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Screening of worldwide cowpea collection to drought tolerant at a germination stage

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ABSTRACT

Global warming has an increasing impact on the availability of water for agriculture. Crops tolerant to high temperatures and drought, such as cowpea (*Vigna unguiculata* L. Walp.), have an added value in the near future. The main objective of this study was to evaluate the effect of drought on seed germination and seedling emergence of cowpea genotypes, in order to screen the most tolerant genotypes. Seeds from 58 cowpea genotypes all over the world were submitted to two stress conditions, induced by PEG-6000 (corresponding to osmotic potentials of −0.75 bars and −1.5 bars). Germination and seedling growth parameters, vigor index and proline content were determined to assess drought tolerance. The results revealed significant differences of all parameters among genotypes after treatments and interaction of both. Water stress caused a general decrease in germination and seedling growth, while an increase in proline content was observed. A high variation of drought responses were detected among genotypes, being possible to select seven genotypes (C11, C18, C44, C46, C47, C50 and C54) as tolerant to drought at germination stage. These results will be useful to select the best suitable parents for insertion in future breeding programs.

1. Introduction

Worldwide agricultural production has been limited by several environmental constraints in the form of abiotic stresses, which affects plants growth, metabolism and development (Eftekhari et al., 2017; Muscolo et al., 2014). Water scarcity is currently one of the most severe limitations of plant development and production (Eftekhari et al., 2017; Jain and Saxena, 2016). The predicted temperature increase and rainfall decrease will be responsible for more frequent drought periods, mainly in the Mediterranean region including the Iberian Peninsula (Kröner et al., 2017). In this climate change scenario, the selection of drought-tolerant plants gain more importance, particularly the selection during germination. Some studies report several physiological characteristics (including seed germination and seedling growth) as indicators of drought tolerance in specific crop genotypes (Bouslama and Schapaugh, 1984; Steiner et al., 2017; Yan, 2015). Seed germination and seedling emergence are potentially the most critical stages susceptible to water stress (Ahmad et al., 2009; Hellal et al., 2018; Li et al., 2011, 2015) and are pivotal steps for crop propagation (Ravelombola et al., 2017). Indeed, water limitation can be responsible for the decline or even complete inhibition of seedling emergence and stand establishment (Kaya et al., 2006; Wu et al., 2011; Yan, 2015). However, tolerance against drought during the germination stage allow an uniform plant stand (Steiner et al., 2017).

Cowpea (*Vigna unguiculata* L. Walp.) is a grain legume with high worldwide economic importance, originated in Africa. Seeds of this legume are an important source of protein and other nutritional components for human diet (Ravelombola et al., 2017; Timko and Singh, 2008) and also an important source to animal fodder (Huang et al., 2012). Like many legumes, cowpea has the ability to fix atmospheric nitrogen through rhizobium symbiosis (Ehlers and Hall, 1996) and is easily grown in low fertility soils (Eloward and Hall, 1987). Some

Abbreviations: ANOVA, analysis of variance; *GR*, germination rate; PC, principal component; PCA, principal component analysis; PEG, polyethylene-glycol; *RL*, root length; SE, standard error; *SL*, shoot length; *VI*, vigor index; *%G*, germination percentage

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reports referred to the ability of cowpea to grow in regions without irrigation and irregular rainfall, being considered as one of the most tolerant legumes to drought (Agbicodo et al., 2009). Taking into consideration the upcoming climate change and increasing protein needs, all these advantages make desirable to increase cowpea production and consumption in European Union. Nowadays, almost all consumed cowpea in Europe is imported from African countries (FAOSTAT, 2018). The establishment success of this crop in such semiarid regions depends on the fast and uniform seed germination under low water availability (Muscolo et al., 2014).

Several methods and efforts have been employed to identify drought tolerant varieties in different crops (Darkwa et al., 2016; Muscolo et al., 2014), including in cowpea (Jain and Saxena, 2016; Muchero et al., 2009). Some studies referred that an *in vitro* screening method based on polyethylene-glycol (PEG) is suitable for selecting tolerant genotypes able to germinate under drought stress conditions (Jain and Saxena, 2016; Kocheva and Georgiev, 2003; Muscolo et al., 2014; Ravelombola et al., 2017) being a good alternative method to field experiments (Steiner et al., 2017). Indeed, the PEG polymer has been used to mimic drought stress effects in plants with limited metabolic interferences (Murillo-Amador et al., 2002). Another important and appropriate methodology for determining drought tolerance levels is proline determination. The accumulation of osmolytes is a plant protection strategy against abiotic stress (Mafakheri et al., 2010). Proline accumulation is one of the first plant responses to water-deficit stress, in order to reduce injury to cells (Anjum et al., 2011). In general, proline concentration has been considered a good indicator of drought tolerance, as higher levels are detected in stress-tolerant plants when compared to susceptible ones (Toscano et al., 2016).

Recently, germination and growth responses to drought stress have been reported in several crops, including legume crops as chickpea (*Cicer arietinum* L.; Dharanguttikar et al., 2015), common bean (*Phaseolus vulgaris* L.; Machado Neto et al., 2006), lentil (*Lens culinaris* Medik.; Muscolo et al., 2014) and soybean (*Glycine max* L. Merr.; Kpoghomou et al., 1990; Vieira et al., 1991). In general, these studies indicated a delay in initial germination and a reduction in the different germination parameters due the low water potential. Until now, few studies regarding cowpea seed germination in drought stress conditions have been developed. This is the first report of cowpea germination under drought conditions and makes use of a large set cowpea seeds from Iberian Peninsula and also from worldwide countries. The main objectives of this work are (1) the evaluation of cowpea responses to drought stress during germination, and (2) the screening of drought-tolerant cowpea genotypes from a world-wide collection. Besides the understanding of mechanisms involved in germination under drought stress, the results will be useful for selecting the best genotypes for enhancing the production of this grain legume in Southern Europe (Iberian Peninsula).

2. Materials and methods

2.1. Plant material

A total of 58 cowpea (*Vigna unguiculata* L. Walp.) genotypes were used for drought tolerance evaluation at germination stage (Table 1) being 29 from Iberian Peninsula, 26 originally collected from 17 different worldwide countries and three used as reference. In a previous study, the majority of the cowpea genotypes were already characterized using single nucleotide polymorphism (SNP) through the Illumina Cowpea iSelect Consortium Array (Carvalho et al., 2017b). Some of the Iberian Peninsula cowpea genotypes were also characterized through the morphological and agronomical parameters (Carvalho et al., 2017a). The references displayed different levels of drought tolerance: Bambey 21 (highly susceptible), CB46 (moderately susceptible) and IT93K-503-1 (highly tolerant), as described by Hamidou et al. (2007) and Muchero et al. (2008, 2010).

Table 1
Cowpea genotypes used in this study with reference to their origin (city and country, when available) and current status.

Code	Origin	Status
C1	Ferreira do Alentejo, Portugal	Landrace
C2	Ansião, Portugal	Landrace
C3	Évora, Portugal	Landrace
C4	Mértola, Portugal	Landrace
C5	Abrantes, Portugal	Landrace
C6	Almeida, Portugal	Landrace
C7	Figueira Castelo Rodrigo, Portugal	Landrace
C8	Pinhel, Portugal	Landrace
C9	Meda, Portugal	Landrace
C10	Trancoso, Portugal	Landrace
C11	Macedo de Cavaleiros, Portugal	Landrace
C12	Penamacor, Portugal	Landrace
C13	Sabugal, Portugal	Landrace
C14	Mogadouro, Portugal	Landrace
C15	Portugal	Variety
C16	Granada, Spain	Landrace
C17	Malaga, Spain	Landrace
C18	Malaga, Spain	Landrace
C19	Orense, Spain	Landrace
C20	Girona, Spain	Landrace
C21	Baleares, Spain	Landrace
C22	Caceres, Spain	Landrace
C23	Pontevedra, Spain	Landrace
C24	Huelva, Spain	Landrace
C25	Jaen, Spain	Landrace
C26	Badajoz, Spain	Landrace
C27	Albacete, Spain	Landrace
C28	Zamora, Spain	Landrace
C29	Cordoba, Spain	Landrace
C30	Sicilia, Italy	Landrace
C31	Puglia, Italy	Landrace
C32	Cuneo, Italy	Landrace
C33	Italy	Landrace
C34	Italy	Landrace
C35	Italy	Landrace
C36	Greece	Landrace
C37	Greece	Landrace
C38	Greece	Landrace
C39	Creta, Greece	Landrace
C40	Nigeria	Cultivar
C41	Angola	Landrace
C42	Benin	Landrace
C43	Egy	Landrace
C44	Ghana	Landrace
C45	Senegal	Landrace
C46	Zambia	Cultivar
C47	Iran	Landrace
C48	Irak	Landrace
C49	Cuba	Landrace
C50	Congo	Landrace
C51	China	Landrace
C52	India	Landrace
C53	Brazil	Cultivar
C54	Bulgaria	Landrace
C55	China	Landrace
References		
Bambey21	Senegal	Cultivar
CB46	California, USA	Cultivar
IT93K-503-1	Nigeria	Breeding line

2.2. Determination of optimal PEG concentration

A pilot experiment was performed in order to determine the optimal polyethylene glycol 6000 (PEG-6000) concentration for cowpea seed germination studies. Three cowpea genotypes (C8, C15 and C40) were tested under four PEG-6000 (Merk Millipore, Germany) concentrations, corresponding to final osmotic potentials of −0.75, −1, −1.5, −2 bars. Germination assays (six days) were performed in an incubator (Binder incubator series D, Germany) in the dark. The temperature was set for 26 ± 1 °C, as previous results showed that cowpea genotypes

had the highest seed germination rate at this temperature (data not shown), which is also in agreement with the optimal temperature reported by Jain and Saxena (2016). Uniform seeds from each cowpea genotype were selected and sterilized for about 3 min, in a 10% sodium hypochlorite solution, to prevent fungal growth. Seeds were then washed with sterile distilled water for about 3 min, four times. Following the description of Jain and Saxena (2016), ten seeds from each genotype were germinated on a two-folded filter paper, placed in a Petri dish (diameter 11 cm), containing 14 mL of PEG-6000 solutions. Distilled water (without PEG-6000) was used as control. Each Petri dish was sealed with Parafilm to avoid evaporation and contaminations. Three replicates of each treatment/genotype combination were performed. Cowpea drought tolerance was evaluated by seed germination rate.

2.3. Germination conditions and experimental design

Germination assays with all cowpea genotypes were performed as previously described from December 2017 to February 2018. Drought stress was induced by two different PEG-6000 concentrations, corresponding to final osmotic potentials of -0.75 and -1.5 bars (hereinafter referred to as stress 1 and 2, respectively). Three replicates of each treatment/genotype combination were performed and separately placed on three different incubator shelves (each shelf was considered as a block). The experiment was run multiple times due to space limitations. After each run, the incubator was sprayed with 75% ethanol solution to limit any microbial growth and contamination.

2.4. Measurements and data collection

A seed was considered germinated if the radicle had one-third of seed length, as described by Ravelombola et al. (2017). The number of germinated seeds was daily recorded for six days. At the end, the seed germination percentage (% *G*) was calculated and the roots and shoots length of five plants was measured (*RL* and *SL*, respectively) and the plants were discarded. Seed germination rate (*GR*) was calculated using the formula $GR = \sum_{ti}^{ni}$ proposed by Silva and Matos (2016), where *ni* is the number of seeds germinated on each observation day and *ti* is the observation day. The vigor index (*VI*) was also calculated following the formula presented by Abdul-Baki and Anderson (1973), $VI = (MRL + MSL) \times \%G$, where *MRL* is the mean of root length and *MSL* is the mean of shoot length.

2.5. Proline determination

For free proline content determination, the roots of five seedlings were frozen in liquid nitrogen and ground to a fine powder. Root tissue (40 mg) was homogenized in 1 mL of 3% (w/v) sulfosalicylic acid and centrifuged at 12,000*g* for 20 min, according to Bates (1973) with some modifications. After centrifugation, the supernatant (0.1 mL) was mixed with 0.4 mL of acid-ninhydrin and 0.4 mL of glacial acetic acid. The resulting mixture was heated for 1 h at 100 °C in a water bath. After reaction interruption by placing the tubes on ice, toluene (0.8 mL) was added and vigorously mixed. The toluene phase (upper phase) absorbance was read at 520 nm, using a spectrophotometer (PowerWave XS2, BioTek Instruments, Inc., Winooski, USA). Free proline content was estimated by referring to a standard curve using L-proline and expressed as μg proline/mg of fresh tissue. Each sample of each combination (treatment/genotype) was used for three technical repetitions.

2.6. Data analysis

Data from germination (*% G, GR and VI*) are presented as the mean of three independent assays ($n = 3$). Growth measurements (root and shoot length) and free proline content were performed from five plants per each plate ($n = 15$) and are presented as the mean of 15 repetitions. Before performing the ANOVA, all measurement data were tested for normality, according to the Kolmogorov-Smirnov and Kruskal-Wallis tests, and homogeneity with the Levene test. Non-homogeneity data were observed in germination percentage, being the data transformed with the formula $arcsin\sqrt{(\%G/100)}$ to obtain homogeneity. Differences between means were analyzed with one-way and two-way ANOVA followed by Tukey's test ($p < 0.05$ was considered significant), using *IBM SPSS Statistics version 20* software (IBM SPSS, Inc., Chicago, USA). The statistical significance in mean values among genotypes was examined with Tukey's multiple comparisons tests after two-way ANOVA using the *GraphPad Prism version 7.01* software (GraphPad, Inc., California, USA). Principal component analysis (PCA) was performed using *Past version 3.19* statistical software (Hammer et al., 2001). The used values were normalized into percentage, taking into account the maximum value obtained from each assay, and was calculated by the ratio of stress 2 and control.

3. Results

3.1. Determination of optimal PEG conditions

A preliminary experiment was performed with the aim to select those PEG-6000 concentrations more suitable for screening cowpea (*Vigna unguiculata* L. Walp.) tolerance to drought at a germination stage. Four PEG-6000 concentrations were chosen based on their osmotic potential (−0.75, −1, −1.5 and −2 bars). In the three tested cowpea genotypes, a germination rate decrease with increasing water stress imposition through PEG-6000 was detected (Fig. 1). For the most severe stress condition (−2 bars), a low seed germination rate was detected, not allowing to discriminate the most susceptible genotypes. For this reason, the use of such PEG-6000 concentration could make difficult to screen the most susceptible genotypes. When imposing osmotic potentials of −0.75 and −1.5 bars, a better genotype discrimination was obtained. As one-way ANOVA revealed significant differences among cowpea genotypes ($F = 27.219$ and 9.296, $p = 0.001$ and 0.015, respectively; Supplementary file 1), these osmotic potentials were further used for determining the drought tolerance level of a set of 55 cowpea genotypes and three references.

3.2. Drought effect on seed germination and growth parameters

The drought tolerance level of cowpea genotypes was firstly assessed by determination of % *G*, *GR*, *RL*, *SL* and *VI* (Table 2; Supplementary file 2). For all evaluated parameters, no significant differences ($p > 0.05$) between replicas were observed.

The seed germination percentage decreased with increasing severity of drought in 50% of the evaluated cowpea genotypes, although only three genotypes (C10, IT93K-503-1 and Bambey 21) presented significant differences ($p < 0.05$; Table 2; Supplementary file 2). The germination (%) of the remaining 50% genotypes was not affected even under severe drought treatment (Table 2). The differences between cowpea genotypes were indeed significant ($p < 0.001$; Table 3), which could be partially related with variations on germination capacity of each genotype (even under control conditions; Table 2). Considering all genotypes together, the differences between stress treatments revealed to be significant ($p < 0.01$; Table 3), suggesting that drought stress imposition affects seeds germination of cowpea. Besides the percentage of germinated seeds, the germination rate is considered as one of the most informative parameters in this type of studies. A drop in germination rate was observed when seeds were exposed to drought stress (Table 2), revealing that seeds take more time to germinate when subjected to drought. This result is also in accordance with the detected reduced germination percentage. Significant differences ($p < 0.001$) were also detected between treatments (control and drought stresses) and among genotypes (Table 3). While some genotypes presented a dramatically decrease on germination rate ($p < 0.001$) with increase of drought stress, others did not reveal significant differences ($p >$

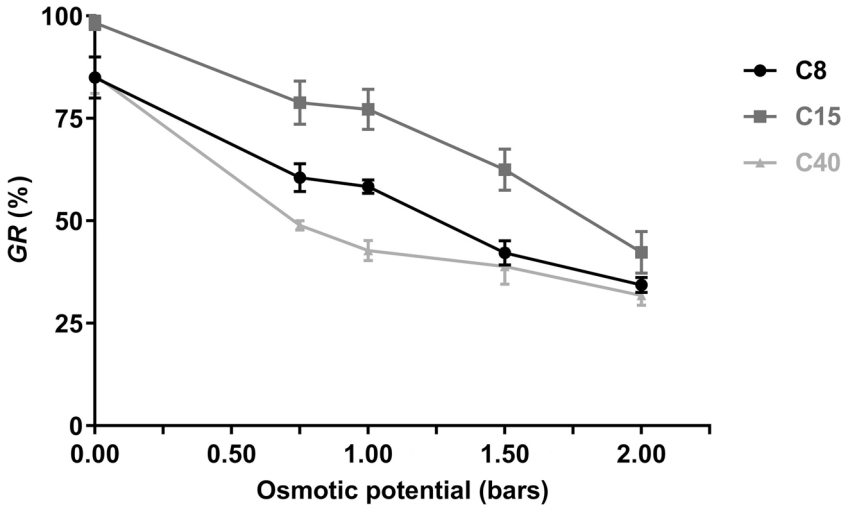

Fig. 1. Seed germination rates (*GR*) of three cowpea genotypes under four drought conditions induced by PEG-6000 (corresponding to osmotic potentials of -0.75, -1, -1.5 and -2 bars) and control (0 bars) with water ($n = 3$).

0.05) between treatments (Table 2).

The results also showed the commitment of seedling emergence with drought stress imposition, since root and shoot growth were generally inhibited under drought stress treatments (Table 2). Indeed, both parameters were significantly ($p < 0.001$) affected by drought stress treatments and genotypes presented significant differences for both parameters ($p < 0.001$; Table 3). A decrease in the root length was generally detected (by the means of all genotypes) when compared to control (23% and 30%, for stress 1 and 2, respectively). Many cowpea genotypes did not show variations in the root length with drought stress, but four genotypes in particular (C6, C20, C25 and Bambey 21) were significantly affected. Similarly, a reduction in shoot length was registered in many cowpea genotypes, corresponding to general decreases (means of all genotypes) of 41% and 59% (for stress 1 and 2, respectively) in relation to control. Interestingly, many genotypes that were significantly affected in their root length, were not significantly affected in their shoot length. This result suggests that root length is more sensitive to drought conditions than shoot length.

As germination and seedling emergence may interfere with plant vigor and ultimately with crop yields, the vigor index was also determined (Table 2). The vigor index decreased significantly ($p < 0.001$) with drought severity and among genotypes (Table 3). However, while many genotypes were significantly affected ($p < 0.001$) by drought stress, some were not affected ($p > 0.05$). The genotypes C11, C54 and IT93K-503-1 (highly tolerant reference) increased their vigor under drought stress conditions (Table 2).

3.3. Drought effect on proline accumulation

Free proline content, in general (means of all genotypes), increased 1.4-fold (stress 1) and 1.7-fold (stress 2) in relation to control condition (Table 4). Differences between stress treatments were significant ($p < 0.01$; Table 3), suggesting that drought stress imposition induces the production of proline in roots. The highest increase of proline was detected in several genotypes, including the tolerant IT93K-503-1 genotype, while others did not reveal significant differences in proline content with drought stress imposition (Table 4; Supplementary file 2). The highly susceptible reference (Bambey 21) genotype had, in all treatments, the lowest proline content (Table 4).

3.4. Screen of genotypes to drought tolerance

Different development measures and proline accumulation, evaluated in the most severe stress condition (stress 2), were normalized in relation with control conditions and used for discriminating cowpea genotypes tolerance/susceptibility with a principal component analysis (PCA; Fig. 2). First two principal components of PCA explained 97.52% of total variation (PC1 = 88.75% and PC2 = 8.77%), being proline (PC1, 0.99) and vigor index and root length (PC2, 0.64) the three most contributive parameters. PCA clustered the genotypes C18, C46, C47, C50, and in particular C44, close to the tolerant reference IT93K-503-1. On the other hand, the C3 and C9 genotypes were grouped close to the susceptible reference genotype Bambey 21. C11 and C54 genotypes, which presented enhanced responses to drought stress, are distant from the remaining genotypes, suggesting a different performance than other genotypes.

4. Discussion

One of the most serious limitations to crops yield is drought. This multifaceted stress condition is differently sensed by plants depending on their growth stage, stress duration and severity (Ahmad et al., 2009). Drought stress during germination can impose a critical limitation to plant development, mainly because seed germination is the most sensitive stage in plant life cycle (Ahmad et al., 2009; Hellal et al., 2018; Muscolo et al., 2014). When seeds are exposed to water stress (or to other unfavorable environmental condition), plants establishment can be compromised (Ahmad et al., 2009; Muscolo et al., 2014). The selection of cowpea (*Vigna unguiculata* L. Walp.) genotypes with increased seed tolerance to drought is a reasonable strategy for the selection of accessions for enhancing cowpea production in a climate change scenario. The use of an *in vitro* screening method on seeds, where drought imposition was artificially imposed by PEG-6000, allowed the assay of 58 cowpea genotypes, which were compared with three susceptible/tolerant genotypes. A wide range of PEG-6000 osmotic potentials was initially tested and presented a dose-dependent detrimental effect on seed germination. This polymer adversely affected the germination and seedling growth of cowpea genotypes, as observed in previous studies (Khodarahmpour, 2011). PEG-6000 concentrations corresponding to osmotic potentials of -0.75 and -1.5 bars were considered adequate to induce stress for the cowpea, while displaying discriminatory resolution among cowpea genotypes. For this reason, both PEG-6000 concentrations were used for imposing drought stress conditions and evaluate the most tolerant drought genotypes.

Considering that tolerant genotypes have higher capacity to germinate and emerge from seeds than susceptible ones, the obtained results could indicate which are the most susceptible and tolerant cowpea genotypes. Previous results obtained under different drought stress

Table 2
Germination and seedling emergence parameters in the 58 studied cowpea genotypes under drought stress conditions. For reference, maximum, minimum, mean, F-value and Tukey's test (significance level of 0.05) values are indicated. Means were analysed with one-way ANOVA followed by Tukey's test (significance level of 0.05) (n = 3 to *%G*, *GR* and *VI* and n = 15 to *RL* and *SL*).

Code	*% G*			*GR*			*RL*			*SL*			*VI*		
	Control	Stress 1	Stress 2	Control	Stress 1	Stress 2	Control	Stress 1	Stress 2	Control	Stress 1	Stress 2	Control	Stress 1	Stress 2
C1	100	100	93.33	0.63	0.34	0.29	11.5	8.89	7.11	5.25	1.79	1.13	1675.00	1067.78	764.00
C2	100	100	86.67	0.76	0.56	0.36	8.57	6.58	5.11	4.15	1.94	1.39	1271.94	852.22	568.89
C3	100	100	93.33	0.64	0.4	0.34	11.51	7.24	5.93	4.02	2.28	1.53	1553.33	951.83	699.78
C4	100	100	100	0.8	0.43	0.47	15.59	8.2	7.24	7.74	2.98	1.98	2332.78	1118	921.67
C5	100	100	100	0.56	0.39	0.33	12.89	8.83	6.38	5.78	2.36	1.43	1866.67	1119.44	780.83
C6	100	73.33	66.67	0.67	0.29	0.23	13.86	5.81	5.75	7.87	1.03	1.75	2172.61	502.83	504
C7	100	93.33	100	0.73	0.43	0.33	13.52	8.84	7.99	7.09	3.17	2.03	2060.67	1093.33	1002.22
C8	100	93.33	80	0.7	0.41	0.28	13.64	9.28	8.06	5.83	2.34	1.89	1947.78	1096.67	811.11
C9	100	93.33	86.67	0.8	0.41	0.33	16.6	11.24	10.51	6.67	3.47	2.01	2327.17	1349.44	1074.67
C10	80	93.33	53.33	0.33	0.31	0.16	11.16	7.91	7.22	4.49	3.24	1.44	1252.44	1057.5	458.44
C11	100	100	100	0.87	0.56	0.44	3.89	13.13	10.22	3.8	4.18	3.06	769.17	1731.67	1328.61
C12	100	100	100	0.93	0.54	0.43	12.41	7.97	9.83	7.44	4.87	3.03	1985.78	1283.89	1286.11
C13	100	100	100	0.83	0.5	0.51	13.83	9.88	9.08	8.71	3.84	2.83	2254.17	1372.5	1191.67
C14	100	93.33	100	1	0.52	0.46	8.86	9.36	9.15	3.15	2.41	2.52	1200.67	1103.73	1166.5
C15	100	100	100	0.93	0.56	0.47	10.35	6.12	6.79	4.13	1.97	1.29	1447.83	809.33	807.44
C16	93.33	86.67	80	0.56	0.47	0.31	10.08	8.78	6.25	3.56	3.69	1.15	1270.00	1118.44	591.33
C17	100	100	100	0.49	0.47	0.38	9.23	8.43	6.2	4.28	3.67	2.42	1351.33	1209.33	861.83
C18	100	93.33	100	0.48	0.41	0.41	7.38	6.73	7.26	3.69	2.62	3.07	1107.33	885.23	1033.5
C19	100	100	93.33	0.9	0.51	0.37	16.28	9.97	7.32	8.67	2.92	2.17	2494.50	1289.00	900.84
C20	100	100	100	0.97	0.51	0.51	11.73	9.06	5.94	6.75	4	2.04	1848.61	1305.83	798.33
C21	100	93.33	100	0.8	0.62	0.58	9.93	6.29	8.36	6.15	2.58	3.18	1607.83	837.72	1153.67
C22	100	100	93.33	0.93	0.53	0.43	18.19	15.96	8.44	9.62	5.67	2.12	2780.67	2162.67	968.00
C23	100	93.33	100	0.8	0.48	0.57	14.34	8.02	7.61	7.78	3.15	2.78	2211.94	1032.22	1039.17
C24	100	100	93.33	1	0.67	0.44	16.58	8.51	10.75	8.32	5.14	2.86	2489.83	1365	1247.61
C25	100	93.33	80	0.8	0.49	0.42	15.5	6.05	5.58	8.1	4.77	2.22	2360.00	989.28	627.56
C26	100	100	100	1	0.71	0.63	11.05	5.35	4.01	7.02	2.83	2.16	1807.67	817.5	617.22
C27	60	73.33	40	0.24	0.27	0.13	8.57	9.21	5	5.65	4.23	1.75	842.67	986.00	267.67
C28	93.33	93.33	100	0.9	0.48	0.57	11.45	12.75	6.12	6.12	4.61	1.51	1625.33	1634.67	763.33
C29	100	80	100	0.9	0.41	0.47	14.33	12.95	9.59	7.79	3.00	2.83	2212.00	1304.33	1242.22
C30	100	93.33	93.33	0.83	0.57	0.47	12.66	9.42	8.48	5.73	4.77	3.11	1838.89	1313.78	1083.44
C31	73.33	53.33	86.67	0.34	0.19	0.32	12.99	8.64	8.03	6.43	2.88	2.32	1370.83	634.22	871.33
C32	100	100	100	0.48	0.52	0.42	12.66	10.02	9.37	3.84	3.79	2	1650.17	1381.00	1136.67
C33	100	100	100	0.93	0.73	0.5	8.66	7.39	8.47	4.34	3.04	3.19	1300.22	1042.50	1165.17
C34	86.67	80	86.67	0.43	0.3	0.34	10.99	10.3	8.15	3.66	2.31	1.68	1271.33	1015.33	859.33
C35	100	100	100	0.76	0.48	0.46	9.22	7.1	7.13	3.47	1.71	1.69	1269.33	880.56	881.50
C36	86.67	93.33	86.67	0.34	0.4	0.28	12.68	9.78	7.3	6.71	3.81	1.53	1650.67	1298.67	762.00
C37	100	100	100	0.83	0.56	0.54	11.81	10.7	9.43	6.6	4.22	3.12	1840.83	1491.83	1254.44
C38	93.33	100	86.67	0.42	0.46	0.34	12.24	10.12	11.23	5.68	4.75	2.57	1659.06	1486.83	1205.5
C39	93.33	80	93.33	0.51	0.31	0.38	13.78	9.52	10.99	7.17	4.45	2.24	1976.33	1090.17	1238.67
C40	100	93.33	86.67	1	0.49	0.4	8.69	8.34	5.67	5.26	3.43	1.81	1394.67	1094.83	653.61
C41	100	100	100	0.97	0.63	0.49	8.91	6.41	8.89	7.9	5.1	3.9	1681.33	1151.33	1278.67
C42	100	100	100	0.57	0.51	0.47	6.71	4.83	5.55	4.94	3.33	2.51	1165.00	816.00	806.00
C43	93.33	93.33	100	0.73	0.41	0.41	6.43	3.41	3.86	2.41	0.69	1.15	838.89	380.78	500.56
C44	66.67	100	100	0.6	0.53	0.49	8.07	9.47	9.25	4.21	4.58	3.99	880.83	1405.33	1324
C45	80	60	53.33	0.28	0.4	0.25	8.5	6.17	8.56	7.64	3.92	4.33	1357.33	636.53	663.33
C46	100	100	100	0.6	0.5	0.5	5.75	5.53	6.05	4.87	4.12	3.22	1061.5	965.72	927.17
C47	100	100	100	0.56	0.48	0.43	9.49	10.93	10.98	3.54	3.00	2.08	1303.33	1393.33	1306.33
C48	100	100	100	0.9	0.47	0.49	9.24	7.31	6.11	2.71	1.75	1.55	1195.33	905.67	766.00
C49	93.33	86.67	80	0.87	0.46	0.39	7.19	7.22	6.93	2.38	2.05	1.22	884.17	810.22	652.09
C50	100	86.67	86.67	0.42	0.43	0.42	6.54	4.59	5.76	3.04	2.6	2.26	958.67	649.33	719.5
C51	100	86.67	93.33	0.8	0.44	0.41	7.35	8.06	7.5	3.04	2.09	1.53	1039	876.22	850.13
C52	100	93.33	93.33	0.5	0.47	0.47	5.57	5.03	4.35	4.91	2.61	2.4	1048.17	722.89	636.00
C53	100	80	100	0.47	0.42	0.41	9.89	7.31	6.95	3.46	3.93	2.02	1334.67	873.17	896.83
C54	93.33	100	100	0.59	0.5	0.44	6.98	6.13	8.73	3.24	2.09	4.00	959.33	822.00	1272.5
C55	100	93.33	93.33	0.63	0.4	0.33	7.43	7.24	6.24	3.31	2.79	1.88	1074	950.67	746.67
Bambey 21	100	46.67	40	0.17	0.21	0.16	9.8	5.24	3.85	3.32	1.24	1.18	1311.67	295.11	198.67
CB46	80	93.33	93.33	0.67	0.66	0.42	8.24	7.51	7.76	4.44	2.74	1.88	1014.67	949.56	902.06
IT93K-503-1	60	93.33	93.33	0.47	0.77	0.49	3.72	4.38	5.64	1.77	1.36	1.42	329.33	530.67	651.78
Maximum	100	100	100	1	0.77	0.63	18.19	15.96	11.23	9.62	5.67	4.33	2780.67	2162.67	1328.61
Minimum	60	46.67	40	0.17	0.19	0.13	3.72	3.41	3.85	1.77	0.69	1.13	329.33	295.11	198.67
Mean	95.29	92.3	91.15	0.68	0.47	0.41	10.6	8.2	7.45	5.34	3.17	2.23	1530.82	1057.1	891.18
SD	9.91	11.63	14.31	0.22	0.12	0.1	3.29	2.37	1.87	1.95	1.14	0.78	521.68	331.16	274.34
F	14.77^{***}	5.36^{***}	7.16^{***}	2.90^{***}	2.28^{***}	4.03^{***}	11.07^{***}	6.88^{***}	4.75^{***}	6.49^{***}	3.64^{***}	4.96^{***}	8.84^{***}	4.80^{***}	5.64^{***}
***Tukey*$_{0.05}$**	0.19	0.17	0.14	0.35	0.44	0.39	3.32	3.03	2.88	2.57	2.00	1.18	589.69	507.85	388.28

% *G* - seed germination percentage, *GR* - seed germination rate, *RL* - root length, *SL* - shoot length and *VI* - vigor index. Control with water; stress 1 and 2 correspond to the use of PEG-6000 osmotic for obtaining a potential of -0.75 bars and -1.5 bars, respectively. **Dark gray or** *** - significant differences at level $p < 0.001$; **gray** - significant differences at level $p < 0.01$; ***light gray*** - significant differences at level $p < 0.05$; **white/clear** - no significant differences.

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Table 3
Statistical analysis of seed germination, seedling emergence and proline content evaluated in the 58 studied cowpea genotypes under drought stress conditions. Means were analysed with one-way and two-way ANOVA followed by Tukey's test (n = 3 to *%G*, *GR*, *VI* and n = 15 to *RL* and *SL* and proline content).

Treatment	Source	*DF*	*F* ratio	*Prob* > *F*
% *G*	Genotype	57	5.968	< 0.001
	Treatment	2	6.330	0.002
	Genotype * Treatment	114	1.523	0.002
GR	Genotype	57	20.427	< 0.001
	Treatment	2	495.982	< 0.001
	Genotype * Treatment	114	4.754	< 0.001
RL	Genotype	57	14.680	< 0.001
	Treatment	2	187.136	< 0.001
	Genotype * Treatment	114	4.472	< 0.001
SL	Genotype	57	9.367	< 0.001
	Treatment	2	415.854	< 0.001
	Genotype * Treatment	114	3.352	< 0.001
VI	Genotype	57	11.893	< 0.001
	Treatment	2	26.131	< 0.001
	Genotype * Treatment	114	4.287	< 0.001
Proline content	Genotype	57	10.865	< 0.001
	Treatment	2	78.039	< 0.001
	Genotype * Treatment	114	2.928	< 0.001

% *G* – seed germination percentage, *GR* – seed germination rate, *RL* – root length, *SL* – shoot length and *VI* – vigor index.

conditions, but using mature plants, revealed Bambey 21 as highly susceptible, CB46 as moderately susceptible and IT93K-503-1 as highly tolerant genotypes (Hamidou et al., 2007; Muchero et al., 2010, 2008). This classification was previously confirmed by us, using different physiological and biochemical approaches on drought-stressed mature plants (unpublished data). The results here presented revealed that a similar trend was observed at germination/seedling stages, suggesting that the mentioned genotypes (Bambey 21, CB46 and IT93K-503-1) could be used as susceptible/tolerant reference genotypes. On the other hand, several studies in other crop species (*e.g.* Beshir et al., 2016; Dodig et al., 2015), including in cowpea (Singh et al., 1999), have revealed a close correspondence of drought tolerance observed in seedlings and reproductive stage plants. Different seed germination and seedling emergence capacities were displayed by distinct cowpea genotypes under stress conditions. As a large proportion of cowpea genotypes did not present any difference on seed germination percentage between treatments, PEG-6000 treatments could not have a strong influence during this stage. According to Mickky and Aldesuquy (2017), the use of PEG-6000 causes a delay in seed germination, as it happens naturally in the drought, but the seed germination percentage is not affected. In the present work, the decrease of seed germination rate was indeed more evident that the decline in germination (%, six days after sowing) and one of the most pronounced parameters under study. This result is in accordance with other cowpea studies, where the seed germination rate (*GR*) also decreased with drought stress induced by PEG-6000 (Araújo et al., 2018; Ferreira et al., 2017; Murillo-Amador et al., 2002). Several cowpea genotypes revealed significant alterations in their germination with PEG-6000 treatments, while others were not so affected, suggesting that distinct genotypes could be differently disturbed by drought. This variability can be considered as a valuable tool for screening cowpea genotypes more tolerant and adapted to climate change. From the assayed cowpea genotypes, 16 cowpea genotypes, including the moderately susceptible CB46, revealed non-significant changes on both germination parameters. These results can be a valuable information about the possible drought tolerant genotypes.

Root length is pointed as another key trait for the selection and differentiation of drought tolerant genotypes, due to the role of roots in providing water and maintaining an adequate water balance in plants.

Table 4
Proline content evaluated in the 58 studied cowpea genotypes under drought stress induced by PEG-6000 and control. For reference, maximum, minimum, mean, *F*-value and Tukey's test (significance level of 0.05) values are indicated. Means (n = 15) were analysed with one-way ANOVA followed by Tukey's test (significance level of 0.05).

	Proline content		
Code	Control	Stress 1	Stress 2
C1	0.49	0.33	0.41
C2	0.21	0.33	0.35
C3	0.73	0.30	0.27
C4	0.15	0.26	0.47
C5	0.21	0.31	0.38
C6	0.15	0.54	0.91
C7	0.14	0.28	0.43
C8	0.29	0.40	0.30
C9	0.17	0.06	0.03
C10	0.23	0.56	0.35
C11	0.65	0.38	0.37
C12	0.14	0.26	0.28
C13	0.17	0.42	0.23
C14	0.17	0.38	0.8
C15	0.58	1.09	0.99
C16	0.19	0.26	0.35
C17	0.26	0.28	0.24
C18	0.11	0.24	0.32
C19	0.16	0.35	0.77
C20	0.18	0.41	0.49
C21	0.19	0.21	0.24
C22	0.13	0.13	0.22
C23	0.12	0.14	0.14
C24	0.14	0.12	0.21
C25	0.20	0.40	0.74
C26	0.16	0.32	0.40
C27	0.13	0.25	0.31
C28	0.15	0.15	0.30
C29	0.15	0.18	0.20
C30	0.11	0.17	0.32
C31	0.29	0.24	0.23
C32	0.54	0.43	0.52
C33	0.37	0.41	0.42
C34	0.17	0.31	0.55
C35	0.42	0.55	0.68
C36	0.19	0.24	0.46
C37	0.26	0.26	0.39
C38	0.19	0.20	0.34
C39	0.16	0.92	0.4
C40	0.31	0.42	0.50
C41	0.30	0.53	0.45
C42	0.34	0.42	0.35
C43	0.59	0.65	1.10
C44	0.38	0.56	0.78
C45	0.36	0.34	0.25
C46	0.25	0.36	0.41
C47	0.42	0.41	0.64
C48	0.30	0.53	0.84
C49	0.16	0.34	0.8
C50	0.32	0.46	0.51
C51	0.13	0.33	0.55
C52	0.20	0.57	0.54
C53	0.15	0.47	0.57
C54	0.08	0.46	0.76
C55	0.23	0.44	0.67
Bambey 21	0.52	0.59	0.42
CB46	0.32	0.27	0.32
IT93K-503-1	0.62	0.89	1.38
Maximum	0.73	1.09	1.38
Minimum	0.08	0.06	0.03
Mean	0.27	0.38	0.48
SD	0.15	0.19	0.25
F	14.77^{***}	5.36^{***}	7.16^{***}
***Tukey*$_{0.05}$**	0.3	0.26	0.31

Control with water; stress 1 and 2 correspond to the use of PEG-6000 osmotic for obtaining a potential of -0.75 bars and −1.5 bars, respectively.
Dark gray or *** – significant differences at level p < 0.001; **gray** – significant differences at level p < 0.01; ***light gray*** – significant differences at level p < 0.05; **white/clear** – no significant differences.

Roots are thus deeply affected when plants are subjected to water stress and are the first plant organs suffering from water stress during seedlings development (Silva and Matos, 2016; Trachsel et al., 2013). Water stress causes decrease of cellular division, increase of rigidification of cell wall resulting in a reduction of root elongation and root-hair development during germination (Muscolo et al., 2014; Silva and Matos, 2016). In general, with increasing of water stress, cowpea seedlings presented a higher decrease of root length than shoot length. This result is in agreement with others studies that verified that the symptoms observed in shoots are normally softer and can be delayed relative to the root (Silva and Matos, 2016). The C6 and Bambey 21 (susceptible reference) genotype (together with C20 and C25 genotypes) were the most affected under drought stress conditions, indicating a higher susceptibility to osmotic stress. In contrast, the drought-tolerant reference (IT93K-503-1) and several other genotypes did not present significant differences between treatments in root length and shoot length, suggesting them as drought tolerant genotypes.

Seedling vigor index is another important parameter that combines seed germination percentage and seedling growth data. The values obtained for this parameter decreased in all genotypes with increasing water stress, except for the tolerant reference (IT93K-503-1) and also C11 and C54 genotypes. In all these cowpea genotypes, the vigor index values increased with water stress conditions, indicating that they display some capacity to tolerate drought. Furthermore, C11 and C54 could be so adapted to water limiting conditions that seem to have a preference for water scarcity during its development. Furthermore, the moderately susceptible reference (CB46) and other four genotypes (C33, C14, C46 and C47) were the least affected by drought stress presenting the lowest decrease of vigor index in the three treatments, being also considered as possible drought tolerant genotypes. On the other hand, a drastic decrease on vigor was observed in the susceptible reference (Bambey 21), similar to decreases observed for other three cowpea genotypes (C6, C25 and C26), followed by others genotypes such as C19, C20 and C22. These results suggest higher susceptibility of these genotypes to drought. Moraes et al. (2005) in common bean (*Phaseolus vulgaris* L.) and Cokkizgin (2013) in pea (*Pisum sativum* L.) also reported a decrease of seedling vigor index with the increasing of PEG-6000 concentrations. The same result was also obtained in cowpea by Jain and Saxena (2016) using PEG-4000.

As proline is one of the compatible solutes that plants accumulate under water stress being the accumulation of this osmolyte correlated with stress tolerance (Anjum et al., 2011). Proline accumulation is commonly associated with the increase of cell osmotic potential, facilitating the water absorption (Ashraf and Foolad, 2007; Toscano et al., 2016), but can also reduce cells injury (Anjum et al., 2011). Our data agree with proline protective role, as proline content generally increased in all genotypes under stress conditions, presenting the tolerant reference (IT93K-503-1) the highest contents. A significant increase in proline with drought imposition was also observed for other 13 cowpea genotypes. Other studies revealed similar increases in other cowpea genotypes (Cavalcanti et al., 2004; Goufo et al., 2017; Merwad et al., 2018), as well in other species, such as in soybean (*Glycine max* L. Merr.; Mwenye et al., 2016) or chickpea (*Cicer arietinum* L.; Mafakheri et al., 2010). On the other hand, in the present study some genotypes did not reveal any difference in the proline content under drought conditions, as the moderately susceptible reference CB46 (together with C8, C32 and C42). In others genotypes, the proline content decreased with drought stress, such as in the highly susceptible reference Bambey 21 (and also in C1, C3, C9, C31 and C45).

Although a common trend is observed for all cowpea genotypes under drought stress (germination and seedling development alterations), each genotype displays a more specific response, probably due to the processes to which they are more susceptible/tolerant. For example, C21 genotype is greatly affected in shoot development, while C33 is significantly affected in seed germination rate. The most susceptible genotypes will be affected in most of evaluated parameters, as detected for Bambey 21, while the most tolerant will be unaffected, like observed for IT93K-503-1. Taking this into consideration, our data suggest that the most tolerant cowpea genotypes were C16, C18, C44, C46, C47, C50, C53, and in a lesser extent C38, C43, C52. In contrast, the most susceptible genotypes seem to be C6, C22, C24, C25, and in a lesser extent C7, C20, C28, C40. A PCA performed with normalized data (ratio between the highest drought stress imposition and control) showed that the reference genotypes Bambey 21 and IT93K-503-1 had divergent

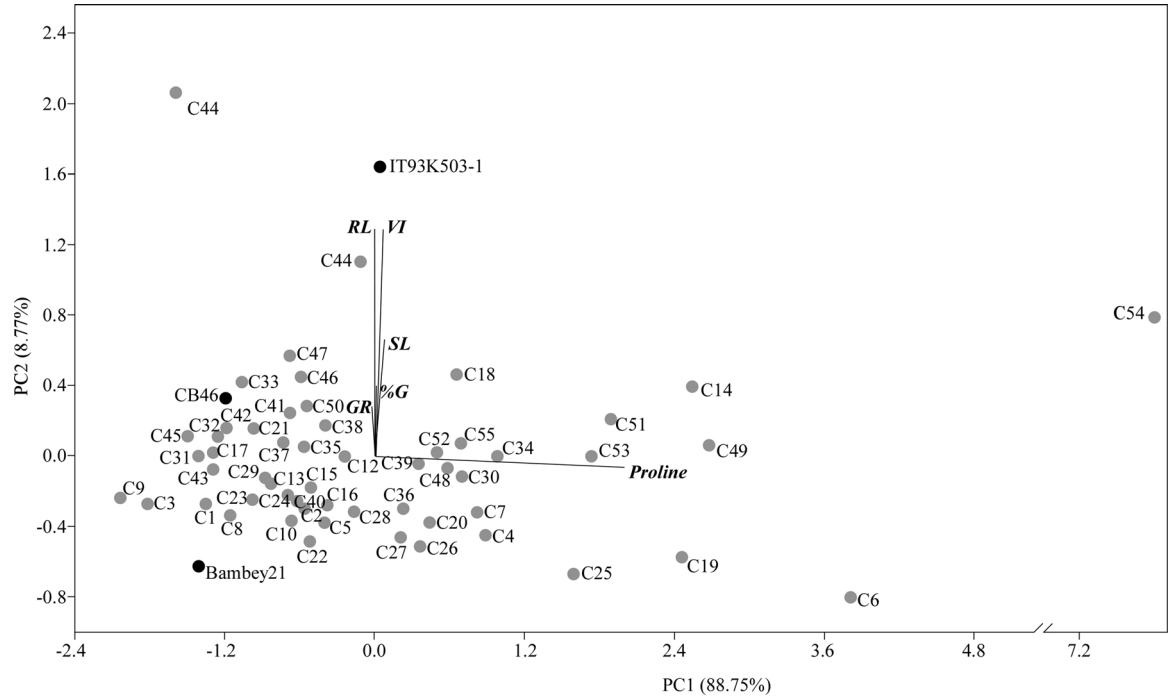

Fig. 2. Principal component analysis with all the cowpea genotypes data, obtained from stress 2 *versus* control. PCA was performed using the results of seed germination percentage (% *G*) and rate (*GR*), root (*RL*) and shoot (SL) length, vigor index (*VI*) and proline content.

drought responses, corroborating the previous studies of Hamidou et al. (2007) and Muchero et al. (2008, 2010). Close to the tolerant reference (IT93K-503-1) was the genotype C44, and also C18, C46, C47 and C50. Regarding, C11 and C54 genotypes, the PCA revealed that they present a different drought response from all the others genotypes under study, presenting a general increase of studied traits, consistent with drought tolerant genotypes. For other hand, the susceptible reference (Bambey 21) was very close to the genotypes C3 and C9.

Most of the evaluated genotypes had been previously included in a genetic diversity study using single nucleotide polymorphisms (SNPs), revealing that they were grouped based on their geographical origin (Carvalho et al., 2017b). The suggested tolerant genotypes C46 (Zambia), C47 (Iran) and C50 (Congo) were considered admixed due they have information from several subpopulations. Probably, these genotypes are the result of introgression of genetic material on other lines and subsequent selection by farmers based on their adaptation to specific environmental conditions, in these cases all tropical and subtropical weather. The other suggested tolerant genotypes (C11, C18 and C44) were from Portugal, Spain and Spain, respectively, and belong to different subpopulations from genetic analysis (Carvalho et al., 2017b). These genotypes could be a source of variability and could be useful for the improvement of new varieties to mitigate the effects of climate change.

5. Conclusions

The selection of cowpea (*Vigna unguiculata* L. Walp.) genotypes well-adapted to upcoming climate change (including drought) is a key step for improving crop production. Drought can inhibit the germination and subsequent seedling growth, impairing the crops establishment. Therefore, the germplasm screening at an early growth stage is a reasonable approach for selecting tolerant genotypes to drought conditions. PEG induction is a simple, cost effective and fast method of drought induction allowing to screen a large number of genotypes. Various seed germination and seedling emergence features could be evaluated to have a complete picture of drought responses in an early stage, but root length, vigor index and proline contents were the most consistent and informative, enabling to infer about genotypes drought tolerance. A response variation was identified in this collection of cowpea genotypes that can be further explored by plant breeders. Our results suggest that C11 (Portugal), C18 (Spain), C44 (Ghana), C46 (Zambia), C47 (Iran), C50 (Congo) and C54 (Bulgaria) cowpea genotypes showed a high drought tolerance at germination stage. These accessions could be further used as parents for developing segregating populations for cowpea drought tolerance and to get of new varieties.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.scienta.2018.11.082.

References

Abdul-Baki, A.A., Anderson, J.D., 1973. Vigor determination in soybean seed by multiple criteria. Crop Sci. 13, 630–633. https://doi.org/10.2135/cropsci1973.0011183X001300060013x.

Agbicodo, E.M., Fatokun, C.A., Muranaka, S., Visser, R.G.F., Linden Van Der, C.G., 2009. Breeding drought tolerant cowpea: constraints, accomplishments, and future prospects. Euphytica 167, 353–370. https://doi.org/10.1007/s10681-009-9893-8.

Ahmad, S., Ahmad, R., Ashraf, M.Y., Ashraf, M., Waraich, E.A., 2009. Sunflower (*Helianthus Annuus* L.) response to drought stress at germination and seedling growth stages. Pak. J. Bot. 41, 647–654.

Anjum, S., Xie, X., Wang, L., 2011. Morphological, physiological and biochemical responses of plants to drought stress. Afr. J. Agric. Res. 6, 2026–2032. https://doi.org/10.5897/AJAR10.027.

Araújo, E.D.D.E., Melo, A.S.D.E., Do, M., Rocha, S., 2018. Germination and initial growth of cowpea cultivars under osmotic stress and salicylic acid. Rev. Caatinga 31, 80–89.

Ashraf, M., Foolad, M.R., 2007. Roles of glycine betaine and proline in improving plant abiotic stress resistance. Environ. Exp. Bot. 59, 206–216. https://doi.org/10.1016/j.envexpbot.2005.12.006.

Bates, L.S., 1973. Rapid determination of free proline for water stress studies. Plant Soil 39, 205–207.

Beshir, H.M., Bueckert, R., Sciences, H., 2016. Effect of temporary drought at different growth stages on snap bean pod quality and yield. Afr. Crop Sci. J. 24, 317–330. https://doi.org/10.4314/acsj.v24i3.8.

Bouslama, M., Schapaugh, W.T., 1984. Stress tolerance in soybeans. I. Evaluation of three screening techniques for heat and drought tolerance. Crop Sci. 24, 933–937. https://doi.org/10.2135/cropsci1984.0011183X002400050026x.

Carvalho, M., Bebeli, P.J., Pereira, G., Castro, I., Egea-Gilabert, C., Matos, M., Lazaridi, E., Duarte, I., Lino-Neto, T., Ntatsi, G., Rodrigues, M., Savvas, D., Rosa, E., Carnide, V., 2017a. European cowpea landraces for a more sustainable agriculture system and novel foods. J. Sci. Food Agric. 97, 4399–4407. https://doi.org/10.1002/jsfa.8378.

Carvalho, M., Muñoz-Amatriaín, M., Castro, I., Lino-Neto, T., Matos, M., Egea-Cortines, M., Rosa, E., Close, T., Carnide, V., 2017b. Genetic diversity and structure of Iberian Peninsula cowpeas compared to world-wide cowpea accessions using high density SNP markers. BMC Genom. 18, 891. https://doi.org/10.1186/s12864-017-4295-0.

Cavalcanti, F.R., Oliveira, J.T.A., Martins-Miranda, A.S., Viégas, R.A., Silveira, J.A.G., 2004. Superoxide dismutase, catalase and peroxidase activities do not confer protection against oxidative damage in salt-stressed cowpea leaves. New Phytol. 163, 563–571. https://doi.org/10.1111/j.1469-8137.2004.01139.x.

Cokkizgin, A., 2013. Effects of hydro and osmo-priming on seed vigor of pea (*Pisum sativum* L.). Agric. For. Fish. 2 (6), 225–228. https://doi.org/10.11648/j.aff.20130206.14.

Darkwa, K., Ambachew, D., Mohammed, H., Asfaw, A., Blair, M.W., 2016. Evaluation of common bean (*Phaseolus vulgaris* L.) genotypes for drought stress adaptation in Ethiopia. Crop J. 4, 367–376. https://doi.org/10.1016/j.cj.2016.06.007.

Dharanguttikar, V.M., Bharud, R.W., Borkar, V.H., 2015. Physiological responses of chickpea genotypes for drought tolerance under induced moisture stress. IJSRP 5, 1–11.

Dodig, D., Zorić, M., Jović, M., Kandić, V., Stanisavljević, R., Šurlan-Momirović, G., 2015. Wheat seedlings growth response to water deficiency and how it correlates with adult plant tolerance to drought. J. Agric. Sci. 153, 466–480. https://doi.org/10.1017/S002185961400029X.

Eftekhari, A., Baghizadeh, A., Yaghoobi, M.M., Abdolshahi, R., 2017. Differences in the drought stress response of *DREB2* and *CAT1* genes and evaluation of related physiological parameters in some bread wheat cultivars. Biotechnol. Biotechnol. Equip. 31, 709–716. https://doi.org/10.1080/13102818.2017.1316214.

Ehlers, J.D., Hall, A.E., 1996. Genotypic classification of cowpea based on responses to heat and photoperiod. Crop Sci. 36, 673–679. https://doi.org/10.2135/cropsci1996.0011183X003600030026x.

Eloward, H.O., Hall, A.E., 1987. Influence of early and late nitrogen fertilization on yield and nitrogen fixation of cowpea under well-watered and dry field conditions. Field Crops Res. 15, 229–244.

FAOSTAT, 2018. Food and Agriculture Organization of the United Nations – Statistics Division. (Accessed 20 June 2018). http://faostat3.fao.org/browse/Q/QC/E.

Ferreira, A.C.T., Felito, R.A., Da Rocha, A.M., De Carvalho, M.A.C., Yamashita, O.M., 2017. Water and salt stresses on germination of cowpea (*Vigna unguiculata* cv. brs tumucumaque) seeds. Rev. Caatinga 30 (4), 1009–1016.

Goufo, P., Moutinho-Pereira, J.M., Jorge, T.F., Correia, C.M., Oliveira, M.R., Rosa, E.A.S., António, C., Trindade, H., 2017. Cowpea (*Vigna unguiculata* L. Walp.) metabolomics:

osmoprotection as a physiological strategy for drought stress resistance and improved yield. Front. Plant Sci. 8, 1–22. https://doi.org/10.3389/fpls.2017.00586.
Hamidou, F., Zombre, G., Braconnier, S., 2007. Physiological and biochemical responses of cowpea genotypes to water stress under glasshouse and field conditions. J. Agron. Crop Sci. 193, 229–237. https://doi.org/10.1111/j.1439-037X.2007.00253.x.
Hammer, O., Harper, D.A.T., Ryan, P.D., 2001. Past: paleontological statistics software package for education and data analysis. Paleontol. Electron. 4 (1), 1–9.
Hellal, F.A., El-Shabrawi, H.M., Abd El-Hady, M., Khatab, I.A., El-Sayed, S.A.A., Abdelly, C., 2018. Influence of PEG induced drought stress on molecular and biochemical constituents and seedling growth of Egyptian barley cultivars. J. Genet. Eng. Biotechnol. 16, 203–212. https://doi.org/10.1016/j.jgeb.2017.10.009.
Huang, K., Mellor, K.E., Paul, S.N., Lawson, M.J., Mackey, A.J., Timko, M.P., 2012. Global changes in gene expression during compatible and incompatible interactions of cowpea (*Vigna unguiculata* L.) with the root parasitic angiosperm *Striga gesnerioides*. BMC Genom. 13, 402. https://doi.org/10.1186/1471-2164-13-402.
Jain, C., Saxena, R., 2016. Varietal differences against PEG induced drought stress in cowpea Chinkita Jain. Octa J. Environ. Res. 4, 58–62.
Kaya, M.D., Okçu, G., Atak, M., Çikili, Y., Kolsarici, Ö., 2006. Seed treatments to overcome salt and drought stress during germination in sunflower (*Helianthus annuus* L.). Eur. J. Agron. 24, 291–295. https://doi.org/10.1016/j.eja.2005.08.001.
Khodarahmpour, Z., 2011. Genetic analysis of tolerance to heat stress in maize (*Zea mays* L.). Afr. J. Agric. Res. 6, 2767–2773. https://doi.org/10.5897/AJAR10.798.
Kocheva, K., Georgiev, G., 2003. Evaluation of teh reaction of two contrasting barley (*Hordeum vulgare* L.) cultivars in response to osmotic stress with PEG 6000. Bulg. J. Plant Physiol. Spec. Issue 2003, 290–294.
Kpoghomou, B.K., Sapra, V.T., Beyl, C.A., 1990. Screening for drought tolerance: soybean germination and its relationship to seedling responses. J. Agron. Crop Sci. 164, 153–159. https://doi.org/10.1111/j.1439-037X.1990.tb00801.x.
Kröner, N., Kotlarski, S., Fischer, E., Lüthi, D., Zubler, E., Schär, C., 2017. Separating climate change signals into thermodynamic, lapse-rate and circulation effects: theory and application to the European summer climate. Clim. Dyn. 48, 3425–3440. https://doi.org/10.1007/s00382-016-3276-3.
Li, F.L., Bao, W.K., Wu, N., 2011. Morphological, anatomical and physiological responses of *Campylotropis polyantha* (Franch.) Schindl. seedlings to progressive water stress. Sci. Hortic. (Amsterdam) 127, 436–443. https://doi.org/10.1016/j.scienta.2010.10.017.
Li, R., Zeng, Y., Xu, J., Wang, Q., Wu, F., Cao, M., Lan, H., Liu, Y., Lu, Y., 2015. Genetic variation for maize root architecture in response to drought stress at the seedling stage. Breed. Sci. 65, 298–307. https://doi.org/10.1270/jsbbs.65.298.
Machado Neto, N.B., Custódio, C.C., Costa, P.R., Dona, F.L., 2006. Deficiência hídrica induzida por diferentes agentes osmóticos na germinação e vigor de sementes de feijão. Rev. Bras. Sementes 28, 142–148. https://doi.org/10.1590/S0101-31222006000100020.
Mafakheri, A., Siosemardeh, A., Bahramnejad, B., Struik, P.C., Sohrabi, E., 2010. Effect of drought stress on yield, proline and chlorophyll contents in three chickpea cultivars. Aust. J. Crop Sci. 4, 580–585.
Merwad, A.R.M.A., Desoky, E.S.M., Rady, M.M., 2018. Response of water deficit-stressed *Vigna unguiculata* performances to silicon, proline or methionine foliar application. Sci. Hortic. (Amsterdam) 228, 132–144. https://doi.org/10.1016/j.scienta.2017.10.008.
Mickky, B.M., Aldesuquy, H.S., 2017. Impact of osmotic stress on seedling growth observations, membrane characteristics and antioxidant defense system of different wheat genotypes. Egypt. J. Basic Appl. Sci. 4, 47–54. https://doi.org/10.1016/j.ejbas.2016.10.001.
Moraes, G.A.F., Menezes, N.L., Pasqualli, L.L., 2005. Comportamento de sementes de feijão sob diferentes potenciais osmóticos. Ciênc. Rural 35 (4), 776–780. https://doi.org/10.1590/S0103-84782005000400004.
Muchero, W., Ehlers, J.D., Close, T.J., Roberts, P.A., 2009. Mapping QTL for drought stress-induced premature senescence and maturity in cowpea [*Vigna unguiculata* (L.) Walp.]. Theor. Appl. Genet. 118, 849–863. https://doi.org/10.1007/s00122-008-0944-7.
Muchero, W., Ehlers, J.D., Roberts, P.A., 2010. Restriction site polymorphism-based candidate gene mapping for seedling drought tolerance in cowpea [*Vigna unguiculata* (L.) Walp.]. Theor. Appl. Genet. 120, 509–518. https://doi.org/10.1007/s00122-009-1171-6.
Muchero, W., Ehlers, J.D., Roberts, P.A., 2008. Seedling stage drought-induced phenotypes and drought-responsive genes in diverse cowpea genotypes. Crop Sci. 48, 541–552. https://doi.org/10.2135/cropsci2007.07.0397.
Murillo-Amador, B., López-Aguilar, R., Kaya, C., Larrinaga-Mayoral, J., Flores-Hernández, A., 2002. Comparative effects of NaCl and polyethylene glycol on germination, emergence and seedling growth of cowpea. J. Agron. Crop Sci. 188, 235–247. https://doi.org/10.1046/j.1439-037X.2002.00563.x.
Muscolo, A., Sidari, M., Anastasi, U., Santonoceto, C., Maggio, A., 2014. Effect of PEG-induced drought stress on seed germination of four lentil genotypes. J. Plant Interact. 9, 354–363. https://doi.org/10.1080/17429145.2013.835880.
Mwenye, O.J., van Rensburg, L., van Biljon, A., van der Merwe, R., 2016. The role of proline and root traits on selection for drought-stress tolerance in soybeans: a review. S. Afr. J. Plant Soil 33, 245–256. https://doi.org/10.1080/02571862.2016.1148786.
Ravelombola, W.S., Shi, A., Weng, Y., Clark, J., Motes, D., Chen, P., Srivastava, V., 2017. Evaluation of salt tolerance at germination stage in cowpea [*Vigna unguiculata* (L.) Walp]. HortScience 52, 1168–1176. https://doi.org/10.21273/HORTSCI12195-17.
Silva, P., Matos, M., 2016. Assessment of the impact of Aluminum on germination, early growth and free proline content in *Lactuca sativa* L. Ecotoxicol. Environ. Saf. 131, 151–156. https://doi.org/10.1016/j.ecoenv.2016.05.014.
Singh, B.B., Mai-Kodomi, Y., Terao, T., 1999. A simple screening method for drought tolerance in cowpea. Indian J. Genet. Plant Breed. 59, 211–220.
Steiner, F., Zuffo, A.M., Zoz, T., Zoz, A., Zoz, J., 2017. Drought tolerance of wheat and black oat crops at early stages of seedling growth. SCAP 40 (3), 576–586. https://doi.org/10.19084/RCA16118.
Timko, M.P., Singh, B.B., 2008. Cowpea, a multifunctional legume. In: Moore, P.H., Ming, R. (Eds.), Genomics of Tropical Crop Plants. Springer Science + Business Media LLC, New York, pp. 227–258. https://doi.org/10.1007/s13398-014-0173-7.2.
Toscano, S., Farieri, E., Ferrante, A., Romano, D., 2016. Physiological and biochemical responses in two ornamental shrubs to drought stress. Front. Plant Sci. 7, 645. https://doi.org/10.3389/fpls.2016.00645.
Trachsel, S., Kaeppler, S.M., Brown, K.M., Lynch, J.P., 2013. Maize root growth angles become steeper under low N conditions. Field Crops Res. 140, 18–31. https://doi.org/10.1016/j.fcr.2012.09.010.
Vieira, R.D., Tekrony, D.M., Egli, D.B., 1991. Effect of drought stress on soybean seed germination and vigor. J. Seed Technol. 15, 12–21.
Wu, C., Wang, Q., Xie, B., Wang, Z., Cui, J., Hu, T., 2011. Effects of drought and salt stress on seed germination of three leguminous species. Afr. J. Biotechnol. 10, 17954–17961. https://doi.org/10.5897/AJB11.2018.
Yan, M., 2015. Seed priming stimulate germination and early seedling growth of Chinese cabbage under drought stress. S. Afr. J. Bot. 99, 88–92. https://doi.org/10.1016/j.sajb.2015.03.195.