Koskosidis A *et al.* (2020)
Notulae Botanicae Horti Agrobotanici Cluj-Napoca 48(1):294-304
DOI:10.15835/nbha48111799
Research Article

Effect of PEG-induced drought stress on germination of ten chickpea (*Cicer arietinum* L.) genotypes

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Abstract

Chickpea (*Cicer arietinum* L.) is one of the most important legume crops worldwide, with its importance mainly relying on its high protein content. Chickpea productivity is strictly threatened by abiotic stresses, of which drought exerts the most crucial role in terms of growth inhibition and yield losses encountered. Given that germination is a critical stage that is negatively affected by drought, the aim of this study was to estimate the genotypic variability among ten chickpea genotypes and to determine the seed germination and seedling growth ability under drought stress conditions. Seeds were subjected to water stress by polyethylene glycol (PEG-6000) at five stress levels (0, 5, 10, 20, 30 and 50% PEG). Germination percentage, seed water content, seed water absorbance, root and shoot development and seedling vigour index were evaluated. The analyses revealed significant genetic variability in relation to genotypic performance under drought stress. Drought significantly affected germination as well as all other associated traits, with the effects of stress being analogous to the stress level applied. Findings point to the fact that seedling vigour index is a suitable selection criterion for drought tolerance as it allowed the classification of genotypes as tolerant, moderately tolerant, moderately susceptible and susceptible. Taken together, the commercial varieties 'Thiva', 'Keryneia' and 'Gavdos' as well as the landrace 'Lemnos' showed an increased drought tolerance at high stress level, indicating their possible exploitation as valuable genetic material for breeding programs or for commercial use.

Keywords: chickpea; drought; germination; tolerance; water stress

Abbreviations: PEG: polyethylene glycol; WU: Seed water absorbance; WC: Seed water content; SVI: seedling vigor index

Received: 03 Feb 2020. Received in revised form: 23 Mar 2020. Accepted: 25 Mar 2020. Published online: 31 Mar 2020.

Introduction

Chickpea (*Cicer arietinum* L.) is an important pulse crop cultivated in over 50 countries (Jumrani and Bhatia, 2014). Chickpea is a grain legume of great dietary value mainly due to its high protein content (Venn and Mann, 2004; Duc *et al*., 2014; Arnoldi *et al*., 2015). Further, its ability for nitrogen fixation contributes significantly to the reduction of fertilizer inputs (Rubiales and Mikic, 2015). Chickpea is classified as a low-water-demanding crop with satisfactory adaptation in dry areas, with crop establishment however being largely depended on seed germination potential under low water availability (Arjenaki *et al*., 2011).

During the last 5000 years, limited water availability is considered as the main factor hampering crop production worldwide (Ceccarelli *et al*., 2010). Drought stress adversely affects crops throughout their lifecycle and causes yield losses, the extent of which varies significantly depending on the duration and intensity of the stress, the genetic background and the developmental stage. Moderate to severe drought reduces plant biomass, grain yield and related yield components of legumes (Siddique *et al*., 2001), while estimates of yield losses due to terminal drought range from 35% to 50% (Sabaghpour, 2003). In chickpea, drought causes yield losses ranging from 30% to 60%, depending on geographical region and length of crop season.

Considering the ongoing climate change as well as the gradual decline of available water resources, research efforts towards developing of drought tolerant germplasm is of paramount importance. Breeding for the trait is routinely based on evaluation of yield performance under water-deficit stress conditions in the field, a procedure which is rather slow and laborious due to the difficulties both in terms of obtaining homogeneous stress conditions and identifying drought tolerant genotypes (Shaheen and Hood-Nowotny, 2005). As an alternative, Richards (1978) underpinned germination phase as the most sensitive stage in chickpea lifecycle and proposed that selection at this phase may form a useful screening criterion for drought tolerance. Further, previous studies underline that seedling vigour index, which combines seed germination rate with morphological traits during early growth of seedlings, is a reliable index for screening drought tolerance at germination phase (Siahsar *et al*., 2010). *In vitro* screening constitutes an alternative selection method that is advantageous over the routine approaches as it is readily applicable and time-effective. To this purpose, stress is commonly conducted through the use of osmotic solutions, among which polyethylene glycol has proven a valuable means of inducing drought stress in the context of screening *in vitro* a large set of genotypes (Kulkarni and Deshpande, 2007). Polyethylene glycol is capable of simulating water stress through decreasing the osmotic potential without entrance into the plant cells (Rai *et al*., 2011). Previous studies on chickpeas (Kalefetoglu *et al*., 2009; Yucel *et al*., 2010) proved the efficiency of PEG to induce drought associated effects on both germination and seedling growth.

The objective of this experiment was to study the effect of PEG-induced drought stress in germination and other traits related to germination and post-germination growth. Specifically, the germination percentage, seed water uptake, seedling water content, root and shoot length and seedling vigour index were employed as parameters for estimating genetic variation among ten chickpea genotypes and for selecting drought tolerant genotypes to be employed for breeding purposes or for commercial use.

Materials and Methods

Plant material

The genetic material consisted of both local and foreign chickpea germplasm. The local material under study consisted of one landrace ('Lemnos'), two elite lines ('Sifnos', 'Line 9/14') and three commercial varieties ('Gavdos', 'Keryneia', 'Thiva'), developed by Hellenic Agricultural Organization-DEMETER/Inst. of Industrial and Forage Crops. The foreign germplasm referred to three elite lines ('CAT16-31', 'CAT16-27', 'CAT16-4'), originating from ICARDA, and one commercial variety, namely 'Macarena'.

Treatments and experimental design

Drought stress was induced by PEG-6000 (M.W. 6000) at different stress levels: i) 0% (control), ii) 5%, iii) 10%, iv) 20%, v) 30% and vi) 50%. According to Michell and Kaufmann (1972), the osmotic potential (ψ_s) of aqueous solutions of polyethylene glycol 6000 (PEG-6000) is curvilinearly related to concentration, thus allowing for calculation of ψ_s, from known concentrations of PEG-6000 through an equation:

$$\psi s = -(1.18 \times 10^{2}) \times C - (1.18 \times 10^{-4}) \times C^2 + (2.67 \times 10^{-4}) \times C \times T + (8.39\ \ 10^{-7}) \times C^2 \times T$$

where C is the concentration of PEG-6000 in g/kg H_2O and T is the temperature in °C (Michell and Kaufmann, 1972).

Based on the abovementioned equation, the osmotic pressure of PEG solutions was estimated as follows: i) 0% (control), ii) 5% (-0.05 MPa), iii) 10% (-0.15 MPa), iv) 20% (-0.5 MPa), v) 30% (-1 MPa) and vi) 50% (-2.7 MPa).

Seeds were surface-sterilized for five minutes in 10% bleach solution and washed three times with sterile water. In order to avoid contamination, seeds were placed in plastic boxes with absorbent paper and treated with fungicide. Seeds were subsequently watered with 12 ml of the appropriate PEG solution. Plastic boxes were placed under natural sunlight and room temperature (app. 25 °C). The experimental design used was a randomized complete block design with four replications for every genotype-stress level combination, each replication consisting of twenty seeds.

Measurements

Evaluation of drought tolerance was performed using the following parameters: germination percentage (%) (1^{st} until 7^{th} day), seed water absorbance (WU) (%) (3^{rd}, 5^{th} and 7^{th} day), seed water content (WC) (%) (10^{th} and 15^{th} day) root and shoot length (cm) (5^{th}, 7^{th}, 9^{th}, 11^{th}, 13^{th} and 15^{th} day) and seedling vigor index (SVI) (15^{th} day). Seeds were considered germinated when the radicle had a length of at least 1 mm. The estimation of abovementioned parameters was performed using the following formulas:

Seed water absorbance (WU) (%) = $\frac{W2 - W1}{W2} \times 100$ (Mujeeb-ur-Rahman *et al*., 2008),
where W_1 = initial seed weight and W_2 = seed weight following water absorbance.

Seed water content (WC) (%) = $\frac{Fresh\ weight - Dry\ weight}{Fresh\ weight} \times 100$ (Black and Pritchard, 2002)

$\text{Seedling vigor index (SVI)} = \text{seedling length (cm)} \times \text{germination percentage}$ (Abdul-Baki and Anderson, 1973), where $\text{seedling length} = \text{root leght} + \text{shootlegnth}$.

Further, correlations between germination and seed characteristics, namely 100 seed weight, WU, WC, shoot length, root length, were estimated.

Statistical analysis

Data were analyzed using ANOVA ($p \leq 0.05$), combining PEG concentrations and genotypes. Genotypes were compared within stress level at specific time intervals, as described above. Additionally, genotypes were compared across stress levels. Differences between means were compared by the least significant difference (LSD) test. All statistical analyses were performed using SPSS statistical software v. 20.

Results

Seed germination was significantly affected by drought stress while, at the same time, differing substantially among genotypes under study (Table 1).

Table 1. Final germination (%), as affected by genotype (G) and PEG concentration (C)

Time	Genotype (G)	PEG Concentration (%) (C)						
		0	5 (-0.05MPa)	10 (-0.15MPa)	20 (-0.5MPa)	30 (1MPa)	50 (-2.7MPa)	(%) Mean (C)
7th day	'Gavdos'	95	96	98	74	31	8	67 ab
	'Keryneia'	94	91	100	96	18	9	67.9 ab
	'Macarena'	96	94	89	35	0	0	52.3 d
	'CAT16-31'	86	91	94	90	0	0	60.2 bcd
	'CAT16-27'	93	96	48	0	28	0	44 e
	'Thiva'	99	100	99	99	30	0	71 a
	'CAT16-4'	98	98	95	10	24	0	54 cd
	'Sifnos'	98	95	88	85	0	0	60.8 bc
	'Line 9/14'	94	91	83	53	11	0	55.2 cd
	'Lemnos'	95	88	79	76,30	79	3	70 a
	Mean (G)	94.6 a	94 a	87 b	61.8 c	22 d	2 e	

Note: At each time interval (days), means followed by the same letter, within each factor, are not significantly different according to LSD ($p \leq 0.05$).

At stress level, the germination percentage ranged from 94.6% to 2% in the controls and plants subjected to high stress level (50% PEG), respectively. At low stress level (5% PEG), no significant differences were noted, while higher stress levels (10, 20, 30 and 50% PEG) resulted in substantial differences in germination percentage. Although certain differences were observed in the performance of genotypes at 10% PEG, most profound decrease in mean germination rate (germination rate across all genotypes at a certain PEG concentration) was recorded at transition from 20% to 30% PEG and was 39.8%. Specifically, at 20% PEG, 'Thiva', 'Keryneia' and 'CAT 16-31' showed a germination percentage above 90%, while at 30% PEG the entire set of genotypes presented considerably lower germination rates (ranging from 0-31%), with the exception of landrace 'Lemnos' which retained a high germination rate (79%). At high stress level (50% PEG), germination was totally repressed in the majority of genotypes, with the exception of landrace 'Lemnos' and cultivars 'Gavdos' and 'Keryneia' that were capable of germination, presenting though low germination rates (3%, 8% and 9%, respectively). Across stress levels, 'Thiva' and 'Lemnos' presented the highest values for mean germination percentage (71% and 70%, respectively), followed by 'Keryneia' and 'Gavdos' (67.9% and 67%, respectively). In contrast, the most severe effect in germination was noted in cultivars 'Macarena' and 'CAT16-27', which showed mean germination rates of 52.3% and 44%, respectively.

As expected, WC and WU increased over time in all genotypes as well as stress levels applied (Tables 2 and 3). At specific time intervals however, WC and WU showed a gradual decrease as PEG concentration increased. In relation to stress levels, the mean values of WC and WU did not differ between control and 5% PEG treatment, whereas at all other stress levels significant differences were recorded. Across stress levels, 'Sifnos' and 'CAT16-31' presented the highest mean WU, while 'Gavdos' and 'Keryneia' showed the highest mean WC. At high stress level (50% PEG), the best performing genotypes were 'CAT16-31' for WU and 'Line 9/14' and 'Keryneia' for WC.

Table 2. Seedling water content (WC) (%), as affected by genotype (G) and PEG concentration (C) after 10 and 15 days of PEG stress

	Genotype (G)	PEG Concentration (%) (C)						
		0	5 (-0.05MPa)	10 (-0.15MPa)	20 (-0.5MPa)	30 (-1MPa)	50 (2.7MPa)	% Mean (C)
10th day	'Gavdos'	63.7	66.5	63.7	57.0	49.4	53.4	59 b
	'Keryneia'	68.5	67.5	67.2	62.8	50.0	52.3	61.4 a
	'Macarena'	65.5	63.7	62.5	53.3	44.1	45.6	55.7 c
	'CAT16-31'	67.7	66.2	66.5	60.5	47.7	51.8	60.1 ab
	'CAT16-27'	60.7	55.7	46.2	42.8	45.4	47.9	49.7 e
	'Thiva'	59.7	60.5	55.8	59.7	46.5	46.4	54.9 cd
	'CAT16-4'	62.2	63.0	56.5	50.0	48.3	46.8	54.5 cd
	'Sifnos'	60.5	58.7	54.7	55.2	44.0	46.2	53.2 d
	'Line 9/14'	65.0	60.0	56.5	51.8	45.0	44.4	53.8 cd
	'Lemnos'	60.5	58.5	58.5	37.5	51.0	50.0	52.7 d
	Mean (G)	63.4 a	62 a	58.8 b	53 c	47.1 d	48.5 d	
15th day								Mean (C)
	'Gavdos'	75.8	77.8	73.0	60.3	49.4	54.1	65 a
	'Keryneia'	71.8	71.0	69.5	71.0	46.9	54.9	64.1 a
	'Macarena'	73.3	70.8	68.5	60.0	43.2	45.9	60.3 bc
	'CAT16-31'	70.0	67.8	68.5	67.8	42.8	50.6	61.3 b
	'CAT16-27'	63.5	59.3	58.5	39.0	43.9	49.3	52.2 g
	'Thiva'	65.3	67.3	55.5	64.5	46.3	48.2	57.8 de
	'CAT16-4'	68.8	71.3	65.0	53.8	48.4	48.3	59.2 bcd
	'Sifnos'	64.8	61.8	60.3	57.8	43.5	46.3	55.7 ef
	'Line 9/14'	70.8	65.3	61.8	55.3	44.3	55.3	58.8 cd
	'Lemnos'	63.8	61.0	60.5	40.2	53.2	50.8	54.8 f
	Mean (G)	68.8 a	67.3 a	64.1 b	56.9 c	46.2 e	50.4 d	

Note: At each time interval (days), means followed by the same letter, within each factor, are not significantly different according to LSD ($p \leq 0.05$).

In relation to traits associated with post-germination growth of seedlings, significant differences were observed both at stress and at genotype level. Specifically, for root length, although no differences were noted in mean values between controls, 5% PEG and 10% PEG, a significant decrease in root length was recorded at 20%, 30% and 50% PEG (Table 4). Across stress levels, 'Thiva' had the highest mean for root length, while landrace 'Lemnos' had the highest root length at 30% PEG. At high stress level (50% PEG) however, most genotypes were incapable of forming roots, with the exception of 'Gavdos', 'Keryneia' and 'Lemnos'. For shoot length, the highest mean values were recorded at 5% PEG, followed by control and 10% PEG treatment (Table 5). At higher stress levels however, a drastic decrease in shoot length was observed, while treatment with 30% and 50% PEG was associated with inability to form shoots in all genotypes tested. With respect to shoot length, 'Keryneia' was the best performing cultivar as it presented the highest mean value at 20% PEG. Based on these findings, 'Thiva' and 'Keryneia' had the highest SVI value across stress levels, followed by 'Gavdos', while 'CAT16-27' ranked in the bottom. According to SVI, genotypes were classified in four distinct classes: i) SVI > 450: 'Thiva', 'Keryneia,' ii) SVI = 350-450: 'Gavdos', iii) SVI = 250-350: 'CAT16-31', 'Macarena', 'CAT16-4' and iv) SVI < 250: 'Sifnos', 'Lemnos', 'Line 9/14' and 'CAT16-27' (Table 6).

In relation to correlations among traits, germination percentage showed significant positive correlation with root length (r = 0.667*), while WC was well correlated with shoot length (r = 0.915**). In contrast, no correlation was found between germination percentage and 100 seed weight (seed size) at any stress level applied (Table 7).

Table 3. Seed water absorbance (WU) (%), as affected by genotype (G) and PEG concentration (C) after 3, 5 and 7 days of PEG stress

Time	Genotype (G)	PEG Concentration (%) (C)						
		0	5 (-0.05MPa)	10 (-0.15MPa)	20 (-0.5MPa)	30 (-1MPa)	50 (-2.7MPa)	% Mean (C)
3rd day	'Gavdos'	54.0	55.8	46.3	47.8	42.5	48.0	48.9 bc
	'Keryneia'	49.7	50.3	52.3	51.7	40.8	35.5	46.7 de
	'Macarena'	48.7	53.8	46.2	45.0	41.2	40.0	45.8 e
	'CAT16-31'	57.3	57.3	61.0	54.0	48.9	48.0	54.4 a
	'CAT16-27'	53.3	51.0	50.0	46.8	47.9	47.4	49.3 b
	'Thiva'	49.5	46.2	49.5	52.8	42.7	42.3	47.2 cde
	'CAT16-4'	55.8	52.8	52.3	43.3	50.0	45.6	49.9 b
	'Sifnos'	58.3	55.5	56.5	52.8	51.6	47.7	53.6 a
	'Line 9/14'	53.0	49.7	52.5	45.5	48.9	45.7	49.2 b
	'Lemnos'	50.0	47.7	52.7	48.3	45.5	44.8	48.2bcd
	Mean (G)	53 a	52 a	51.9 a	48.8 b	46 c	44.5 d	
5th day								Mean (C)
	'Gavdos'	56.8	57.0	50.8	48.5	45.4	47.5	50.9 cde
	'Keryneia'	55.8	52.8	53.5	50.3	39.8	37.9	48.3 f
	'Macarena'	50.8	54.3	49.3	46.5	38.3	38.2	46.2 g
	'CAT16-31'	57.8	61.2	59.5	53.7	50.6	51.8	55.7 b
	'CAT16-27'	55.5	53.3	50.8	44.5	46.0	46.8	49.4 ef
	'Thiva'	53.5	51.0	51.3	54.8	45.0	46.3	50.3 de
	'CAT16-4'	61.5	55.5	56.5	46.3	48.5	46.1	52.3 c
	'Sifnos'	63.3	60.3	58.5	59.5	53.4	52.6	57.8 a
	'Line 9/14'	56.5	55.5	54.5	51.0	48.0	45.6	51.9 cd
	'Lemnos'	54.8	54.0	54.0	47.7	51.5	44.9	51.1 cde
	Mean (G)	56.6 a	55.5 a	53.9 b	50.3 c	46.6 d	45.8 d	
7th day								Mean (C)
	'Gavdos'	56.3	58.5	50.5	46.5	47.0	47.6	51 cd
	'Keryneia'	58.8	58.3	57.8	57.0	40.6	39.0	51.9 bc
	'Macarena'	56.5	57.3	52.0	48.0	41.0	40.0	49.2 d
	'CAT16-31'	62.0	62.8	63.3	56.2	48.2	52.1	57.4 a
	'CAT16-27'	57.8	53.0	51.0	45.0	45.5	48.5	50.1 cd
	'Thiva'	52.5	52.5	52.8	53.8	41.2	43.9	49.4 d
	'CAT16-4'	62.8	57.5	60.5	49.3	45.7	45.0	53.4 b
	'Sifnos'	63.5	61.3	61.3	60.7	49.8	51.9	58 a
	'Line 9/14'	58.3	61.0	56.5	52.0	47.7	44.5	53.3 b
	'Lemnos'	53.3	52.0	51.7	43.2	47.7	46.1	48.9 d
	Mean (G)	58.2 a	57.4 a	55.7 b	51.2 c	45.4 d	45.9 d	

Note: At each time interval (days), means followed by the same letter, within each factor, are not significantly different according to LSD ($p \leq 0.05$).

Table 4. Root length (cm), as affected by genotype (G) and PEG concentration (C)

Time	Genotype (G)	PEG Concentration (%) (C)						
		0	5 (-0.05MPa)	10 (-0.15MPa)	20 (-0.5MPa)	30 (-1MPa)	50 (-2.7MPa)	Mean (C)
15th day	'Gavdos'	2.900	3.664	3.735	3.167	0.680	0.592	2.456 bc
	'Keryneia'	4.377	3.201	4.060	4.600	0.752	0.585	2.929 b
	'Macarena'	3.674	5.367	2.820	3.126	0.000	0.000	2.498 bc
	'CAT16-31'	2.987	3.237	3.615	2.958	0.030	0.000	2.138 c
	'CAT16-27'	2.695	1.500	1.385	0.000	0.935	0.000	1.086 d
	'Thiva'	4.767	5.127	5.262	7.455	1.025	0.000	3.94 a
	'CAT16-4'	3.487	4.558	3.035	1.327	1.015	0.000	2.237 c
	'Sifnos'	3.462	2.615	3.481	2.552	0.055	0.000	2.028 c
	'Line 9/14'	2.235	1.715	1.490	2.047	0.225	0.000	1.285 d
	'Lemnos'	2.335	1.975	4.020	0.295	2.482	0.253	1.893 c
	Mean (G)	3.292 a	3.296 a	3.29 a	2.753 b	0.72 c	0.143 d	

Note: At each time interval (days), means followed by the same letter, within each factor, are not significantly different according to LSD ($p \leq 0.05$)

Table 5. Shoot length (cm), as affected by genotype (G) and PEG concentration (C)

Time	Genotype (G)	PEG Concentration (C)						
		0	5 (-0.05MPa)	10 (-0.15MPa)	20 (-0.5MPa)	30 (-1MPa)	50 (-2.7MPa)	Mean (C)
15th day	'Gavdos'	1.765	4.183	3.918	0.584	0.000	0.000	1.742 b
	'Keryneia'	3.006	2.650	3.865	3.505	0.000	0.000	2.171 a
	'Macarena'	2.212	3.379	2.290	0.815	0.000	0.000	1.449 c
	'CAT16-31'	1.596	2.200	3.005	1.547	0.000	0.000	1.391cd
	'CAT16-27'	0.680	0.458	0.225	0.000	0.000	0.000	0.227 e
	'Thiva'	1.405	2.188	1.145	2.145	0.000	0.000	1.147cd
	'CAT16-4'	2.153	3.248	1.185	0.035	0.000	0.000	1.103 d
	'Sifnos'	0.895	0.438	0.560	0.530	0.000	0.000	0.404 e
	'Line 9/14'	1.033	1.093	0.600	0.200	0.000	0.000	0.487 e
	'Lemnos'	0.580	0.752	0.270	0.100	0.000	0.000	0.284 e
	Mean (G)	1.532 b	2.059 a	1.706 b	0.946 c	0.000 d	0.000 d	

Note: At each time interval (days), means followed by the same letter, within each factor, are not significantly different according to LSD ($p \leq 0.05$).

Table 6. Seedling vigor index (SVI) as affected by genotype (G) and PEG concentration (C)

Time	Genotype (G)	PEG Concentration (C)						
		0	5 (-0.05MPa)	10 (-0.15MPa)	20 (-0.5MPa)	30 (-1MPa)	50 (-2.7MPa)	Mean (C)
15th day	'Gavdos'	441.0	762.0	749.5	276.3	45.0	4.5	379.7b
	'Keryneia'	702.0	537.5	792.5	779.3	23.0	7.0	473.5 a
	'Macarena'	569.0	818.8	451.8	151.0	0,0	0.0	331.8 bc
	'CAT16-31'	395.0	492.0	623.2	404.5	0,0	0.0	319.1 bc
	'CAT16-27'	315.7	190.0	74.7	0,0	51.2	0.0	105.3 f
	'Thiva'	609.5	731.5	635.5	948.7	61.0	0.0	497.7 a
	'CAT16-4'	549.0	763.7	397.0	23.5	57.0	0.0	298.4 c
	'Sifnos'	424.5	288.8	355.7	262.0	0.0	0.0	221.8 d
	'Line 9/14'	301.7	246.8	185.2	153.2	10.3	0.0	149.5 ef
	'Lemnos'	280.0	238.5	333.8	30.7	201.5	1.5	181 de
	Mean (G)	458.7b	507 a	459.9 b	302,9 c	44.9 d	1.3 e	

Note: At each time interval (days), means followed by the same letter, within each factor, are not significantly different according to LSD ($p \leq 0.05$).

Table 7. Correlations between germination percentage and traits related to germination and early seedling growth

Traits and correlation		Germination	Seed weight	WU	WC	Shoot length	Root length
Germination	Pearson Correlation	1	-0.295	-0.095	0.386	0.359	0.667*
	Sig. (2-tailed)		0.408	0.795	0.271	0.308	0.035
	N	10	10	10	10	10	10
Seed weight	Pearson Correlation	-.295	1	-0.073	0.225	0.190	-0.001
	Sig. (2-tailed)	.408		0.842	0.532	0.598	0.998
	N	10	10	10	10	10	10
WU	Pearson Correlation	-0.095	-0.073	1	0.099	-0.046	-0.220
	Sig. (2-tailed)	0.795	0.842		0.785	0.900	0.542
	N	10	10	10	10	10	10
WC	Pearson Correlation	0.386	0.225	0.099	1	0.915**	0.447
	Sig. (2-tailed)	0.271	0.532	0.785		0.000	0.196
	N	10	10	10	10	10	10
Shoot length	Pearson Correlation	0.359	0.190	-0.046	0.915**	1	0.626
	Sig. (2-tailed)	0.308	0.598	0.900	0.000		0.053
	N	10	10	10	10	10	10
Root length	Pearson Correlation	0.667*	-0.001	-0.220	0.447	0.626	1
	Sig. (2-tailed)	0.035	0.998	0.542	0.196	0.053	
	N	10	10	10	10	10	10

* Correlation is significant at the 0.05 level (2-tailed).
** Correlation is significant at the 0.01 level (2-tailed).

Discussion

Drought is a major abiotic factor limiting plant growth, development and productivity in vast majority of crop species worldwide. Nearly one third of cultivated areas is subjected to water scarcity, while according to recent estimates, based on climatic projections, the unavailability of water resources is expected to intensify (Ceccareli *et al.*, 2010). Considering the obvious necessity to focus on breeding for drought tolerance, the identification of traits related to drought tolerance and water uptake (Valladares *et al.*, 2004) is becoming increasingly important. Germination as being one of the most critical stages, due to its correlation with seedling establishment and early growth (Kaydan and Yagmur, 2008), has been proposed as a suitable criterion for screening drought tolerance. In this framework, this study was aimed at determining the response of ten chickpea genotypes to PEG-induced drought stress at germination phase as an approach to select for drought tolerant genotypes.

As far as germination potential is concerned, our results are in accordance to those of relevant studies and provide further evidence that drought significantly affects germination, with the effects of stress being more severe as the intensity of stress increases (Jamaati-e-Somarin and Zabihi-e-Mahmoodabad, 2011; Wu *et al.*, 2011; Haouari *et al.*, 2013; Foti *et al.*, 2018). For chickpea, it has been proposed that the threshold osmotic potential is -0.8 MPa (Yucel *et al.*, 2010), while other studies report even lower threshold levels (Kalefetoglu *et al.*, 2009). Contrasting such reports, 70 % of genotypes employed in this study were able to germinate at -1 MPa, thus surpassing the osmotic potential threshold of -0.8 MPa. Further, a portion of them, namely 'Gavdos', 'Keryneia' and 'Lemnos', was capable of germinating, yet at low rates, under severe osmotic stress conditions (-2.7 MPa). It is worth noting however, that genotypes presented significant variations in relation to germination potential at different stress levels applied. In this line, 'Thiva' ranked at the top across treatments while, at the same time, exhibiting a high germination rate (> 99%) at 20% PEG, followed by a drastic decline at 30 % PEG and total repression of germination (0%) at 50% PEG. On the other hand, landrace 'Lemnos' exhibited a high germination rate (79%) at 30% PEG, which was pinpointed as the critical stress level where germination rate is most profoundly decreased, therefore providing evidence for its drought tolerance as well as for its possible exploitation as valuable genetic material for breeding activities.

Given that water availability affects germination potential (Hodge *et al*., 2009), via regulating the synthesis of hydrolytic enzymes involved in processes of seedling tissue synthesis and radicle elongation (Canas *et al*., 2006), WC and WU are directly affected by drought stress (Muscolo *et al*., 2014). Among genotypes with high germination rate, 'Keryneia' and 'Gavdos' presented the highest values for WC and WU, thus indicating a more efficient water uptake. However, landrace 'Lemnos' followed by 'Thiva', despite their high germination rates, ranked very low at WC and WU, therefore suggesting a different varietal pattern in terms of water demands to initiate the germination process.

In relation to traits related to post-germination, and in particular root and shoot length, their response differs substantially under drought stress, with shoot tissues being more susceptible to water deficiency (Okcu *et al*., 2005; Abd Allah *et al*., 2010). In our study, at 30% PEG-induced stress, all genotypes were incapable of shoot formation, whereas under the same stress conditions they managed to develop roots. Further, germination percentage showed positive correlation only with root length (r = 0.67*). Such findings are indicative of the fact that shoot length was more affected than root length and are in agreement with previous studies suggesting that the decreased osmotic potential leads to a more drastic inhibition of shoot tissue elongation (Kalefetoglu *et al*., 2009; Yucel *et al*., 2010). It is interesting that the majority of genotypes showed high length either for roots or shoots, with the exception of cultivars 'Thiva' and 'Keryneia' which exhibited high values both for shoot and root length. Consequently, 'Thiva' and 'Keryneia' ranked at the top in relation to SVI, followed by 'Gavdos'. In contrast, landrace 'Lemnos', which showed an increased germination potential, presented low SVI due to its low shoot and root elongation rate. Based on SVI, genotypes were classified in four categories: cultivars 'Thiva' and 'Keryneia' were characterised as drought tolerant (SVI > 450), 'Gavdos' as moderately tolerant (SVI: 350-450), 'CAT16-31', 'Macarena' and 'CAT16-4' as moderately susceptible (SVI: 250-350) and 'Sifnos', 'Lemnos', 'Line 9/14' and 'CAT16-27' as susceptible (SVI < 250).

In total, findings of this study underline that the determination of genotypic response to drought stress may be readily pursued at germination phase in chickpea and further indicate that the classification of genotypes in terms of drought tolerance may be performed on the basis of traits associated to seed germination and post-germination growth of seedlings. Given that previous studies have proven a good correlation between *in vitro* and field germination potential under drought stress in wheat, providing similar ranking for genotypic performance in *in vitro* and field conditions (Khakwani *et al*., 2011), such findings are of outmost importance. Upon verification of the fact that the deduced classification of genotypes is well correlated to their field performance under drought conditions, this approach may be exploited for selecting drought tolerant chickpea genotypes at early growth stages.

Conclusions

Overall findings revealed the existence of significant genetic variability in relation to drought tolerance among chickpea genotypes evaluated. Further, the results underline the fact that SVI may be employed as an appropriate criterion for screening drought tolerance in chickpea. To this respect, cultivars 'Thiva' and 'Keryneia', followed by 'Gavdos', exhibited superior performance in terms of drought tolerance, thus suggesting their possible use for cultivation in dry areas. The aforementioned varieties, along with landrace 'Lemnos', may further used in pure line selection or hybridization breeding programs targeted at the development of varieties suitable for areas where water scarcity is a major constraint.

Acknowledgements

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors. The authors wish to thank Dr. Dimitra Loka for her valuable comments on the manuscript.

Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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