Root Phenotyping For Drought Tolerance in Bean Landraces From Calabria (Italy)

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Abstract

Common bean (Phaseolus vulgaris) is cultivated throughout Latin America and Africa, and for the European community, in Italy and Spain, areas are mainly subjected to drought stress which is predict to worsen by regional climatic models. The aims of this work were to identify the drought-tolerant and drought-sensitive bean landraces using drought tolerance and phenotypic plasticity indexes and to dissect the root morphological and 2D-architecture traits related to drought tolerance. Thirty-one landraces from diverse gene pools and areas of the Calabria region (South Italy), with different habits and morphological traits, were screened for drought tolerance in a hydroponic system. Root phenotyping was conducted by image analysis. Drought tolerance screening identified two landraces as drought tolerant and sensitive, respectively. Under drought stress, the drought-tolerant landrace exhibited several interesting root traits such as a higher root length, surface area and, above all, the fineness of the whole root systems and, with emphasis, of the higher order roots. Drought stress induced plastic root responses in both bean landraces but with contrasting patterns. The drought-tolerant landrace exhibited a dimorphic-rooted strategy, which could be included in future utility for bean breeding programmes in drought-prone environments.

Introduction

Common bean (Phaseolus vulgaris) is the most important food legume for the human diet providing proteins, vitamins and minerals, especially in East and southern Africa and the Americas (Beebe 2012). In Europe, Italy and Spain are the main producer regions (FAOSTAT 2012).

In developing countries, bean production is frequently subjected to drought (Thung and Rao 1999) that drastically reduces up to 60 % its yield (Beebe et al. 2011). Extensive irrigation systems mitigate the negative impact of drought stress in developed countries. However, the high consumption of irrigation water together with increasing frequency, intensity and duration of drought stress predicted by regional climatic models in Europe (IPCC 2007) highlights the importance to select high yielding drought-tolerant bean cultivars. In bean, genetic improvement for drought tolerance is based on worldwide germplasm genetic variability for adaptation to water deficiency (Munoz-Perea et al.~ 2006, Beebe et al. 2008, Porch et al. 2009). In this context, Durango and Mesoamerica races belonging to Middle American gene pool provide high levels of drought tolerance in common bean (Munoz-Perea et al. 2006, Beebe~ et al. 2008) as well as in the wild species, *P. acutifolius* (Martinez-Rojo et al. 2007). However, a wide investigation in other wild species or landraces from different gene pools could be useful to identify the morpho-physiological traits related to drought tolerance. In particular, landraces adapted to marginal areas could provide a valuable genetic source for identifying drought-related traits for bean adaptation.

Until now, the selection for drought tolerance in bean was mainly based on yield and its components, phenotypic plasticity and morpho-physiological traits (Beebe et al. 2013). Among the latter, root traits appeared to be more related to drought tolerance (Sponchiado et al. 1989, Mohamed et al. 2002, 2005) compared to the aboveground part, especially in bean (White and Castillo 1992). Nevertheless, root system has been less frequently considered as a source of drought tolerance traits in bean breeding programmes (Beebe et al. 2013). Rooting depth and distribution are generally taken into account for drought tolerance improvement (Sponchiado et al. 1989, Mohamed et al. 2002, 2005), although recently, an array of root traits or phenes have been also reported (Songsri et al. 2008, Henry et al. 2012, Lynch and Brown 2012). For example, the root length ratio (RLR, root length per unit of the plant's dry mass) is considered a better trait than root length for describing the plant's potential for soil resource acquisition under stress conditions (Ryser 1998) because it avoids the 'allometric effects' (Coleman et al. 1994) or the 'apparent plasticity' (Weiner 2004). The RLR is constituted by root mass ratio (RMR, root mass per unit of the plant's dry mass), the allocation component, and root fineness (RF, root length per unit root volume) and tissue density (RTD, root dry mass per unit root volume), the structural components (Ryser 1998). Plants may produce longer roots either by increasing biomass allocation or root fineness and/or reducing root tissue density, leaving biomass allocation unchanged. Under drought stress condition, changes in the RTD were related to drought tolerance in sugar beet (Romano et al. 2013) as well as the RF was considered a functional trait for drought-tolerant herbaceous tallgrass prairie species (Tucker et al. 2011) because it correlated with the root's ability to take up water (Peman et al. 2006, Hernandez et al. 2010, Rewald et al. 2011).

The morphology of single root types has scarcely been considered for the bean adaptation strategy to dry conditions. It is well known that different root types can be characterized by diverse anatomical, morphological and physiological features (Waisel and Eshel 2002) which determined different water uptake capacity among the root classes (Rewald et al. 2011). Furthermore, the genetic variation in the growth rates of distinct root classes could be important for the plant's adaptation to drought stress (Guo et al. 2008, Hund et al. 2009). For example, different responses among root types in P uptake (Rubio et al. 2004), tolerance

to combined P/drought stress (Ho et al. 2005) and rot resistance (Roman-Aviles et al. 2004) have been observed in common bean.

In this framework, the aims of this work were (i) the identification of the drought-tolerant and drought-sensitive landraces in a bean collection from Calabria (South Italy) by specific drought indexes and (ii) the dissection of root morphological traits mainly that related to the 2D-architecture traits such as root orders, whorls and tips.

Considering that the Mediterranean area is very susceptible to the effects of climate changes especially in terms of frequency and magnitude of drought stress (IPCC 2007), the results of this study could allow to identify potential drought tolerant parents and root traits related to drought tolerance useful for the future bean breeding programmes.

Materials and methods

Screening for drought tolerance

Plant material, growth condition and drought stress treatment

Thirty-one bean genotypes from different areas of Calabria region (South Italy) were selected based on variation for gene pools, site of origin and habit (Mercati et al. 2013). Seeds were kindly provided by Agenzia Regionale Sperimentazione e Servizi in Agricoltura (ARSSA – Calabria, Italy) (Table 1). A drought-tolerant American genotype, CO46348 (Brick et al. 2008), kindly provided by Prof. Brick MA (Colorado State University, USA), and a commercial Italian variety (Cannellino nano) were used as testers.

Seeds of bean genotypes were surface-sterilized for 2 min in 10 % NaOCl and germinated in the dark at 25 °C, for 2 d, in rolls paper soaked with 0.5 mM $CaSO_4$ and then placed in growth chamber for 4 d under artificial light. Six days after seeding, six seedlings of uniform size of each genotype were transferred to the growing units (PVC tubes, 4 cm diameter 9 50 cm height) each seedling in a separate tube. According to Fan et al. (2003), the aerated nutrient solution (pH 6) contained 3 mM KNO_3, 2 mM $Ca(NO_3)_2$, 0.5 mM $MgSO_4$, 0.5 mM $(NH_4)_2HPO_4$, 0.05 mM Fe-EDTA, 0.05 mM KCl, 0.025 mM H_3BO_3, 2 lM $MnSO_4$, 2 lM $ZnSO_4$, 0.5 lM $CuSO_4$ and 0.5 lM $(NH_4)_6Mo_7O_{24}$. The nutrient solution was renewed every 2 days, and the pH was daily adjusted to 6.0 by 0.1 N KOH. The growing units were then placed in growth chamber at 25/18 °C and 70 % relative humidity and a 14/10 h light/dark cycle (PPFD above shoot 300 lE m^2 s^1).

The drought stress was simulated by the variation of the osmotic potential by adding the polyethylene glycol (PEG) in the nutrient solution. In particular, at phenological phase V3 (first true leaf), in drought stress plants (D), 224 g l^1 of PEG 8000 (Sigma Aldrich P2139, St. Louis, MO, USA) was added to the nutrient solution to reach an osmotic potential of 0.6 MPa calculated using the equation of Michel (1983). The final PEG concentration was gradually achieved by the addition of 81.2 g l^1 PEG every 6 h. In control plants (C), the nutrient solution was renewed with the same above composition.

Growth parameters and leaf morphology

At 0, 8, 24, 48, 56, 72, 144, 168 and 192 h from PEG treatment, the stem length (StL, cm) and leaf area (LA, cm^2) were measured in each seedling. Stem elongation rate (StER, cm h^1) and leaf area expansion rate (LAER, cm^2 h^1) were calculated by the linear regression slopes of StL vs time and LA vs time.

After 192 h of PEG treatment, the seedlings were harvested and separated in leaves and stems, which were placed in an oven at 70 °C for 2 days to determine the leaf (LDW, g) and stem dry weight (StDW, g), respectively. The shoot dry weight (ShDW, g) was calculated by the sum of StDW and LDW. Finally, the leaf mass per area (LMA, g cm^2) was calculated by LDW/LA ratio (Matıas et al.
2012).

Leaf relative water content

After 192 h of PEG treatment, one terminal leaflet of each genotype from C and D plants was collected and immediately weighed (LFW, g), dipped in deionised water and left in the dark for 48 h. Afterwards, they were again weighed to measure the leaf turgid weight (LTW, g) and then placed in an oven at 70 °C for 48 h to determine the dry weight (LDW). The relative water content (RWC, %) of the leaf was calculated according to Gonzalez and Gonzalez (2003):

RWC = (LFW-LDW)/ (LTW-LDW)

Phenotypic plasticity and drought stress indexes

The ‘response coefficient’ (RC) as phenotypic plasticity index (Poorter and Nagel 2000) was calculated for each genotype with the following ratio:

$$RC = V_C/V_D$$

where V_C and V_D represent the average values of traits obtained under control and drought stress conditions. The ShDW, LDW, StER, LAER, RWC and LMA were the traits took in account.

The RC values equal to 1 indicated no response to drought stress, while RC<1 and RC>1 indicated the tolerance and susceptibility to drought stress, respectively.

The drought tolerance index (DTI) (Fernandez 1992, Ober et al. 2004) and the drought tolerance efficiency (DTE) (Fischer and Wood 1981) were calculated according to the following formulas:

$$DTI = (DM_D/DM_C)/(ADM_D/ADM_C)$$

$$DTE = DM_D/DM_C$$

where DM_D and DM_C are the dry weights of each genotype, and ADM_D and ADM_C are the average values of dry weights of shoot and leaves of all genotypes under drought and control conditions, respectively.

Root phenotyping

Plant material, growth condition and drought stress treatment

The #25 and #44 landraces, drought tolerant and drought sensitive, respectively, were used in additional experiments on which growth condition and drought stress treatment were the same described in the screening for drought tolerance.

Morphological root analysis

After 192 h from PEG treatment, the seedlings of each genotype and drought stress treatment were harvested and separated in shoots and roots. The root systems were stained with 0.1 % toluidine blue solution for 5 min and then scanned at a resolution of 300 dpi (WinRhizo STD 1600, Instruments Regent Inc., Canada). WinRhizo Pro v. 4.0 software package (Instruments Regent Inc., Chemin Sainte-Foy, Quebec, Canada) was used to measure the following parameters: length (L, cm), surface area (SA, cm^2) and volume (V, cm^3) of the basal (B), tap (T), basal lateral (BL) and tap lateral (TL) roots. Further, the root length distribution among the following root classes diameter, as defined by Bohm (1979), was obtained: very fine (VF, 0–0.5 mm), fine (F, 0.5–1 mm) and large (L, >1mm). Images were used to count the number (N, n.) of the basal roots, the laterals of the basal and tap roots and the root whorls. Shoot (ShDW, g) and root (RDW, g) dry weights were measured after oven-drying at 70 °C for 48 h. Plant dry weight (PDW, g) was calculated by the sum of ShDW and RDW.

Based on the above measurements, root length ratio (root length/whole plant dry weight, cm g^1), root mass ratio (root dry weight/whole plant dry weight, g g^1), root fineness (root length/root volume, cm cm^3) and root tissue density (root dry weight/root volume, g cm^3) were calculated.

Statistical analysis

A completely randomized design with three replicates for genotype and treatment has been utilized. All data were tested for normality (Kolmogorov–Smirnoff test) and homogeneity of variance (Levene median test) and, where required, the data were transformed.

All parameters were analysed by two-way analysis of variance with the genotype and treatment level (control and drought stress) as main factors. Subsequently, Tukey’s test was used to compare the means of all parameters of each genotype and treatment level. For the RC values, the statistical significance was obtained by the probability level ($P < 0.05$) of the Genotype X Treatment (G X T) interaction.

Statistical analysis of the data was carried out using SPSS Statistics v. 15.0 (IBM Corp., Armonk, NY, USA) while the graphics were prepared using SigmaPlot v. 8.0 (Jandel Scientific, San Rafael, CA, USA).

Results

Screening for drought tolerance

The screening for drought tolerance among bean genotypes was carried out by two different approaches: the 'phenotypic plasticity' and the 'tolerance indexes'.

The first approach took into account the 'response coefficient' as phenotypic plasticity index (Poorter and Nagel 2000) while ANOVA (genotype 9 environment interaction) represented a statistical evaluation of the genotype's plasticity (Valladares et al. 2006) in response to drought stress. The phenotypic plasticity was calculated considering different drought-related traits such as growth parameters (shoot and leaf dry weight, stem elongation and leaf area expansion rates), plant water status (leaf relative water content) and leaf morphology (leaf mass per area).

The shoot dry weight significantly varied among the bean genotypes (0.122–1.078 g; $P < 0.001$) and was significantly reduced by drought stress ($P < 0.001$), (Table 2). G X T interaction of the ShDW was also statistically significant ($P < 0.05$), (Table 2), indicating a different response to drought stress among bean genotypes. In particular, the #5, #31, #44, #50, #61, #85, #90, #99 and #100 genotypes exhibited a sharp reduction of ShDW (higher RC value) under drought stress indicating a 'maladaptive fitness' to drought (Table 2). Conversely, the PEG-treated #12, #25, #83 and #91 genotype increased their ShDW (RC value <1) compared to control (Table 2) showing an 'adaptive fitness' to drought stress. A similar pattern was also observed for the leaf dry weight except for the #50 genotype where the LDW reduction was not statistically significant (Table 2).

The StER was reduced by drought stress ($P < 0.001$) and was different among the bean genotypes ($P < 0.001$), but the GxT interaction was not statistically significant ($P = 0.134$), (Table 3). Conversely, the leaf area expansion rate showed a significant G X T interaction ($P = 0.004$) indicating a drought susceptibility (high RC values) in the #1, #5, #24, #29, #31, #44, #45, #49, #50, #59, #61, #85, #99 and # 100 genotypes and a drought tolerance (lower RC values) in the #12, #25, #51, #70, #78, #91, #92 and #CO genotypes (Table 3). Finally, leaf relative water content was significantly influenced by PEG treatment only ($P < 0.001$), (Table 3), whereas the LMA significantly varied among the bean genotypes ($P = 0.01$) and increased in response to PEG treatment ($P < 0.001$), (Table 3). However, the G X T interaction was not statistically significant indicating that the RC<1 for LMA was similar among all the bean genotypes (Table 3).

Besides the phenotypic plasticity, the drought tolerance indexes for selecting drought-tolerant genotypes in the bean germplasm collection were also determined. In particular, drought tolerance index (Fernandez 1992, Ober et al. 2004) and drought tolerance efficiency (Fischer and Wood 1981) for both shoot and leaf dry weights have been calculated. The DTI and DTE results for shoot dry weight indicated #25, #91, #83, #12, #51, #53 and #18 genotypes as highly tolerant to drought stress, while the #5, #44, #31, #90, #61, #99 and #85 resulted as susceptible ones (Table 4). The same pattern was also observed with leaf dry weight (Table 4).

The biplot of DTI against shoot or leaf dry weights at control proposed by Ober et al. (2004) showed the #25 and #44 genotypes as the landrace pair with the largest difference in DTI (Fig. 1).

Root phenotyping

Significant differences in many root traits between #25 and #44 landraces in response to drought stress have been observed (Tables 5–7). The root length was not modified by both landrace and drought treatment but a statistically significant LxT interaction was evident (Table 5). In particular, the PEG-treated #25 landrace sharply increased its root length (+140 %) while the #44 decreased this root trait (79 %) compared to the control (Table 5). A similar pattern for root surface area and dry weight was also observed (Table 5).

The RLR and RMR of landrace pair were not statistically influenced by both landrace and drought treatment (Table 5), although an increase (+70 % for the RLR and +33 % for the RMR) and decrease (58 % for the RLR and 27 % for the RMR) in PEG-treated #25 and #44 landraces, respectively, were observed compared to the control (Table 5). A similar statistically significant pattern has been observed for root fineness which was increased (+38 %) and reduced (44 %) in the #25 and #44 reduced it by 44 % landraces, respectively (Table 5). Conversely, root tissue density was only influenced by landrace (0.113 and 0.064 g cm^3 for #25 and #44, respectively; Table 5).

The root length of the very fine diameter class of the #25 landrace increased from 521 to 1441 cm while in the #44, it decreased under water deficiency (Table 6). Further, the root length of the VF diameter class was five times higher in the #25 when compared

to the #44 under PEG treatment (Table 6). A similar trend was also observed for the Fi diameter class, but not for the root length of the L diameter class which was not modified by both landrace and PEG treatment (Table 6).

At first true leaf stage, the #25 and #44 landraces exhibited a root system constituted by tap, basal, laterals of basal and laterals of tap roots. These root types showed a wide variability between bean landraces and in the response to PEG treatment. In particular, tap root length of the #25 was longer than that of #44; on the other hand, tap lateral roots length was not statistically modified by both landrace and PEG treatment (Table 7). Conversely the previous results, the basal and its lateral roots were sharply affected
by LxT interaction. In particular, the length of basal and its lateral roots of the PEG-treated landraces #25 landrace was strongly increased (+102% and +288% for the B and BL roots, respectively) being reduced in the #44 one (49 % and 90 % for the B and BL roots, respectively) compared to the control (Table 7). In contrast with that of tap lateral roots, the number of basal lateral roots showed a statistically significant LxT interaction (Table 7). In particular, the #25 landrace sharply increased the basal lateral root number in response to drought stress when compared to the #44 landrace (Table 7). Conversely, root whorl number was not modified by both landrace and PEG treatment (Table 7).

Discussion

The phenotypic plasticity of drought-related traits of bean collection, used as first drought screening approach in this work, allowed to categorize the #5, #44, #61 and #85 genotypes as drought susceptible and the #12, #25 and #91 as drought tolerant (comparing Tables 2 and 3). Further, these results confirmed that the leaf area expansion (Navea et al. 2002, Emam et al. 2010), stem elongation rate (Emam et al. 2010), leaf relative water content (RosalesSerna et al. 2004, Turkan et al. 2005) were negatively influenced by drought stress in bean genotypes differently to the LMA (Matıas et al. 2012) and consequently they could be used as drought-related traits.

The drought tolerance indexes, the second drought screening approach, together with the results of phenotypic plasticity indicated the #5 and #44 genotypes as drought sensitive and the #25 and #91 as drought tolerant. In particular, the most tolerant bean landrace appeared to be the #25 which was previously identified as Mesoamerican genotype (Mercati et al. 2013). The Mesoamerican gene pool had previously been identified as potential source of valuable drought-adaptation genes (Miklas et al. 2006). However, for selecting genotypes with contrasting in response to drought stress, Ober et al. (2004) suggested '..the genotype pairs that show similar yield potential but contrasted in DTI provide experimental material for dissection of morphological and physiological traits that confer drought tolerance..'. According to Ober et al. (2004), the biplot of DTI against shoot or leaf dry weight at control was carried out. The results indicated the #25 and #44 as drought-tolerant and drought-susceptible bean landraces, respectively, showing the largest difference in DTI (Fig. 1). Hence, this bean landrace pair has been selected for dissecting the root morphological and architectural traits underlying drought tolerance and sensitivity.

Root phenotyping is an important tool for improving the bean tolerance to abiotic stress (Beebe et al. 2013). In particular, root growth was closely related to drought tolerance in bean (Sponchiado et al. 1989), and the morphophysiological mechanisms in response to drought stress were mainly expressed in root compared with shoot (Sanders and Markhart 1992, White and Castillo 1992). The results indicated that the #25 and #44 bean landraces were different in drought-induced plastic response of the whole root system: the #25 increased root length, surface area and dry weight while the #44 decreased them (Table 5). A similar strategy has been already reported in bean. Indeed, the drought-tolerant SEA5, BAT477 and SER16 genotypes exhibited a longer root system in greenhouse compared to the drought-sensitive BAT881 and MD23-24 (Rao et al. 2006). Further, Grzesiak et al. (1997a,b) demonstrated that the drought-tolerant bean genotypes exhibited higher root dry weight and length. Finally, a genetically modified bean plant harbouring a HVA1 gene encoding a type III LEA protein which confers drought tolerance, showed a higher root length (Kwapata et al. 2012). As suggested by Lynch and Brown (2012) which wrote 'several classes of root traits have the potential to be deployed in crop breeding programs to improve soil resource acquisition', the present work deeply analysed the root morphology of the #25 and #44 drought-tolerant and drought-sensitive bean landraces, respectively, adopting two different approaches.

The first approach considered relative root length rather than its absolute value to avoid the 'allometric effects' (Coleman et al. 1994) or the 'apparent plasticity' (Weiner 2004). In this respect, different authors (Ryser 1998, Sorgona et al. 2005, 2007, Romano et al. 2013) considered the root length ratio, that is, the root length relative to the plant biomass, as a morphological trait closely related to the plant ability for acquiring soil resources under abiotic stress conditions. The results on RLR supported the previous findings that the #25 landraces increased its root length in absolute terms under PEG treatment differently from the #44 (Table 5). The 'RLR' trait consists in the biomass allocation (root mass ratio) and the structural parameters (fineness and tissue density) whose response patterns changed root length (Ryser 1998, Sorgona et al. 2005, 2007, Romano et al. 2013). Hence, the

plants could improve root length by increasing the biomass allocation or efficiently utilizing this biomass to increase the root fineness and/or reducing the root tissue density. In this respect, the results indicated that the #25 landrace increased the biomass allocation to roots and especially the root fineness, compared to the #44 under drought stress (Table 5). Root fineness seems to be an adaptive trait in the #25 because it is induced under PEG treatment, in contrast with RTD which was a genotypeconstitutive trait (Table 5). The sharp increase of root fineness in the drought-tolerant #25 landrace allowed to consider this root trait interesting for bean adaptation to drought stress. Indeed, it is well known that the increased root system fineness allowed a higher root-soil contact increasing the root's ability to take up water (Rewald et al. 2011), increased radial conductivity (Huang and Eissenstat 2000) and allowed a greater root hydraulic conductance per leaf unit surface area (Peman et al. 2006) or per stem cross section area (Hernandez et al. 2010). The results of the root length distribution among the diameter classes confirmed the higher root fineness of the drought-tolerant #25 landrace compared to the #44 (Table 6).

The 'within-root analysis', the second root phenotyping approach, took into account the morphological change of different root types that make up the whole bean root system, providing early information on two-dimensional root architecture. The within-root analysis pointed out a different 2-D root architecture between the two bean landraces and a plastic response of these important traits to drought stress (Table 7). Indeed, the #25 in contrast to the #44 exhibited a tap-rooted strategy characterized by a predominance of tap roots in no stress condition. Conversely, under drought stress, it showed a dimorphic-rooted strategy, increasing basal and basal lateral roots and maintaining a higher length of tap root. This root strategy already observed in combined P/drought stress (Ho et al. 2005) remains an open question because the basal more than tap roots play an important role for the acquisition of immobile soil resources, such as phosphate, but not for the mobile ones, such as water (Ho et al. 2005, Lynch and Brown 2012). Furthermore, the association between drought tolerance and basal root growth observed in the #25 landrace appeared to be in contrast with previous results where a tap-rooted strategy was observed in bean (Sponchiado et al. 1989, Sanders and Markhart 1992, Ho et al. 2005, referred by Beebe et al. 2013). Probably these contrast findings could be due to the different growing media utilized (hydroponic vs solid media) (Sponchiado et al. 1989, Sanders and Markhart 1992, Ho et al. 2005). In solid growing media, the lack of water is a lesser important limiting factor than 'mechanical stress' for root growth under dry condition (Bengough et al. 2011). In hydroponic system, the different water uptake ability among root types/ orders is probably more important than the spatial localization of the root axis for plant adaptation to drought stress. Among different root types, the lateral or higher order roots were reported as the preferential entry point of water (Rewald et al. 2011). Hence, the #25 landrace, increasing the length and surface area of the basal lateral roots and, at lesser extent, of the tap lateral roots, could have more potential ability to water uptake than the #44 landrace, and consequently to improve its adaptation to the drought stress.

Finally, root tips and whorls number are root phenes correlated with plant drought tolerance. Indeed, the tips are the root region with higher water uptake due to the presence of root hair and non-lignified tissues (Guo et al. 2008, Paula and Pausas 2011), while higher root whorl number determined an improvement of soil exploration (Lynch and Brown 2012). The #25 landrace, increasing the basal lateral root number, confirmed again its root system as more interesting for selecting new bean genotypes adapted to water-limited condition.

Conclusion

The bean germplasm collection, here evaluated, represents a useful genetic tool for studying the responses to drought stress in this important crop-legume. Based on phenotypic plasticity of different traits and the tolerance indexes, DTI and DTE, it has been possible to identify diverse bean genotypes at different drought tolerance levels. However, the #25 and #44 landrace pair, defined as drought tolerant and drought sensitive, respectively, appeared to be the best genotypes for dissecting the morpho-physiological mechanisms underlying drought tolerance. Drought stress imposed by PEG treatment induced contrasting root plasticity patterns in both bean landraces. In particular, the #25 landrace exhibited a wide array of root traits such as a higher root length, surface area and, mainly, the root fineness together with a dimorphic-rooted strategy that conferred it a higher drought tolerance than the #44 landrace. These root traits may be of large interest for bean breeding programmes in drought-prone environment.

However, as in this work the evaluation for bean drought responses has been carried out in hydroponic system, further experiments have been planned for the validation of morpho-physiological responses to the drought stress in field trials.

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Table 1 – Description, characteristics and code of the 31 calabrian bean landraces, the drought tolerant American genotype and the commercial Italian variety used in this study.

Code (ID#)	Common Name	Site of origin[§]	Habitus	Gene pool[‡]
1	Fagiolo uncino	CS	Climbing	A
5	Posa di montagna	RC	Climbing	A
12	Serpente	CS	Climbing	A
14	Suraca larga	Unknown	Climbing	A
17	A fava	CS	Climbing	C
18	Reniforme	CS	Climbing	U
24	Sbraca pasta	RC	Dwarf	A
25	Fasolu vasciu	CS	Dwarf	M
29	A cavolo	Unknown	Dwarf	A
31	Cannellino bianco	CS	Climbing	A
37	Fasolu quarantino	CS	Dwarf	A
40	Cocò gialla	CZ	Dwarf	A
44	Fagiolo ciuncu 2008	CS	Dwarf	A
45	Cannellino nano	Italy	Dwarf	A
49	Vovolacu	CZ	Climbing	A
50	Core di Gesù	CZ	Climbing	A
51	Mangiatutto a granella	CS	Climbing	A
53	Sarrisa	CZ	Climbing	A
59	Selvaggia	CZ	Climbing	U
61	Vravalacu	CZ	Climbing	A
67	Povarella	CZ	Dwarf	A
70	Fagiolo cursuni dall'occhio	CS	Dwarf	U
78	Azzicca	CS	Climbing	A
83	Zicca o valana	RC	Climbing	A
85	Fag. bianco piccolo	RC	Climbing	M
87	Azzicca a caciumbalo	CS	Climbing	A
90	Cervineddu	CS	Climbing	A
91	Nicolisa	CZ	Climbing	A
92	Fagiolino	RC	Climbing	M
99	Favarula nera	CS	Climbing	C
100	Posa rossa di settembre	RC	Climbing	A
101	Monachella	CZ	Climbing	A
CO	CO46348	USA	Climbing	A

[§]Site of origin: CS (Cosenza), CZ (Catanzaro), VV (Vibo Valentia), RC (Reggio Calabria)
[‡]Gene pool (Mercati et al., 2013): A, Andean; M, Middle American; C, *P. coccineous*; U, Unknown.

Table 2 Shoot and leaf dry weight and phenotypic plasticity (RC, Response Coefficient) of bean genotypes grown for 8 days with (D) or without 6% PEG (C) in nutrient solution.

Genotypes (ID#)	**Shoot dry weight** (g)			**Leaf dry weight** (g)		
	C	*D*	*RC*	*C*	*D*	*RC*
1	0.524	0.360	1.45	0.310	0.191	1.62
5	0.399	**0.180**	2.22	0.265	**0.094**	2.81
12	0.297	0.307	0.97	0.176	0.192	0.92
14	0.276	0.238	1.16	0.141	0.119	1.19
17	0.705	0.630	1.12	0.368	0.311	1.18
18	0.527	0.482	1.09	0.226	0.150	1.51
24	0.287	0.232	1.24	0.176	0.137	1.28
25	0.310	0.403	0.77	0.211	0.266	0.79
29	0.448	0.282	1.59	0.306	0.182	1.68
31	0.996	**0.480**	2.08	0.619	**0.267**	2.32
37	0.376	0.330	1.14	0.251	0.220	1.14
40	0.343	0.283	1.21	0.224	0.187	1.20
44	0.404	**0.186**	2.18	0.232	**0.075**	3.10
45	0.420	0.293	1.43	0.239	0.141	1.69
49	0.571	0.438	1.30	0.349	0.240	1.45
50	0.581	**0.389**	1.49	0.339	0.226	1.50
51	0.326	0.312	1.04	0.196	0.187	1.05
53	0.320	0.294	1.09	0.210	0.190	1.10
59	0.592	0.463	1.28	0.369	0.264	1.40
61	0.443	**0.144**	3.08	0.223	0.076	2.96
67	0.446	0.391	1.14	0.276	0.234	1.18
70	0.151	0.122	1.24	0.089	0.062	1.43
78	0.350	0.303	1.16	0.220	0.192	1.15
83	0.302	0.336	0.90	0.183	0.193	0.95
85	0.491	**0.278**	1.77	0.294	**0.139**	2.12
87	0.448	0.361	1.24	0.282	0.204	1.38
90	0.670	**0.340**	1.97	0.451	**0.219**	2.06
91	0.510	0.578	0.88	0.327	0.354	0.92
92	0.369	0.324	1.14	0.241	0.207	1.16
99	1.078	**0.597**	1.81	0.537	**0.209**	2.57
100	0.635	**0.440**	1.44	0.395	**0.236**	1.68
101	0.313	0.283	1.11	0.163	0.142	1.15
CO	0.496	0.359	1.38	0.291	0.186	1.57
	G	7.12***		G	4.20***	
Statistic[§]	T	38.42***		T	43.62***	
	GxT	1.55*		GxT	1.48*	

Bold: statistical difference between the mean of each bean germplasm at drought condition respect to the control plants ($p<0.05$, test of Tukey);

[§]Statistical analysis: ANOVA two-way (G: genotypes; T: treatment with PEG; GxT: genotypes x treatment interaction); *0.05>P<0.01; **0.01>P<0.001; ***0.001>P; NS not significant

Table 3 Stem elongation rate (StER), leaf area expansion rate (LAER), relative water content (RWC) and leaf mass per area (LMA) and relative phenotypic plasticity (RC, Response Coefficient) of bean genotypes grown for 8 days with (D) or without PEG (C) in nutrient solution.

Genotypes (ID#)	**StER** ($cm\ h^{-1}$)			**LAER** ($cm^2\ h^{-1}$)			**RWC** (%)			**LMA** ($g\ cm^2$)		
	C	*D*	*RC*	*C*	*D*	*RC*	*C*	*D*	*RC*	*C*	*D*	*RC*
1	0.249	0.087	2.86	0.67	**0.169**	3.96	0.95	0.83	1.14	0.00061	0.00097	*0.63*
5	0.287	0.1	2.87	0.546	**0.122**	4.47	0.94	0.77	1.22	0.00064	0.00131	*0.49*
12	0.271	0.219	1.24	0.49	0.43	1.14	0.92	0.93	0.99	0.00042	0.00056	*0.75*
14	0.142	0.09	1.58	0.411	0.278	1.48	0.97	0.65	1.49	0.00046	0.0059	*0.08*
17	0.413	0.217	1.90	0.523	0.163	3.21	0.92	0.7	1.31	0.00067	0.00123	*0.54*
18	0.221	0.167	1.32	0.581	0.244	2.38	0.95	0.66	1.44	0.00069	0.00133	*0.52*
24	0.192	0.087	2.21	0.656	**0.314**	2.09	0.88	0.72	1.22	0.00044	0.00108	*0.41*
25	0.103	0.137	0.75	0.849	0.732	1.16	0.88	0.84	1.05	0.00045	0.00067	*0.67*
29	0.067	0.024	2.79	1.364	**0.325**	4.20	0.88	0.78	1.13	0.00047	0.00177	*0.26*
31	0.504	0.323	1.56	0.552	**0.179**	3.08	0.99	0.81	1.22	0.00058	0.00063	*0.92*
37	0.029	0.015	1.93	0.887	0.607	1.46	0.92	0.79	1.16	0.00046	0.00058	*0.79*
40	0.145	0.071	2.04	0.851	0.569	1.49	0.89	0.79	1.13	0.00048	0.00063	*0.76*
44	0.117	0.076	1.54	1.047	**0.191**	5.48	0.92	0.58	1.59	0.00052	0.0011	*0.47*
45	0.173	0.111	1.56	0.681	**0.244**	2.79	0.91	0.58	1.57	0.00054	0.0008	*0.67*
49	0.186	0.212	0.88	0.594	**0.177**	3.35	0.94	0.82	1.15	0.00048	0.00075	*0.64*
50	0.326	0.178	1.83	0.643	**0.143**	4.50	0.96	0.71	1.35	0.00062	0.00087	*0.71*
51	0.162	0.122	1.33	0.528	0.451	1.17	0.91	0.9	1.01	0.00051	0.00056	*0.91*
53	0.017	0.009	1.89	0.593	0.29	2.04	0.88	0.64	1.37	0.00054	0.00083	*0.65*
59	0.295	0.166	1.78	0.898	**0.22**	4.08	0.91	0.77	1.18	0.00053	0.0011	*0.48*
61	0.328	0.064	5.12	0.885	**0.099**	8.94	0.9	0.6	1.5	0.00053	0.00177	*0.30*
67	0.261	0.143	1.82	0.467	0.245	1.91	0.92	0.82	1.12	0.00066	0.00075	*0.88*
70	0.038	0.024	1.58	0.336	0.246	1.36	0.84	0.83	1.01	0.00061	0.0011	*0.55*
78	0.161	0.086	1.87	0.205	0.154	1.33	0.87	0.9	0.97	0.00055	0.00064	*0.86*
83	0.12	0.108	1.11	0.334	0.178	1.88	0.93	0.6	1.55	0.0005	0.0013	*0.38*
85	0.386	0.178	2.17	0.439	**0.137**	3.20	0.95	0.77	1.23	0.00061	0.00098	*0.62*
87	0.186	0.097	1.92	0.479	0.201	2.38	0.9	0.84	1.07	0.00091	0.00105	*0.87*
90	0.486	0.256	1.90	0.62	0.253	2.45	0.96	0.81	1.18	0.00052	0.00088	*0.59*
91	0.077	0.079	0.97	1.031	0.705	1.46	0.91	0.94	0.97	0.00059	0.00076	0.78
92	0.315	0.267	1.18	0.912	0.753	1.21	1.08	0.91	1.19	0.0005	0.00056	*0.89*
99	0.394	0.242	1.63	0.795	**0.261**	3.04	0.94	0.75	1.25	0.00061	0.00128	*0.48*
100	0.3	0.119	2.52	0.837	**0.095**	8.81	0.93	0.82	1.13	0.00063	0.00119	*0.53*
101	0.181	0.155	1.17	0.299	0.202	1.48	0.91	0.78	1.17	0.00072	0.00076	*0.95*
CO	0.321	0.239	1.34	0.721	0.539	1.34	0.93	0.82	1.13	0.00052	0.00079	*0.66*
Statistic[§]	G	8.38***		G	4.51***		G	1.12		G	1.84*	
	T	59.79***		T	139.63***		T	78.64***		T	66.38***	
	GxT	1.33		GxT	1.98**		GxT	0.97		GxT	1.41	

Bold: statistical difference between the mean of each bean genotype at drought respect to the control plants ($p<0.05$, test of Tukey);

[§]Statistical analysis: ANOVA two-way (G: genotype; T: treatment with PEG; GxT: genotype x treatment interaction); *0.05>P<0.01; **0.01>P<0.001; ***0.001>P; NS not significant

Table 4 Drought tolerance index (DTI) and Drought tolerance efficiency (DTE) of bean genotypes grown for 8 days with or without PEG in nutrient solution.

Genotypes (ID#)	***Shoot dry weight basis***		***Leaf dry weight basis***	
	DTI	**DTE**	**DTI**	**DTE**
25	0.97	130	0.86	126
91	0.85	113	0.74	108
83	0.83	111	0.72	106
12	0.77	103	0.75	109
51	0.72	96	0.65	95
53	0.69	92	0.62	91
18	0.68	91	0.45	66
101	0.68	90	0.59	87
17	0.67	89	0.58	84
92	0.66	88	0.59	86
37	0.66	88	0.60	88
67	0.65	88	0.58	85
78	0.65	86	0.60	87
14	0.65	86	0.58	84
40	0.62	83	0.57	83
24	0.60	81	0.53	78
87	0.60	81	0.49	72
70	0.60	80	0.48	70
59	0.58	78	0.49	71
49	0.57	77	0.47	69
CO	0.54	72	0.44	64
45	0.52	70	0.40	59
100	0.52	69	0.41	60
1	0.51	69	0.42	62
50	0.50	67	0.46	67
29	0.47	63	0.41	59
85	0.42	57	0.32	47
99	0.41	55	0.27	39
61	0.39	52	0.23	34
90	0.38	51	0.33	48
31	0.36	48	0.29	43
44	0.34	46	0.22	32
5	0.34	45	0.24	35

Table 5 Morphology of whole root system of the landraces of *P. vulgaris* (#25 and #44) grown for 8 days with (D) or without PEG (C) in nutrient solution. *AL*: average landraces; *AT*: average treatments.

		Landraces *(ID#)*				
		25	**44**	*AT*	***Statistics*[§]**	
Root length (cm)	C	667^{B}	1872^{A}	1269^{P}	***L***	0.00016^{ns}
	D	1603^{A}	388^{B}	996^{P}	***T***	0.5^{ns}
	AL	1135^{x}	1130^{x}		***LxT***	9.78*
Root surface area (cm^2)	C	67^{A}	155^{A}	111^{P}	***L***	0.052^{ns}
	D	145^{A}	42^{B}	93^{P}	***T***	0.293^{ns}
	AL	106^{x}	98^{x}		***LxT***	8.372*
Root dry weight (g)	C	0.044^{A}	0.065^{A}	0.054^{P}	***L***	2.297^{ns}
	D	0.079^{A}	0.021^{B}	0.050^{P}	***T***	0.111^{ns}
	AL	0.061^{x}	0.043^{x}		***LxT***	10.346*
Root length ratio ($cm\ g^{-1}$)	C	1869^{A}	4650^{A}	3259^{P}	***L***	0.47^{ns}
	D	3184^{A}	1961^{A}	2572^{P}	***T***	0.37^{ns}
	AL	2526^{x}	3305^{x}		***LxT***	3.13^{ns}
Root mass ratio ($g\ g^{-1}$)	C	0.12^{A}	0.15^{A}	0.13^{P}	***L***	0.42^{ns}
	D	0.16^{A}	0.11^{A}	0.13^{P}	***T***	0.016^{ns}
	AL	0.142^{x}	0.130^{x}		***LxT***	2.83^{ns}
Root fineness ($cm\ cm^{-3}$)	C	1634^{AB}	1818^{AB}	1726^{P}	***L***	2.77^{ns}
	D	2253^{A}	1039^{B}	1646^{P}	***T***	0.067^{ns}
	AL	1943^{x}	1428^{x}		***LxT***	5.09*
Root tissue density ($g\ cm^{-3}$)	C	0.109^{A}	0.068^{B}	0.088^{P}	***L***	8.00*
	D	0.117^{A}	0.060^{B}	0.088^{P}	***T***	0.000085^{ns}
	AL	0.113^{x}	0.064^{y}		***LxT***	0.220^{ns}

Different letters among the means indicated significant difference at $p < 0.05$ (Tukey's test).

[§]Statistical analysis: ANOVA two-way (L: landraces; T: treatment with PEG; LxT: landrace x treatment interaction); *0.05>P<0.01; **0.01>P<0.001; ***0.001>P; NS not significant.

Table 6 Root length distribution among the diameter classes (Very Fine, VF: 0 – 0.5mm; Fine, Fi: 0.5 – 2.0mm; Large, L: > 2.0 mm) of calabrian landraces of *P. vulgaris* (#25 and #44) grown for 8 days with (D) or without PEG (C) in nutrient solution. *AL*: average landraces; *AT*: average treatments.

Landraces (ID#)	**VF (0-0.5 mm)**			**Fi (0.5-2.0 mm)**			**L (>2.0 mm)**		
	C	D	*AL*	C	D	*AL*	C	D	*AL*
25	521^{B}	1441^{A}	981^{x}	103^{A}	153^{A}	128^{x}	4.25^{A}	6.93^{A}	5.59^{x}
44	1623^{A}	288^{B}	955^{x}	112^{A}	57^{B}	84^{y}	7.02^{A}	8.90^{A}	7.96^{x}
AT	1072^{P}	864^{P}		107^{P}	105^{P}		5.64^{P}	7.92^{P}	
				***Statistics*[§]**					
	L	0.0048^{ns}		***L***	6.345*		***L***	1.334^{ns}	
	T	0.319^{ns}		***T***	0.0145^{ns}		***T***	1.238^{ns}	
	LxT	9.436*		***LxT***	9.275*		***LxT***	0.0381^{ns}	

Different letters among the means indicated significant difference at $p < 0.05$ (Tukey's test).

[§]Statistical analysis: ANOVA two-way (L: landraces; T: treatment with PEG; LxT: landraces x treatment interaction); *0.05>P<0.01; **0.01>P<0.001; ***0.001>P; NS not significant.

Table 7 Length and number of different root types [Tap, Tap Lateral, Basal and Basal Lateral] and root whorls of calabrian landraces of *P. vulgaris* (#25 and #44) grown for 8 days with (D) or without PEG (C) in nutrient solution. *AL*: average landraces; *AT*: average treatments.

		Landraces *(ID#)*				
		25	**44**	*AT*	***Statistics*[§]**	
Tap length (cm)	C	34^{A}	27^{B}	30^{P}	***L***	6.89*
	D	30^{A}	14^{B}	22^{P}	***T***	4.32^{ns}
	AL	32^{x}	21^{y}		***LxT***	1.10^{ns}
Tap lateral length (cm)	C	266^{A}	388^{A}	327^{P}	***L***	0.0182^{ns}
	D	316^{A}	170^{A}	243^{P}	***T***	0.917^{ns}
	AL	291^{x}	279^{x}		***LxT***	2.325^{ns}
Basal length (cm)	C	88^{B}	138^{A}	113^{P}	***L***	1.159^{ns}
	D	178^{A}	70^{B}	124^{P}	***T***	0.161^{ns}
	AL	133^{x}	104^{x}		***LxT***	8.436*
Basal lateral length (cm)	C	278^{B}	1318^{A}	798^{P}	***L***	0.0218^{ns}
	D	1079^{A}	133^{B}	606^{P}	***T***	0.362^{ns}
	AL	679^{x}	726^{x}		***LxT***	9.698*
Tap lateral number (n)	C	272^{A}	205^{A}	238^{P}	***L***	0.0123^{ns}
	D	211^{A}	263^{A}	237^{P}	***T***	0.000636^{ns}
	AL	241^{x}	234^{x}		***LxT***	0.797^{ns}
Basal lateral number (n)	C	329^{B}	969^{A}	649^{P}	***L***	0.561^{ns}
	D	766^{A}	374^{B}	570^{P}	***T***	0.226^{ns}
	AL	547^{x}	671^{x}		***LxT***	9.723*
Basal number (n)	C	5.7^{A}	7.7^{A}	6.7^{P}	***L***	0.0769^{ns}
	D	8.7^{A}	7.3^{A}	8.0^{P}	***T***	1.231^{ns}
	AL	7.2^{x}	7.5^{x}		***LxT***	1.923^{ns}
Root whorls (n)	C	2.7^{A}	2.7^{A}	2.7^{P}	***L***	0.667^{ns}
	D	3.0^{A}	3.7^{A}	3.3^{P}	***T***	2.667^{ns}
	AL	2.8^{x}	3.2^{x}		***LxT***	0.667^{ns}

Different letters among the means indicated significant difference at $p < 0.05$ (Tukey's test).

[§]Statistical analysis: ANOVA two-way (L: landraces; T: treatment with PEG; LxT: landrace x treatment interaction); *0.05>P<0.01; **0.01>P<0.001; ***0.001>P; NS not significant.

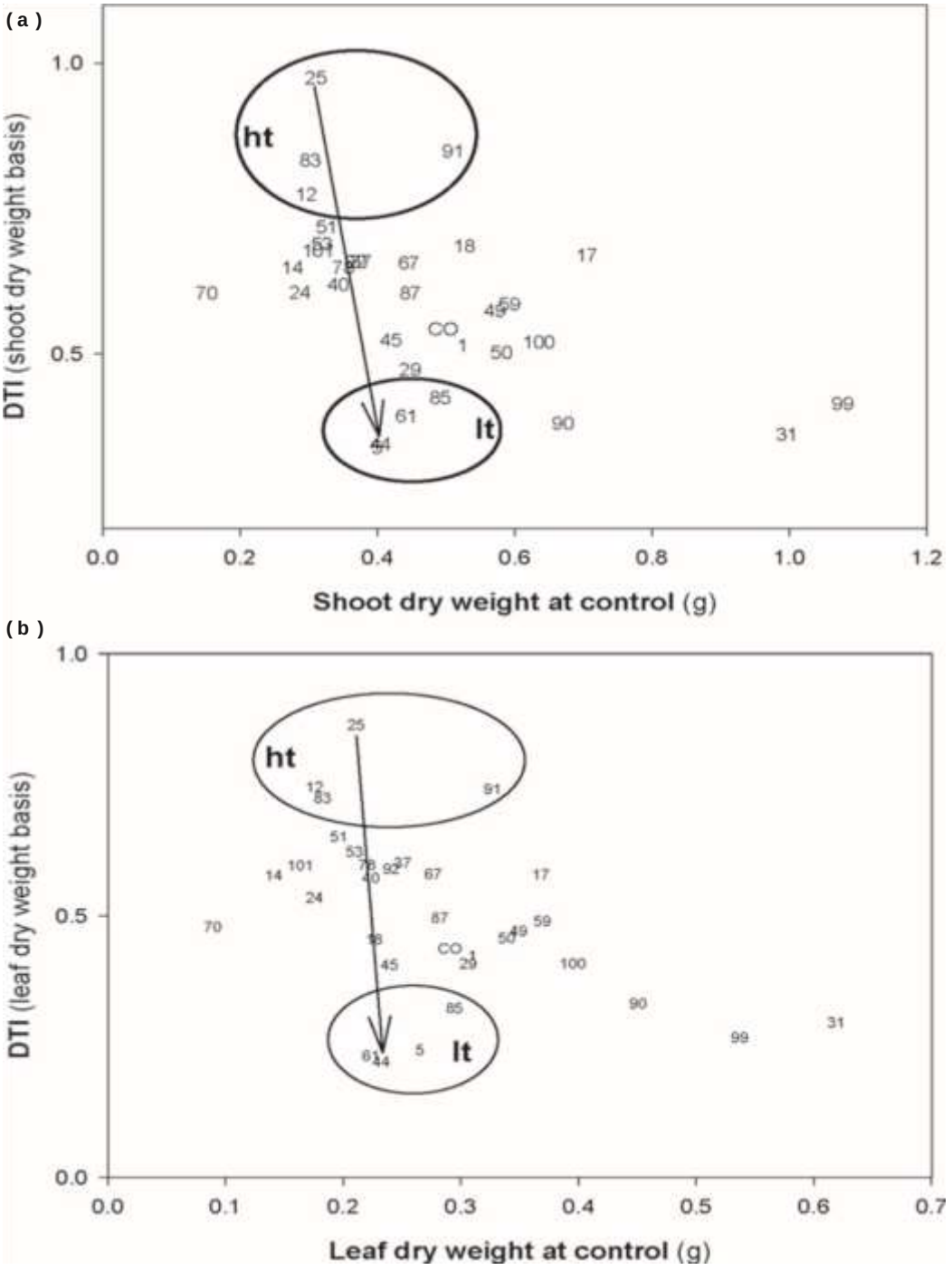

Fig. 1 Drought tolerance index based on shoot (a) and leaf dry weight (b) of bean genotypes plotted against the shoot (a) and leaf dry weight (b) obtained in the absence of PEG treatment. The circles defined by 'ht' and 'lt' contained the drought-tolerant and drought-sensitive, respectively, bean genotypes. The arrows indicated the genotypes pairs which showed similar biomass at control but the widest difference of
DTI.