622	76	53	2515	1047
DS74573	76	35	2369	663
EH 06006-6	78	34	2793	679
ILB938/2	65	31	1938	397
Melodie/2	65	32	1471	476
WS99501	61	27	1592	348
SE	3		162	
LSD (5%)	8		462	
Treatment				
Well watered	72		2154	
Water limited	36		618	
SE	1		81	
LSD (5%)	4		231	
P-value				
Treatment	***		***	
Accession	***		***	
Treatment x Accession	ns		ns	

Figure 1(on next page)

Root and shoot dry weights.

Total of 89 accessions of which 37 faba bean accessions from wet zones, 45 from dry zones, and 7 from Ethiopia. Error bars show LSD.

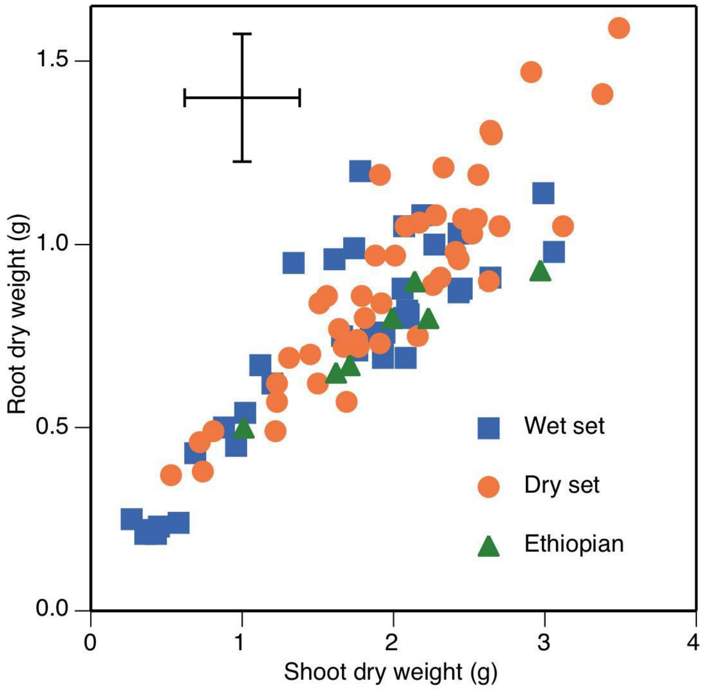

1.5
1.0
0.5
0.0
Root dry weight (g)
0
1
2
3
4
Shoot dry weight (g)
Wet set
Dry set
Ethiopian

Figure 2 (on next page)

Tap root, lateral and second order lateral root lengths of 8 accessions of faba bean at 19 days after initiation of treatment.

Total root length is the sum of the three classes. Error bars show LSDs of, bottom to top, taproot, lateral, second order lateral, and total root length.

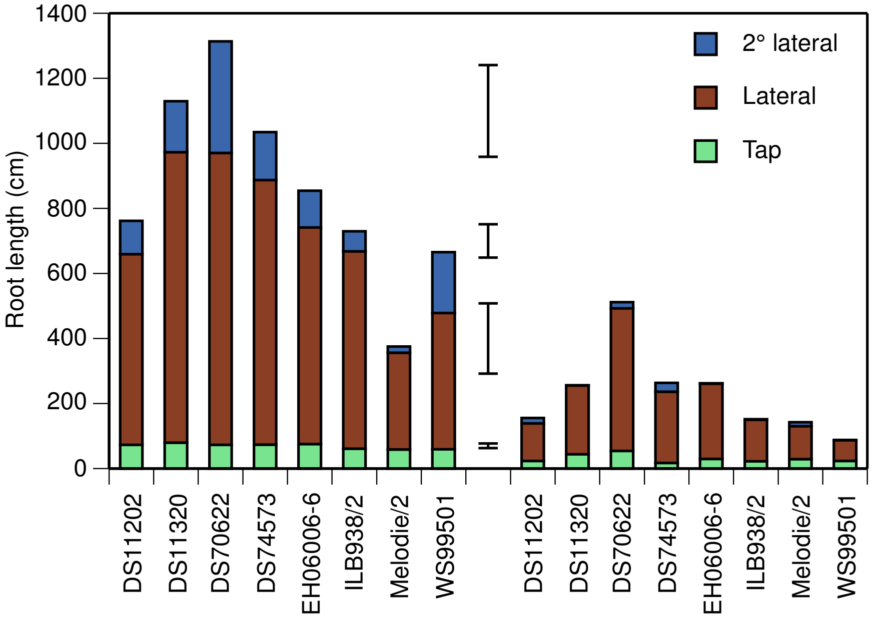
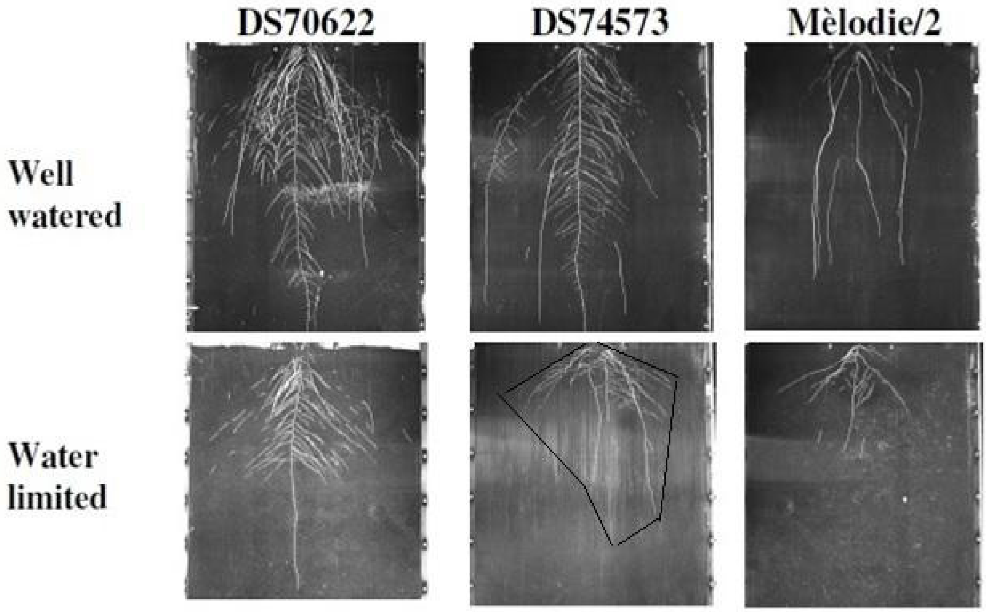

1400
1200
1000
800
600
400
200
0
Root length (cm)
2° lateral
Lateral
Tap
DS11202
DS11320
DS70622
DS74573
EH06006-6
ILB938/2
Melodie/2
WS99501
DS11202
DS11320
DS70622
DS74573
EH06006-6
ILB938/2
Melodie/2
WS99501

Figure 3(on next page)

Examples of GROWSCREEN-Rhizo root images. t length.

DS70622, DS74573 and Mèlodie/2 roots 19 DAT. The outlined root area at DS74573 at water limited exemplifies the convex hull area of the root system. Scale: 1:18.


DS70622
DS74573
Mèlodie/2
Well watered
Water limited