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Response of Lentil Genotypes Under PEG-induced Drought Stress: Effect on Germination and Growth

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Abstract: Drought has a negative impact on plant growth and is responsible for considerable crop yield loses worldwide. Given the importance of improving yield under drought, the ability to select tolerant genetic material is a prerequisite in all relevant plant breeding activities. Lentil is an economically important crop which often suffers from inadequate soil moisture. In this study, seed germination potential and seedling growth were determined in various genotypes exposed to drought as a means to explore the possibility of identifying drought-tolerant germplasm at an early stage. Drought stress experiments were carried out using six lentil cultivars, representing local and imported germplasm. Stress was induced by varying concentrations of polyethylene glycol (PEG6000: 0%, 5%, 10% and 20%). Genotype performance was assessed on a daily basis and referred to germination percentage (%), seed water absorbance (%), seedling water content (%), shoot and root length (cm) and number of seedlings with abnormal genotype. Our findings revealed that drought stress substantially affects parameters associated to germination and growth, with its effect being analogous to the stress level applied. Genotypic differences also were evident, with cultivars Elpida, Samos and Thessalia proving as the most tolerant and cultivar Flip 03-24L as the least tolerant genotypes under severe drought stress. Overall findings provide evidence that identifying drought tolerant germplasm might be accomplished by scoring seed germination and early growth potential under water deficit conditions. Such possibility is of outmost importance for a time- and cost-efficient selection of drought tolerant lentil genotypes to be exploited in breeding programs.

Keywords: Abiotic Stress Tolerance, Drought, Lentil, PEG6000, Early Selection

1. Introduction

Lentil (*Lens culinaris* Medik.), originating from the near East and central Asia, is a prehistoric domesticated crop species that is mostly cultivated in the Mediterranean area [1]. Lentil is considered as one of the most important legumes in several countries worldwide, including Greece, as its seeds are a valuable protein source for human and animal consumption, whereas the entire plant biomass is further used as fodder for livestock [2, 3]. Moreover, its nitrogen fixing ability places lentil among the most suitable candidates for use in dryland cropping systems in rotation with cereals.

Its cultivation worldwide is mostly restricted to arid and semi-arid areas [4]. Drought reduces both lentil growth and yield as it affects major aspects of plant growth, development and metabolism [5-7]. Although irrigation has a positive impact on yield [8, 9], manifested as improved seed and biomass yield as well as harvest index [10, 11], restricting it to a minimum is crucial for a sustainable and economically viable crop production. To this end, the most practical means to cope with inadequate soil moisture is through the use of drought tolerant varieties. However, breeding efforts for the improvement of drought tolerance are seriously hampered by the polygenic nature of the trait, the wide environmental variation associated with it as well as the lack of suitable methods to efficiently select for the trait. Consequently, confidently identifying drought tolerant genetic material constitutes an area that certainly necessitates further research efforts.

Screening for genotypic tolerance traditionally relies on the estimation of yield reduction under drought stress, usually applied at plant's most critical growth stages.

However, such an approach is rather laborious and inefficient due to the difficulty encountered in achieving controlled drought stress in field conditions but also due to the exhausting number of genotypes required for the genetic improvement of such complex trait [12, 13]. Alternatively, screening for drought tolerance at germination phase has proven to provide an accurate estimation of yield and growth potential under inadequate soil moisture [14-16]. In this framework, the current study is aimed at determining seed germination potential of six lentil cultivars subjected to different levels of drought stress. Drought conditions were simulated by varying concentrations of polyethylene glycol (PEG), a molecule whose ability to mimic drought stress is well established [16-19]. The *in vitro* seed germination potential, as well as growth processes associated with it, was employed as a short-cut approach to identify and select drought-tolerant genotypes at early growth stages.

2. Materials and Methods

2.1. Plant Material

Drought stress experiments were carried out using six lentil genotypes, both of local and foreign origin, whose adaptation to suboptimal environmental conditions is not well established. More specifically, the local cultivars Thessalia, Athina, Samos and Elpida were conventionally bred at ELGO-"Demeter", Institute of Industrial and Fodder Plants, Larissa, Greece, whereas cultivars Flip03-24L and Flora were imported from ICARDA and France, respectively.

2.2. Stress Treatments and Experimental Design

Seeds were surface-sterilized for 5 min in 20% hypochlorite/H_2O solution supplemented with Tween-20, while gently mixing, and washed 4x with excess of sterile water. Drought stress was performed by decreasing the osmotic potential with the addition of PEG 6000. Sterilized seeds were allowed to germinate and grow in plastic trays containing different solutions: i) sterile distilled H_2O, ii) 5% PEG, iii) 10% PEG and iv) 20% PEG. Plants grown in dH_2O were included as controls. Trays were regularly monitored for the level of containing solution and, when necessary, H_2O was added in order to retain PEG concentration at constant levels. Plants were grown under controlled conditions (25°C, 16 h light/8 h dark) for a period of 18 days.

The experimental layout was that of a randomized complete block design with four replications, of 50 seeds each, for every genotype-stress level combination. The experimental plot (tray) consisted of four rows, with the two middle rows used to provide material for the measurements.

2.3. Measurements

Genotype performance under conditions of PEG-induced drought stress was assessed on a daily basis and referred to the following traits: germination percentage (%) at seven time intervals (1^{st} until 7^{th} day), seed water absorbance (WU) (%) (4^{th}, 6^{th} and 8^{th} day), seedling water content (WC) (%) (10^{th} and 17^{th} day), shoot and root length (cm) (5^{th}, 7^{th}, 9^{th}, 11^{th}, 13^{th} and 3^{rd}, 5^{th}, 7^{th}, 9^{th} and 11^{th} day respectively) and number of seedlings with abnormal genotype (1^{st} until 18^{th} day).

Seeds were considered germinated when the radicle reached a length of at least 2 mm. Seed water uptake (WU) was expressed as a percentage according to the formula

$$WU\ (\%) = (W2 - W1) / W1 \times 100 \quad (1)$$

where W1 = initial seed weight and W2 = seed weight after water absorbance [20]. For estimation of WU, the weight of twenty seeds (five from each replication) of each cultivar was taken into account. Seedling water content (WC) was expressed as a percentage according to the formula

$$WC\ (\%) = (FW\text{-}DW / FW) \times 100 \quad (2)$$

where FW = fresh weight, DW = dry weight [21].

For estimation of dry weight, twenty seedlings (five from each replication) of each cultivar were incubated at 70 °C for a period of 2 days.

2.4. Statistical Analysis

Data were treated by ANOVA according to the experimental design. The genotypes were compared within stress level applied at abovementioned time intervals. Comparisons were further conducted for genotypes across drought stress levels as well as for drought stress levels across genotypes. The significance of differences between pairs of means was assessed by the Student's LSD. All statistical analyses were performed using JMP statistical software v. 8.

3. Results

Overall findings revealed that drought stress substantially affects parameters associated to germination and early growth. As expected, the effect of drought in general was analogous to the level of stress induced, therefore leading to most profound effects at the high stress level for all traits under study.

Germination was considerably affected by the osmotic potential as well as by the genotype (Table 1). Germination of all cultivars commenced at the first day, while both control and stressed plants reached an almost 100% final germination at the seventh day. For the first four days, the germination rate was in most cases correlated with the level of stress applied, with the high stress level leading to significantly decreased germination. To the contrary, extending the stress period for three more days did not significantly alter germination percentage. Although severely affected at the first day of stress like all others, based on germination percentage at the highest level of stress during the first four days, Thessalia, Samos and Elpida appeared as the most tolerant cultivars. Such superiority was also adequately reflected in their mean response to all stress levels. At the other end, Flip 03-24L, although of lower germinability to start with, consistently proved the least tolerant during the entire period of observation. The germination rates of the six genotypes at each stress level are comparatively depicted in Figure 1.

Table 1. *Germination (%) as affected by genotype (G) and PEG concentration (C) at seven different time intervals (1st until 7th day) of drought stress.*

Time (d)	Genotype (G)	PEG concentration (%) (C)				
		0	5	10	20	
1th						Mean (G)
	Thessalia	85.75b	81.50a	80.00a	27.00bc	68.562b
	Athina	87.00ab	87.00a	78.00a	29.00ab	70.250ab
	Flora	82.00bc	77.50a	81.50a	48.00a	72.25a
	FLIP 03-24L	76.25c	75.50a	46.00b	9.00c	51.69b
	Samos	94.00a	83.50a	80.00a	29.50ab	71.75a
	Elpida	84.50b	71.50a	83.00a	36.00ab	68.75ab
	SED	3.39	8.22	11.23	9.29	SED (G) = 4.43
	Mean (C)	84.91a	79.41ab	74.75b	29.75c	SED (C) = 3.62
2th						Mean (G)
	Thessalia	96.00ab	94.50a	96.00a	92.00a	94.625a
	Athina	94.00b	94.00a	94.00a	84.00a	91.50ab
	Flora	86.00c	86.00ab	84.00ab	84.00a	85.00b
	FLIP 03-24L	85.75c	85.50ab	75.50b	46.00b	73.1875c
	Samos	99.00a	93.50ab	92.00a	81.00a	91.375ab
	Elpida	93.50b	84.50b	85.00ab	86.00a	87.25ab
	SED	1.81	4.36	6.06	7.14	SED (G) = 2.79
	Mean (C)	92.38a	89.99ab	87.75b	78,83c	SED (C) = 2.28
3th						Mean (G)
	Thessalia	97.50a	95.00a	95.00a	98.25a	96.4375a
	Athina	96.00ab	96.00a	95.00a	94.50a	95.375a
	Flora	92.50c	92.00a	90.00b	91.00a	91.375a
	FLIP 03-24L	93.25bc	92.50a	90.50b	66.50b	85.6875b
	Samos	99.00a	97.00a	96.00a	88.00a	95.00a
	Elpida	97.00a	92.00a	90.25b	94.50a	93.4375a
	SED	1.48	3.15	1.74	6.89	SED (G) = 1.97
	Mean (C)	95.87a	94.08a	92.79a	88.79b	SED (C) = 1.61
4th						Mean (G)
	Thessalia	98.50ab	99.00a	98.00a	98.00a	98.375a
	Athina	98.00ab	97.00ab	98.00a	98.50a	97.875a
	Flora	93.00c	92.00b	96.50a	91.50a	93.25b
	FLIP 03-24L	95.00bc	94.50ab	90.00b	79.50b	89.75c
	Samos	99.50a	98.50a	99.50a	94.00a	97.875a
	Elpida	97.00ab	95.00ab	98.00a	95.00a	96.25ab
	SED	1.08	2.50	1.51	3.53	SED (G) = 1.20
	Mean (C)	96.83a	96.00a	96.66a	92.75b	SED (C) = 0.98
5th						Mean (G)
	Thessalia	98.50a	99.50a	98.50a	98.00a	98.625a
	Athina	99.00a	93.50b	98.50a	98.00a	97.25ab
	Flora	97.00a	95.50ab	97.00a	93.00a	95.625b
	FLIP 03-24L	97.00a	96.50ab	92.50b	85.00b	92.75c
	Samos	100.00a	99.50a	99.50a	94.50a	98.375a
	Elpida	97.00a	96.50ab	98.00a	97.00a	97.125ab
	SED	0.96	2.20	1.26	2.86	SED (G) = 1.01
	Mean (C)	98.08a	96.83a	97.33a	94.25b	SED (C) = 0.92
6th						Mean (G)
	Thessalia	98.50ab	99.50a	98.50a	97.75a	98.5625a
	Athina	99.00ab	97.00ab	99.00a	95.50ab	97.625ab
	Flora	97.00bc	93.50b	97.00ab	91.00bc	94.625cd
	FLIP 03-24L	96.00c	95.50ab	92.50c	87.25c	92.8125d
	Samos	100.00a	99.50a	99.50a	94.00ab	98.25ab
	Elpida	99.00ab	96.50ab	95.00bc	95.00ab	96.375bc
	SED	0.77	2.30	1.24	2.99	SED (G) = 0.98
	Mean (C)	98.25a	96.91a	96.91a	93.41b	SED (C) = 0.80
7th						Mean (G)

Time (d)	Genotype (G)	PEG concentration (%) (C)				
		0	5	10	20	
	Thessalia	99.00ab	99.50a	98.50ab	98.00a	98.75a
	Athina	99.00ab	97.00ab	99.50ab	96.50ab	98.00a
	Flora	98.25ab	93.50b	97.50b	93.00bc	95.56b
	FLIP 03-24L	96.75b	96.00ab	92.50c	89.00c	93.56c
	Samos	100.00a	99.50a	100.00a	97.50ab	99.25a
	Elpida	98.75ab	96.50ab	98.50ab	98.00a	97.93a
	SED	0.90	2.30	1.09	2.32	SED (G) = 0.87
	Mean (C)	98.63a	97.00ab	97.75a	95.33b	SED (C) = 0.72

Note: At each time interval (days), means followed by the same letter, within each factor, are not significantly different according to LSD (p≤0.05). SED stands for Standard Error of the Difference between any two means in the group

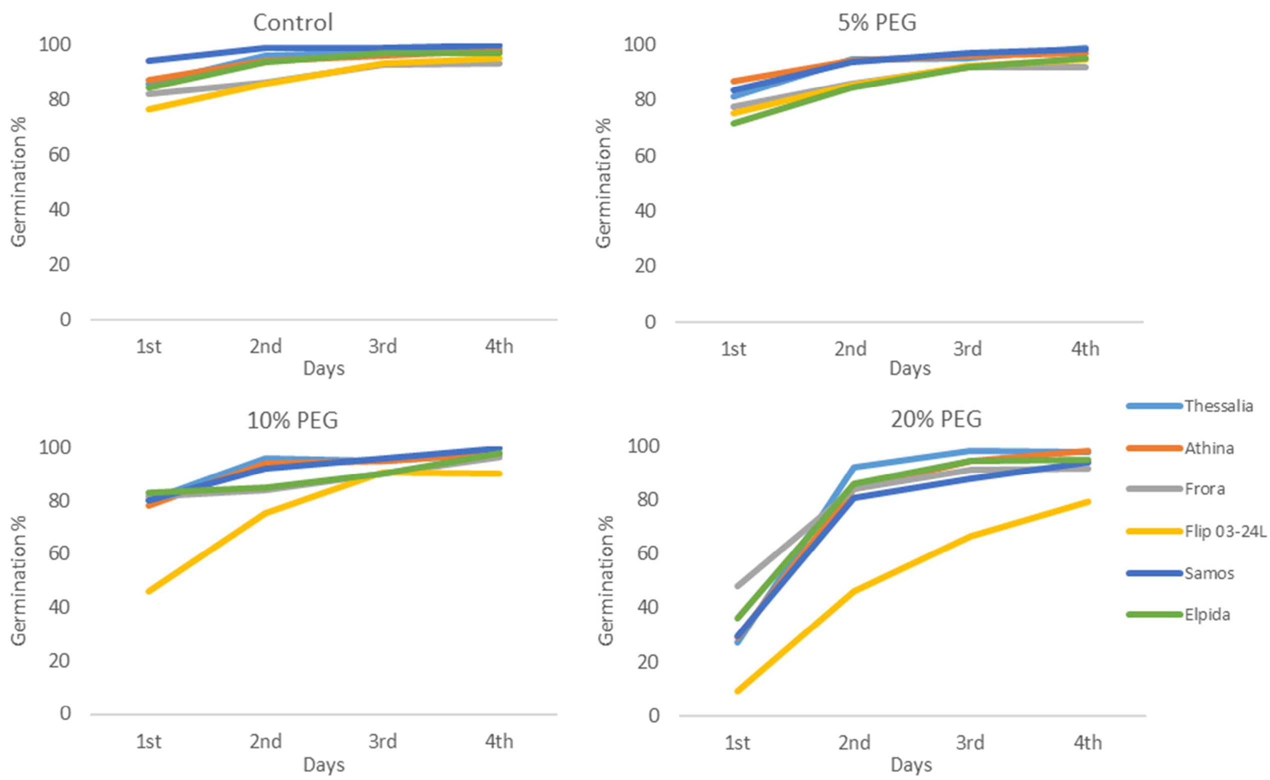

Figure 1. *Germination percentage (%) of six lentil cultivars at four different levels of salinity stress and four time intervals (1st until 4th day).*

As expected, both WU and WC increased over time in all cultivars and treatments. In all cases however, the results point to a decreasing trend of WU and WC upon stress, with their decrease being depended on the intensity of stress applied (Tables 2 and 3). At high stress levels, Thessalia and Flip 03-24L showed the lowest and highest decrease in WU respectively. In addition, the analysis revealed statistically significant differences in root and shoot length among cultivars and stress level (data not shown) as can also be seen in Figures 2 and 3. In general, the increased concentrations of PEG resulted in a significant reduction of length for both tissue types. Under conditions of low stress level (5% PEG) however, such reduction was in some cases not statistically significant. In contrast, the high level of stress resulted in a drastic reduction of both root and shoot elongation rate in all cultivars studied. At this stress level, the most significant reduction in root length was noted in Samos, while the lowest reduction was found in Elpida. As far as shoot length is concerned, the lowest reduction was observed in Elpida, whereas Flip 03-24L was the most severely affected cultivar. Despite its impact on several other traits, PEG-induced drought stress did not lead to the emergence of seedlings with abnormal phenotype.

Table 2. *Seed water absorbance (WU) (%) as affected by genotype (G) and PEG concentration (C) after 4, 6 and 8 days of drought stress.*

Time (d)	Genotype (G)	PEG concentration (%) (C)				
		0	5	10	20	
4th						Mean (G)
	Thessalia	65.47b	65.90b	63.07bc	59.00a	63.35a

Time (d)	Genotype (G)	PEG concentration (%) (C)				
		0	5	10	20	
	Athina	65.34b	61.59c	59.69d	59.73a	61.58ab
	Flora	68.53a	69.75a	66.40a	56.72a	65.35a
	FLIP 03-24L	69.03a	70.33a	64.30ab	56.41a	65.01a
	Samos	64.84b	63.32c	61.28cd	39.88b	57.32b
	Elpida	69.27a	67.52b	65.76a	57.45a	64.99a
	SED	1.37	1.02	1.03	2.63	SED (G) = 0.91
	Mean (C)	67.08a	66.40a	63.41b	54.86c	SED (C) = 2.55
6th						Mean (G)
	Thessalia	68.31d	69.68bc	63.92b	66.63b	67.13b
	Athina	69.71c	70.47abc	70.10a	65.27b	68.88ab
	Flora	72.98b	72.91a	66.61bc	70.36a	70.71a
	FLIP 03-24L	74.70a	71.94ab	65.32c	59.56c	67.87ab
	Samos	69.86c	69.93bc	69.74ab	54.88d	66.10b
	Elpida	66.85e	68.64c	69.09ab	63.10bc	66.92b
	SED	0.51	1.34	1.60	1.74	SED (G) = 0.68
	Mean (C)	70.40a	70.59a	67.46b	63.29c	SED (C) = 2.55
8th						Mean (G)
	Thessalia	75.71ab	75.92abc	74.43b	67.73b	73.44ab
	Athina	74.90ab	73.55c	73.62b	62.83c	71.22b
	Flora	77.17a	77.59a	78.42a	71.02a	76.05a
	FLIP 03-24L	77.47a	76.86ab	70.75c	65.65b	72.68b
	Samos	76.55ab	76.63ab	70.88c	67.09b	72.78ab
	Elpida	73.95b	74.49ab	71.14c	62.86c	70.60b
	SED	1.42	1.22	0.69	1.21	SED (G) = 0.65
	Mean (C)	75.95a	75.84a	73.20b	66.19c	SED (C) = 0.53

Note: At each time interval (days), means followed by the same letter, within each factor, are not significantly different according to LSD (p≤0.05). SED stands for Standard Error of the Difference of any two means in the group

Table 3. *Water content (WC) (%) of lentil seedlings as affected by genotype (G) and PEG concentration (C) after 10 and 17 days of drought stress.*

Time (d)	Genotype (G)	PEG concentration (%) (C)				
		0	5	10	20	
10th						Mean (G)
	Thessalia	94.58b	93.11c	92.27c	93.35bc	93.32c
	Athina	96.21ab	94.79b	94.58b	93.79b	94.84b
	Flora	97.57a	97.44a	96.43a	96.88a	97.07a
	FLIP 03-24L	94.62b	93.91bc	94.01b	91.70c	93.55c
	Samos	94.54b	93.53bc	93.59bc	93.02bc	93.66c
	Elpida	92.03c	90.83d	90.46d	88.82d	90.53d
	SED	0.77	0.50	0.47	0.68	SED (G) = 0.30
	Mean (C)	94.92a	93.93b	93.55b	92.92c	SED (C) = 0.24
17th						Mean (G)
	Thessalia	96.44b	95.82b	94.79b	92.84c	94.97d
	Athina	97.05b	97.03b	95.92b	94.67b	96.17b
	Flora	98.86a	98.92a	98.55a	96.81a	98.28a
	FLIP 03-24L	96.76b	96.07b	95.17b	94.56bc	95.64c
	Samos	97.06b	96.02b	95.47b	94.47bc	95.75bc
	Elpida	94.51c	93.93c	91.08c	91.06d	92.64e
	SED	0.57	0.37	0.40	0.58	SED (G) = 0.24
	Mean (C)	96.78a	96.29b	95.16c	94.06d	SED (C) = 0.20

Note: At each time interval (days), means followed by the same letter, within each factor, are not significantly different according to LSD (p≤0.05). SED stands for Standard Error of the Difference of any two means in the group

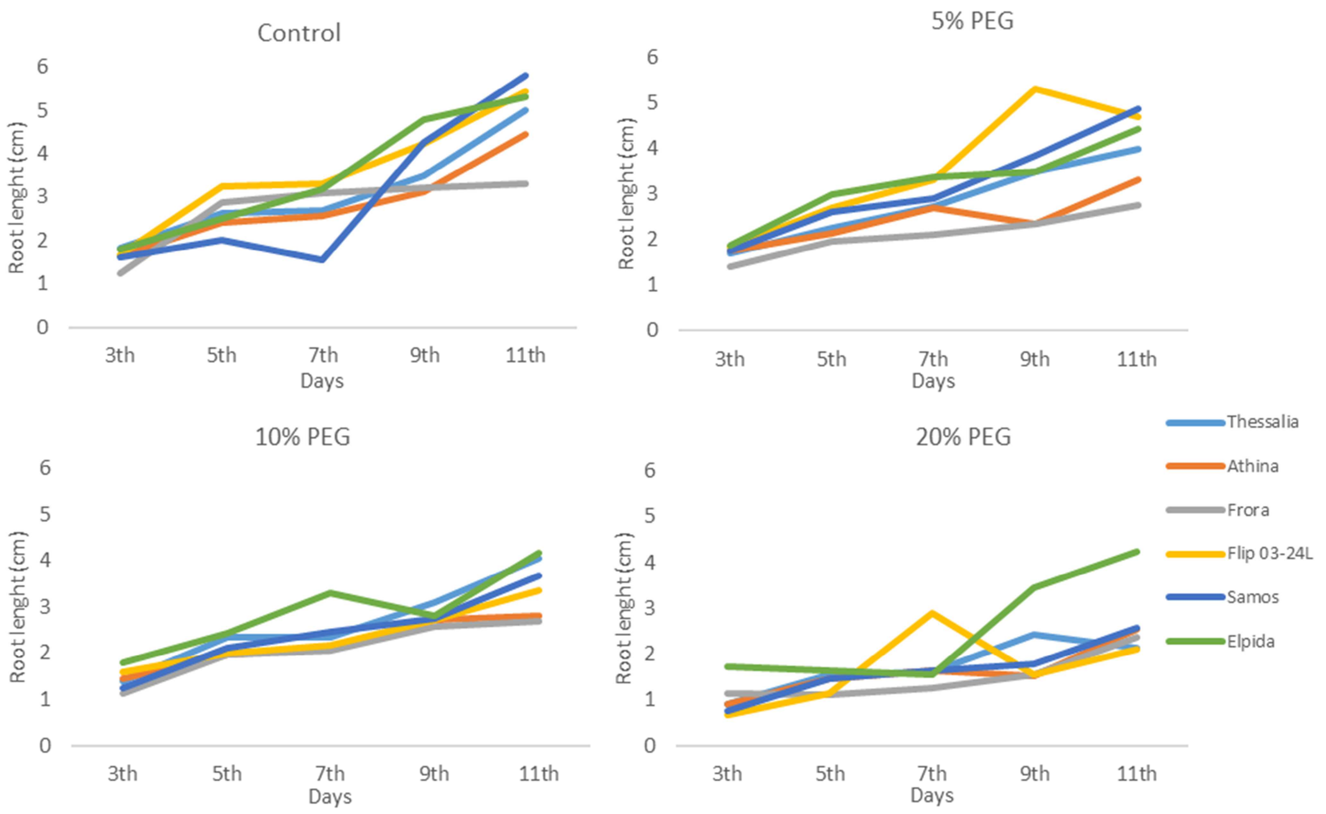

Figure 2. *Root length (cm) of six lentil cultivars at four levels of PEG-induced drought stress and five time intervals (3th, 5th, 7th, 9th and 11th day).*

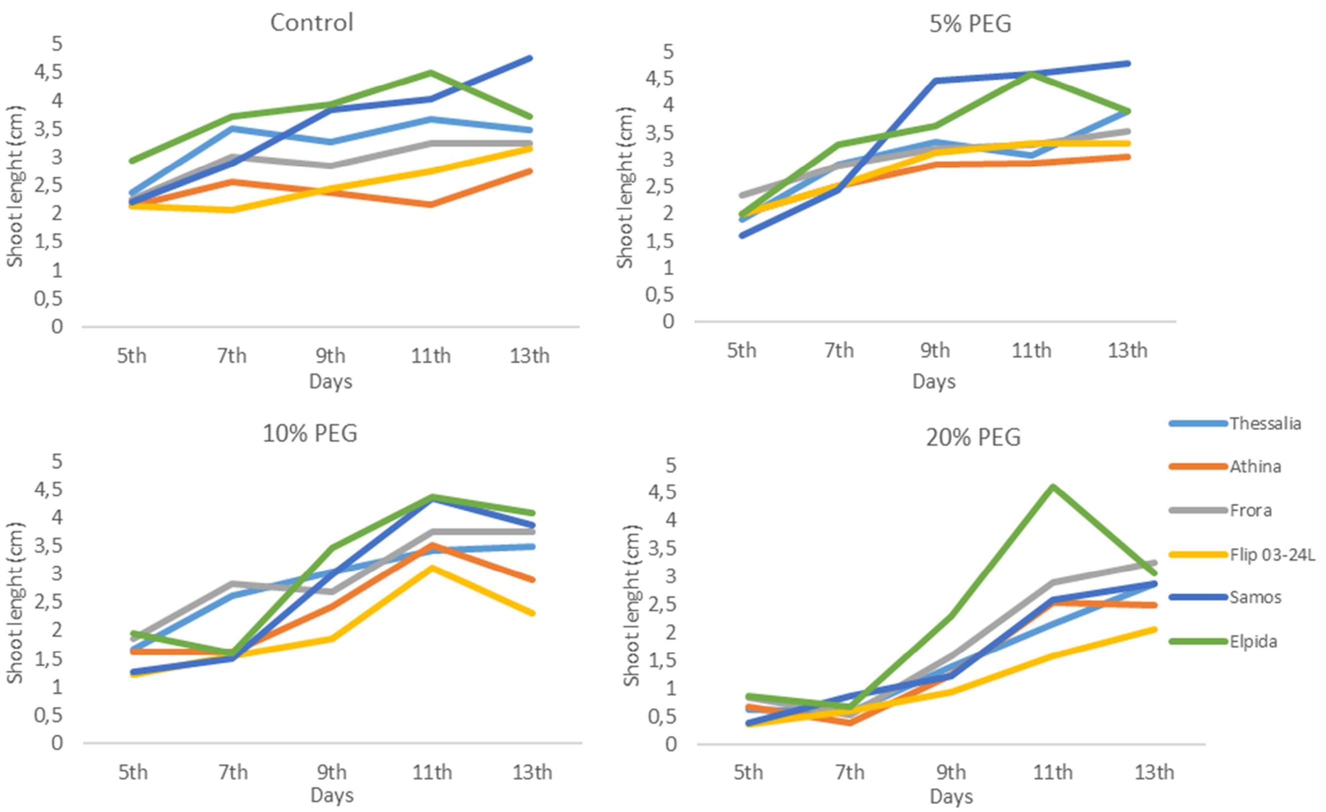

Figure 3. *Shoot length (cm) of six lentil cultivars at four levels of PEG-induced drought stress and five time intervals (5th, 7th, 9th, 11th, 13th day).*

4. Discussion

Although an autumn crop, lentil often suffers from the lack of adequate soil moisture due to its cultivation in arid and semi-arid zones but also due to climate changes and insufficient rainfall in early spring thereof. Given that drought negatively affects major aspects of growth and productivity, the development of drought tolerant varieties is the most sustainable and economical approach to maintain sufficient yield under suboptimal moisture conditions. The success in such an endeavour however is strongly depended on the availability of selection methodologies for an unambiguous screening of drought tolerant genotypes. Given that germination phase is most critical for plant lifecycle, seed germination potential in water deficit stress has proven as a valuable parameter in estimating genetic tolerance to drought [14, 15, 22, 23]. In this study, we pursued the evaluation of six lentil cultivars, subjected to different levels of PEG-induced drought stress, on the basis of seed germination potential and other traits associated to germination and early growth.

In general, findings point to the conclusion that drought stress substantially affects parameters associated to germination and seedling development, with the effect of drought being in most cases analogous to the level of stress induced. These results are consistent with those of relevant studies [7, 11, 24, 25] and provide further evidence for the suitability of PEG for inducing drought stress in lentil. Germination percentage is generally considered as an indicative criterion for proper seedling establishment as well as yield and growth potential under conditions of water deficiency [15]. Although at the end of the observation period the germination percentage reached almost 100% for all genotypes under stress, germination was considerably affected by the level of stress when examining the first four days. The most severe effect in germination rate was recorded in cultivar Flip 03-24L, whereas Thessalia, Samos and Elpida manifested the higher values for this trait. Seed water absorbance is a trait highly related to germination as it involves the activation of enzymes that stimulate hydrolysis of starch reserves into sugars which serve as energy source for radicle emergence and tissue elongation [26-28]. In accordance with seed germination data, WU presented a trend of reduction which was inversely related to the level of stress applied. In this regard, at high stress level Thessalia and Flip 03-24L were the best and worst performing cultivars respectively as far as WU is concerned.

Following germination phase, during which radicle emergence is included, growth of shoot and root axis are considered as important traits for estimating tolerance to drought [7]. In relation to elongation rate of roots and shoots, the results indicate that PEG-induced drought stress negatively affects length of both tissue types. The observed length reduction however was often negligible at low stress level and became more drastic at high level of stress. Most severe reduction was observed in cultivars Samos and Flip 03-24L for root and shoot length respectively, indicating their sensitivity which is most probably attributed to a reduced rate of cell division. In contrast, the lowest reduction in root and shoot length of Elpida is indicative of its better tolerating ability under water deficit conditions. The observed association between shoot and root length has been also reported in previous studies and has been proposed to allow for an indirect selection for underground traits (root growth) that are more difficult to measure [7]. As far as WC is concerned, the results point to a decreasing trend upon increased level of stress. However, the results for this trait do not provide the possibility to accurately classify cultivars according to their performance.

Overall results support the conclusion that cultivars Elpida, Samos and Thessalia are mostly capable of resisting inhibition of germination and early development under water deficit conditions. It is worth noting that previous studies focusing on the assessment of cultivar performance under conditions of salinity stress, classified Elpida and Thessalia as salt sensitive cultivars [29]. To the contrary, cultivar Samos, whose ability to withstand severe salinity has been previously established [29], showed an analogous tolerance to drought. Such differential response to drought and salinity has been also reported in previous studies [11, 30] and is most probably indicative of the fact that the mechanisms underlying resistance to drought and salinity in lentil are distinct. Indeed, recent studies on lentil accessions subjected to drought and salinity revealed a differential metabolic response, which is attributed to the induction of distinct mechanisms underlying the observed genotypic variation. In this regard, the study pointed ornithine and asparagine and alanine and homoserine as stress-specific biomarkers for drought and salinity stress respectively [31].

Moreover, this study highlights the possibility of using this methodology for the selection of drought tolerant material at early growth stages employing high level drought stress (PEG: 20%) and preferably combining it with WU data. Obviously however, further validation and correlation with yield performance under conditions of water deficit in the field is a prerequisite in order to assure robustness of such data. At this point, it is important to mention that the genotypes tested were well adapted in drought conditions as having been extensively selected and grown in relevant environments. It is therefore well justified to expect that early breeding germplasm would manifest greater genetic variability for the trait of drought tolerance and therefore such selection methodology should prove quite useful.

In view of the fact that plants' response to environmental stimuli may be dealt with a different perspective at the post-genomic era, the efficacy of early selection procedures may be further strengthened by the development of functional markers (i.e. specific metabolic compounds and genes), whose expression is linked to tolerance. This approach is anticipated to enable the reliable identification of drought-tolerant genotypes to meet current and future challenges.

5. Conclusions

In total, findings underline the superiority of Elpida, Samos and Thessalia in tolerating drought stress, thus providing evidence for its possible exploitation either for direct cultivation under water deficit conditions or for use as tolerant breeding germplasm. Furthermore, overall findings support the conclusion that the identification and selection of drought tolerant lentil germplasm may be readily pursued through the determination of seed germination and early growth potential under stress conditions. Provided that the observed tolerance is further validated under field drought conditions, such short-cut screening approach allows for a time- and cost-efficient selection of suitable germplasm material to be exploited in relative breeding programs.

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