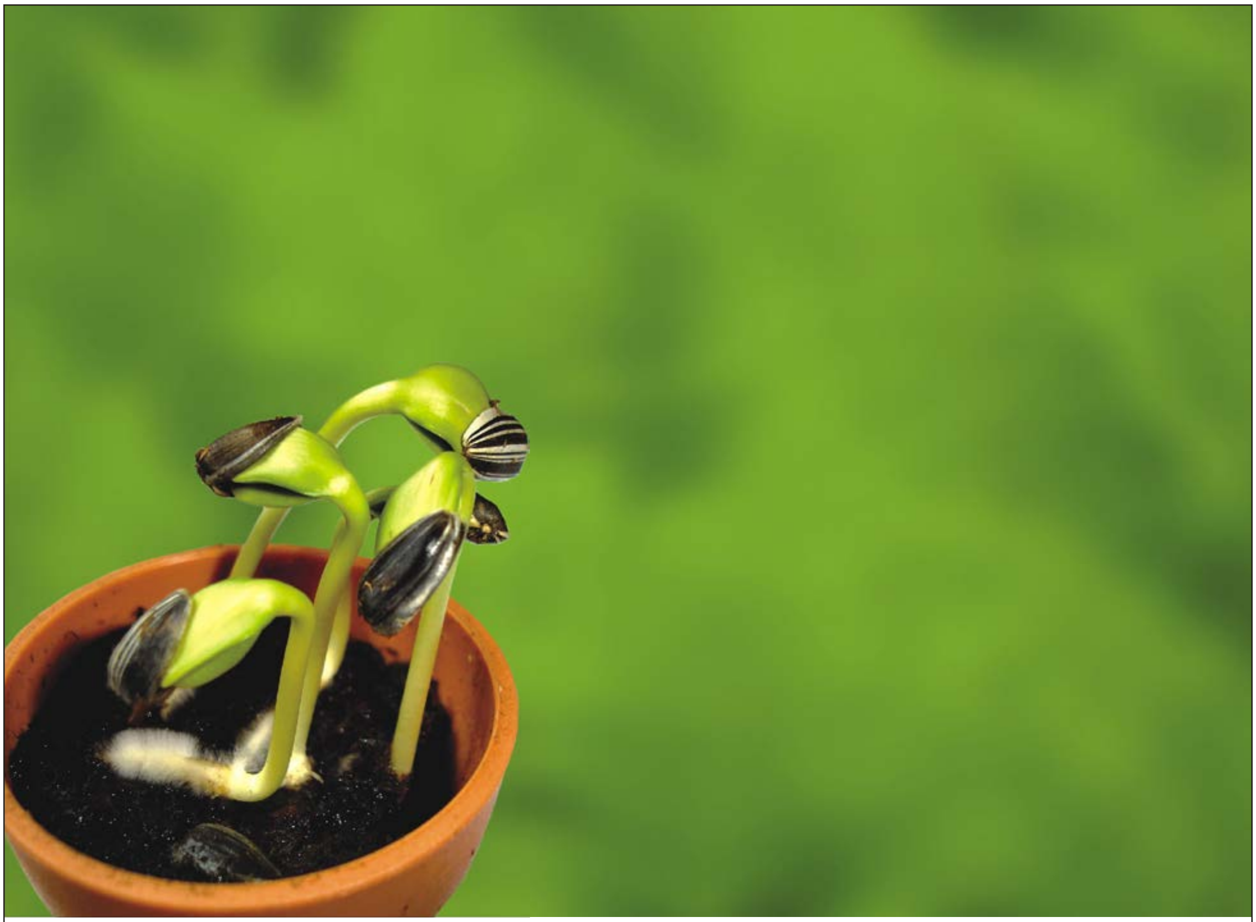

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Research Article
Effect of Water Deficit during Germination and Flowering Period of Grass Pea (*Lathyrus sativus* L.)

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Abstract

Background and Objective: Grass pea (*Lathyrus sativus* L.) is a multipurpose grain legume with an indeterminate growth habit. The objective of this study was to identify adaptation mechanisms of grass pea to water deficits. **Materials and Methods:** In the germination experiment, the used osmotic potential were: 0 (distilled water); -0.03, -0.1, -0.7 and -1.0 MPa, osmotic shock induced by increasing concentrations of polyethylene glycol (PEG). After treatment for 7 days, germination percentage, root and shoot length, seedling fresh and dry weight were measured. In the second experiment, water deficit was imposed on plants of grass pea by withholding water from first flowering. **Results:** Seeds of *L. sativus* were able to germinate at all concentrations of PEG treatments. Higher concentrations of PEG (-1.0 MPa) were less inhibitor effect on seedling shoot and root elongation. This study showed that under water deficit, grass pea avoids dehydration through a reduction in leaf area and fresh biomass. Water deficit reduced dry matter, seed yield, seed number, seed size, flowers number and harvest index, respectively, when compared with the controls. **Conclusion:** Conclusively, grass pea showed a better germination under osmotic stress; therefore, it seems to be a suitable candidate for breeding programs.

Key words: *Lathyrus sativus* L., germination, polyethylene glycol, osmotic potential, water deficit

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The environmental stress such as drought is serious obstacle for horticulture and field crops in all around the world, especially in arid and semiarid regions. Drought is considered as a worldwide problem currently which is expected to elaborate in the near future[1]. Environments with water restriction and salt excess produce harmful effects on seeds, such as a significant reduction in the germination percentage and rate in the root length and fresh mass and in the shoot of seedlings[2]; they may even cause a complete inhibition of the germination process of seeds[3]. In plant production, the most important factors limiting crop productivity are environmental stresses. Disruption of plant water management caused by drought, salinity or low temperature is a major yield decreasing factor[4]. The use of plants that are more resistant to environmental stresses, particularly in the germination and early seedling growth stage is gaining importance day by day[5]. Several *Lathyrus* species and in particular *Lathyrus sativus* (grass pea) have great agronomic potential as grain and forage legume, especially in drought conditions[6]. It can survive harsh environmental conditions, making the crop suitable for drought prone areas[7]. It is superior in yield, protein value, nitrogen fixation and drought, flood as well as salinity tolerance than other legume crops. Grass pea not only has potential as an agriculturally important crop for animal feed and human food, it can also be useful for studies of plant drought resistance[8].

In Tunisia, the genus *Lathyrus* is cultivated mainly for grain production (food) and also used as fodder (animal feed) by farmers. Grass pea (*Lathyrus sativus* L.) is a legume crop with a unique adaptation features to abiotic stresses such as drought or poor soil conditions[6] and as a result is considered a promising source of genes of interest. It is one of the relatively infrequent grain legumes. Also, high protein content makes this species interesting as a forage crop[9-11]. Although rich in protein, the utilization of grass pea grain is limited by the presence of water soluble, non-protein amino acid a-N-oxalyl diaminopropionic acid (a-ODAP) which causes lathyrism in animal[12].

While grass pea crops are known to be well adapted to drought but details of growth and yield responses to water deficit during germination and the reproductive period are not fully understood. The objective of this study was to determine the effect of water stress on germination and during reproductive period as well as on some morpho-physiological parameters of *Lathyrus sativus* due to osmotic effect and to optimize the best priming treatment for these stress conditions.

MATERIALS AND METHODS

Design of simulated drought conditions: Water stress conditions were simulated by polyethylene glycol-6000 (PEG) at one of four concentrations. Table 1 shows how osmotic potential decreases with increasing PEG-6000 concentration:

$$OP = (-1.18\times10^{-2})^{-}C-(1.18\times10^{-4})\times C+(2.67\times10^{-4})\times C\times T+(8.39\times10^{-7})\times C^{2}T$$

Where:

C = PEG concentration

T = Temperature

Plant materials and growing conditions: In the first experiment, to determine the tolerance of the germination to the water stress, the seeds of grass pea were imbibed in the distilled water and in solutions of Polyethylene Glycol (PEG-6000). For each experiment, 20 seeds were soaked in PEG 6000 solution of different water potentials (-1.0, -0.7, -0.1 and -0.03 MPa) were prepared. The number of seeds germinated was counted daily for 7 days. Distilled water was used as a control (0 MPa). The PEG solutions were prepared dissolving different concentrations of PEG 6000 in deionized water. Seeds were germinated at a constant temperature of 25±1°C, in a controlled chamber. Samples of 80 seeds (4 replicates of 20 seeds each) were placed in Petri dishes containing a single filter paper moistened with 10 mL of PEG solution. After treatment for 7 days, all grass pea seedlings were sampled. Germination percentage, root and shoot length, water content, seedling fresh and dry weight and content of sugar were measured in the study.

In the second experiment, growth conditions and stress treatments of grass pea (*Lathyrus sativus* L.) were conducted in a greenhouse at the Experimental Station of INRAT in Tunisia (35°87 N, 9°96 E) in January, 2018. When plants reached flowering, drought was imposed to stress pots by withholding water for a week, while non-stressed plots continued receiving irrigation. The experiment design was split-plot with 6 replications.

Measurement of germination and agronomic parameters

Experiment 1: Germination: The seedling fresh and dry weight expressed as mg/plant and length of root and

Table 1: Amounts of polyethylene glycol used for producing of different levels of osmotic stresses

Water stress (Mpa)	0	-0.03	-0.1	-0.7	-1.0
*Amount of PEG 6000 (g PEG/l H_2O)	0	5.3	15.3	63.9	80.3

*According to Michel and Kaufmann[13] at temperature of 25°C shoot (mm) were determined after 7th day of germination test in all the germinated seedlings. The seedling dry weight was determined after drying at 80°C for 24 h.

The germination process was evaluated during 7 days; germinated seeds were counted every day (February, 2018):

- The final Germination Percentage (GP) was calculated in the 7th day by using the following equation:

$$GP = \frac{\text{Total number of germinated seeds}}{\text{Total seed}} \times 100$$

- Fresh weight of seedling after 24, 42, 48 and 92 h at different osmotic potentials
- Dry weight of hypocotyl and radicle
- Radicle length and hypocotyl length of seedling
- Water content (WC) at different osmotic potentials, the WC percentage was calculated by using the equation[14]:

$$100 \times \frac{FW - DW}{FW}$$

- **Determinations of total sugars:** Total sugars were determined by phenol-sulfuric acid reaction, the analysis consisted in addiction of 2.5 mL of sulfuric acid and 0.5 mL 5% (w v-1) phenol solution over 0.5 mL of diluted sample. Afterwards, it was kept at room temperature for 20 min. After the reaction time, the optical density was determined at 480 nm in spectrophotometer[15]

Experiment 2: Flowering period: In the second experiment, observations on yield and yield attributing characters: Plant height (cm), number of pods per plant, number of seeds per pod, number of seeds per plant, fresh weight of biomass per plant (g), dry weight of biomass per plant (g), number of flowers per plant were recorded in May, 2018.

Statistical analysis: The data was statistically tested by a one-way ANOVA followed by a Tukey's test. The analysis of the significance of difference for each parameter was performed on the basis of mean values and a level of significance at $p \leq 0.05$. Each parameter was measured in at least five reiterations in control, PEG treatment and water deficit groups.

RESULTS

Germination of grass pea at different osmotic potentials of PEG

Seed germination: The result showed that germination percentage was not significantly decreased by PEG solutions. Contrary, it increased considerably with decrease of osmotic potential. The maximum of germination was obtained after 3 days in all treatments. Decreasing osmotic potential from -0.7 to -1.0 MPa in the media caused a significant reduction in germination percentage. Seed germination was 96% under control and 84% under stress conditions (Fig. 1).

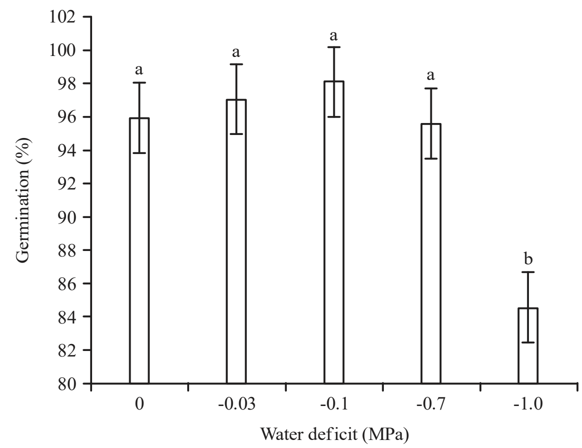

Fig. 1: Germination percentages of grass pea at different osmotic potentials of PEG

Values are Mean±SE (n = 5), one-way ANOVA was used to test differences among treatments, different letters indicate significant differences among concentrations of PEG 6000 at $p<0.05$ (Tukey's test), control treatment was distilled water

Seedling fresh weight: Decreasing water potential by PEG concentration caused a reduction in seedling fresh weight. Differences determined among the treatments were significant. When early seedlings were exposed 24 h from water stress to optimal conditions (-1.0 MPa), they were accompanied by a decrease in fresh weight of about 0.12 mg (from 0.36-0.24 mg) (Fig. 2).

Seedling dry weight: Dry weights of hypocotyl and radicle of *L. sativus* grown in different osmotic potential are presented in Fig. 3. Generally, drought conditions generated by PEG significantly influenced dry weight of radicle and hypocotyl of grass pea. The highest and lowest dry weight were observed at control treatment and at -1.0 MPa (Fig. 3). However, at the different osmotic potential the dry weight deceased significantly with increasing drought levels ($p<0.05$) compared to the control (Fig. 3).

Water content: The water content of *Lathyrus* shoots decreased under osmotic stress (Fig. 4). A significant reduction in water content of grass pea was observed at lower osmotic potential (-1.0 MPa), as far as in the experiments with different osmotic potentials of PEG (Fig. 4).

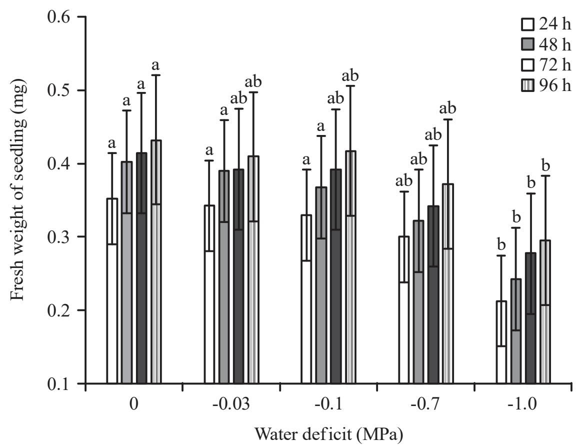

Fig. 2: Seedling fresh weight of field pea at different osmotic potentials of PEG

Values are Mean±SE (n = 5), one-way ANOVA was used to test differences among treatments, different letters indicate significant differences among concentrations of PEG 6000 at p<0.05 (Tukey's test), control treatment was distilled water

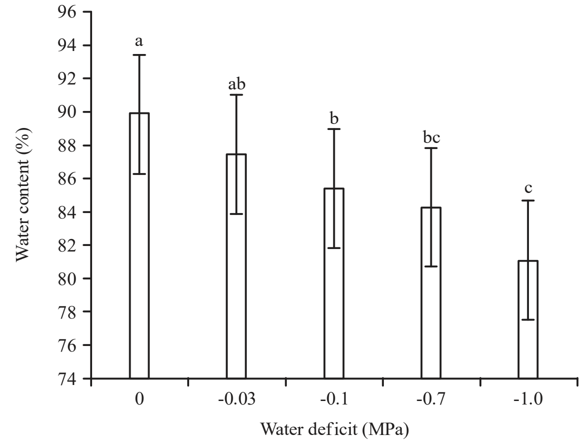

Fig. 4: Water content of grass pea at different osmotic potentials of PEG

Values are Mean±SE (n = 5), one-way ANOVA was used to test differences among treatments, different letters indicate significant differences among concentrations of PEG 6000 at p<0.05 (Tukey's test), control treatment was distilled water

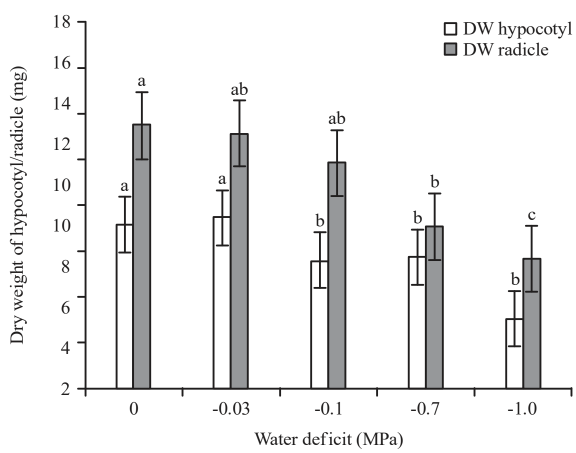

Fig. 3: Dry weight of radicle and hypocotyl of grass pea at different osmotic potentials of PEG

Values are Mean±SE (n = 5), one-way ANOVA was used to test differences among treatments, different letters indicate significant differences among concentrations of PEG 6000 at p<0.05 (Tukey's test), control treatment was distilled water

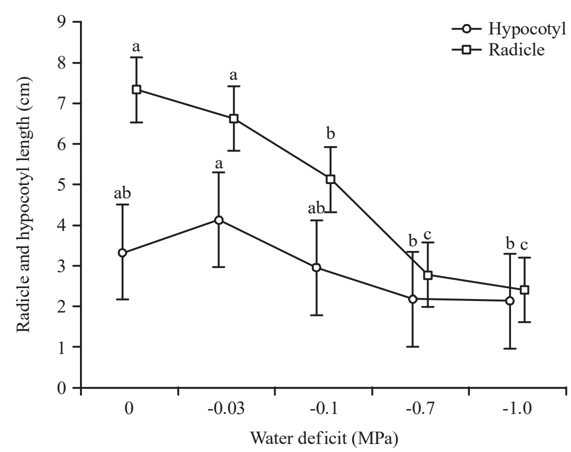

Fig. 5: Length of hypocotyl and radicle of grass pea at different osmotic potentials of PEG

Values are Mean±SE (n = 5), one-way ANOVA was used to test differences among treatments, different letters indicate significant differences among concentrations of PEG

Length of hypocotyl and radical: For the length of hypocotyl and radicle of grass pea at different osmotic potentials, the solutions of -0.7 and -1.0 MPa had a stronger negative effect than the -0.03 and -0.1 MPa solutions (Fig. 5). The highest radicle was recorded in control (7.3 cm), whereas the lowest one was noted at -1.0 MPa (2.9 cm) (Fig. 5).

Content of sugar: The results show that, along with a decrease in osmotic potential, the accumulation of sugar increased significantly in both the radicle and the hypocotyl of *L. sativus* (Fig. 6). The contents of sugar increased with decreasing of osmotic potential with that in the radicle being significantly higher than that in the hypocotyls. The maximum content of sugar in hypocotyl of grass pea seedling was 5.2 μmol g^{-1} DW observed at -1.0 MPa compared to the minimum 3.5 μmol g^{-1} DW at control. Decreasing of osmotic potential affected the content of sugar in radicle, the maximum was 15.2 μmol g^{-1} DW at -0.7 MPa treatment and the lowest was 5.8 μmol g^{-1} DW at control (Fig. 6).

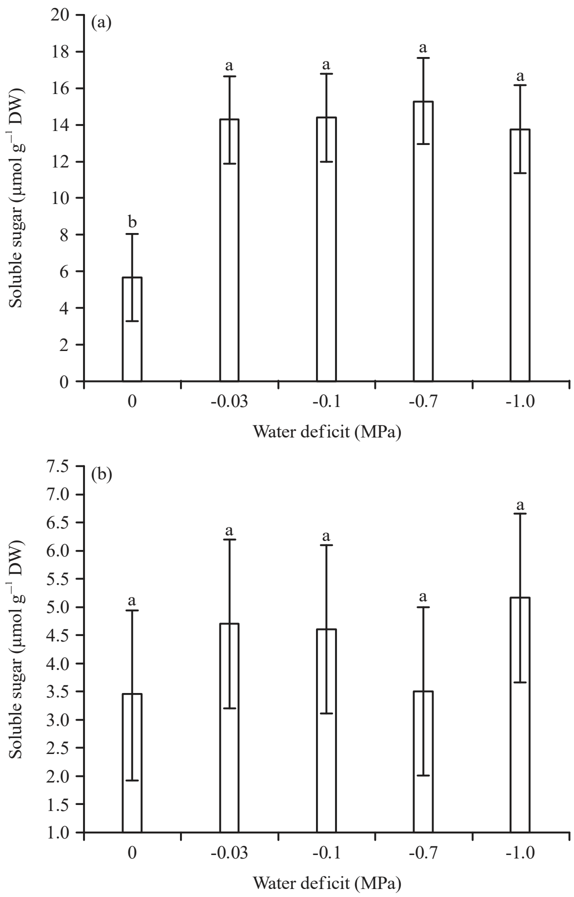

Fig. 6(a-b): Effects of PEG induced water deficit on the content of sugar in (a) Radicle and (b) Hypocotyl of grass pea seedling

Values are the means of 5 replicates means followed by different letters are significantly different at p≤0.05 according to Duncan's method

Table 2: Effect of water deficit on yield, harvest index and yield components in *Lathyrus sativus*

	Treatments		
Components	Control	Stressed	LSD (p<0.05)
DM (g plant^{-1})	1.34^{a}	0.82^{b}	0.001*
Seed yield (g plant^{-1})	1.07^{a}	0.50^{b}	0.007*
Pod number (plant^{-1})	5.10^{a}	2.40^{b}	0.001*
Pod size (cm)	3.52^{a}	2.45^{b}	0.001*
Seed number (pod^{-1})	2.70^{a}	1.70^{b}	0.004*
Seed number (plant^{-1})	10.20^{a}	3.90^{b}	0.000*
Flowers number (plant^{-1})	3.44^{a}	1.01^{b}	0.011*
Harvest index	0.80^{a}	0.61^{b}	0.004*

*Significant at 5%, values are means (n = 8)

Effect of drought during flowering stage: Generally, drought conditions generated by withholding water significantly influenced the vigor of grass pea plants. However, the water deficit caused a significant decrease in plant height, number of branches and leaf area of *Lathyrus sativus* (Fig. 7). The highest plant was obtained at control with 66 cm compared to stressed plant with 45 cm. Contrary, application of water deficit stress during the flowering stage increased sharply root length of grass pea plants about 28 cm compared to their respective controls with 18 cm. The main effect of drought was significant for number of branches and leaf area by reduction of the mentioned traits (Fig. 7). Water deficit decreased flower production by 70 % compared with the control (Table 2). Under stress conditions, the harvest index was 0.61 instead of 0.80 in the control treatment.

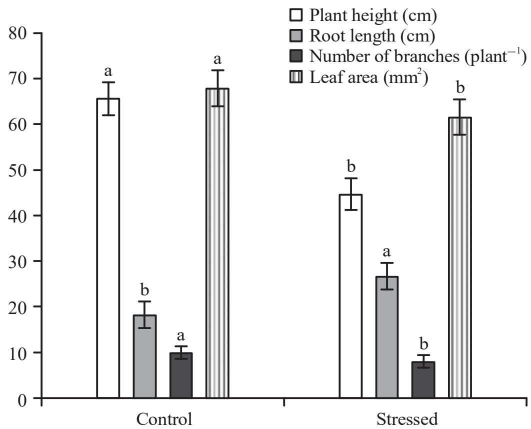

Fig. 7: Variation of plant height, root length, number of branches and leaf area of *Lathyrus sativus* with the water deficit

Different letters indicate significant difference among levels of nitrogen treatment at p<0.05

DISCUSSION

In this study, all determined germination and seedling parameters (fresh and dry weight, radical length, hypocotyl length and water content) decreased significantly in response to water stress with PEG of grass pea. Seed germination and early seedling growth were potentially the most critical stages for water stress[16]. The results showed clearly that the pea seed germination and seedling growth were inhibited by osmotic effect during the seedling development. Contrary, Piwowarczyk *et al.*[17] found that osmotic stress had no influence on seed germination of any of the accessions of *Lathyrus* tested. The depressive effects of an increased water deficit upon germination percentage and following seedling emergence have been extensively shown in many crops[18-20]. Jiang *et al.*[21] showed that grass pea compared to the common garden pea has better adaptation to drought stress. This is a common behavior of plants and in fact, these early phases of the life cycle are generally the most sensitive to stress[19,22,23].

Concentrations of total soluble sugars increased sharply in PEG-treated grass pea seedling compared to their respective controls, which was consistent with some plants under water stress such as maize (*Zea mays* L.)[24] and wheat (*Triticum aestivum* L.)[25]. The significant reduction of pod number and the growth inhibition of *L. sativus* caused by water deficit was due to interference with plant growth processes like cell division and cell enlargement, inhibition in nutrient uptake, reduction in dry matter production due to inhibition of metabolic processes such as photosynthesis and respiration[26]. The sensitivity of flowers and pods to water deficit varies between species and genotypes as well as growth environment[27]. Water deficit reduced flower production in an indeterminate soybean by reducing node numbers[28,29]. Mirzaei *et al*.[30] reported that whatever drought stress is closer to the pod formation stage, its effect will be higher on the number of pods and results showed that part of the yield loss in stress conditions is related to the reduction of number of seeds per pods and number pods. Usually, when water deficit stress is applied after the flowering stage, it causes the reduction of pod number per plant by shortening the flowering period, the reproductive growth duration and finally their fertility of some flowers and their abscission[31]. Pandey *et al*.[32] showed that during water deficit stress at flowering stage, the seed yield decreases due to reducing seeds weights. Moreover, Kage *et al*.[33] demonstrated that dry matter partitioning and biomass disposition are strongly connected with plant productivity under drought stress conditions. Decrease of dry matter was noted in many plant species under drought conditions[19,34]. For this reason, *L. sativus* is gaining interest as grain legume crops in Mediterranean-type environments. However, grass pea as highly resistant crops may serve as potential sources of new and useful genetic traits, such as resistance genes.

CONCLUSION

Grass pea plants were found to be more susceptible during flowering as compared to drought stress at germination. Water deficit caused by PEG treatment resulted in significantly decreased dry weight of radicle and hypocotyl, water content and length of hypocotyl and radicle of grass pea. The yield component's was also affected by drought stress as dry matter and number of pod and seed per plant declined. However, basic research on the mechanisms of drought resistance by grass pea is essential to understand how this plant combats water deficit stress.

SIGNIFICANCE STATEMENT

Despite the importance of legumes for sustainable production of plant proteins, the progress achieved in Tunisia remains modest. Considering the drought tolerance and resistance to biotic stress of grass pea are valuable traits that can be beneficial and become an added commercial value to many other crops. These results indicate that tolerance of *L. sativus* under water deficit could be used as an adaptive trait for improving drought-resistant crops.

REFERENCES

1. Dai, A., 2013. Increasing drought under global warming in observations and models. Nature Climate Change, 3: 52-58.
2. Yan, M., 2015. Seed priming stimulate germination and early seedling growth of Chinese cabbage under drought stress. S. Afr. J. Bot., 99: 88-92.
3. Attia, H., N. Karray and M. Lachaal, 2009. Light interacts with salt stress in regulating superoxide dismutase gene expression in *Arabidopsis*. Plant Sci., 177: 161-167.
4. Jaleel, C.A., P. Manivannan, A. Wahid, M. Farooq, H.J. Al-Juburi, R. Somasundaram and R. Panneerselvam, 2009. Drought stress in plants: A review on morphological characteristics and pigments composition. Int. J. Agric. Biol., 11: 100-105.
5. Fallahi, H.R., P. Rezvani-Moghaddam, M.B. Amiri, M. Aghhavani-Shajari and R.Y. Biuki, 2015. The study of nutritional management of mother plant and seed priming by biofertilizers on improve salinity tolerance of wheat (*Triticum aestivum* L.) cv. Sayonz at germination period. J. Agroecol., 6: 689-700.
6. Vaz Patto, M.C., B. Skiba, E.C.K. Pang, S.J. Ochatt, F. Lambein and D. Rubiales, 2006. *Lathyrus* improvement for resistance against biotic and abiotic stresses: From classical breeding to marker assisted selection. Euphytica, Vol. 147. 10.1007/s10681-006-3607-2.
7. Massawe, F., S. Mayes and A. Cheng, 2016. Crop diversity: An unexploited treasure trove for food security. Trends Plant Sci., 21: 365-368.
8. Choudhary, A., P. Pandey and M. Senthil-Kumar, 2016. Tailored Responses to Simultaneous Drought Stress and Pathogen Infection in Plants. In: Drought Stress Tolerance in Plants, Hossain, M.A., S.H. Wani, S. Bhattacharjee, D.J. Burritt and L.S.P. Tran (Eds.)., Vol. 1, Springer International Publishing, Germany, pp: 427-438.
9. Polignano, G.B., P. Uggenti, V. Bisignano and E. Alba, 2003. Patterns of variation in *Lathyrus sativus* and some related species. Agric. Med. (Italy). 133: 81-88.
10. Crino, P., G.B. Polignano and S. Tavoletti, 2004. Grass pea, a potentially important crop in Mediterranean agriculture. Grain Legumes, 40: 6-7.

11. Polignano, G., 2007. Conservation, analysis of genetic diversity and maintenance of grasspea and other species (*Lathyrus* spp.) in Italy. Plant Genet. Resour. Newslett. Bull. Ressour. Phytogenet. Noticiario Recur. Fitogenet., 152: 73-76.
12. Kaul, A.K., M.Q. Islam and A. Hamid, 1986. Screening of Lathyrus germplasm of Bangladesh for BOAA Content and some Agronomic Characters. In: Lathyrus and Lathyrism, Kaul, A.K. and D. Combes (Eds.)., Third World Medical Research Foundation, New York, pp: 130-141.
13. Michel, B.E. and M.R. Kaufmann, 1973. The osmotic potential of polyethylene glycol 6000. Plant Physiol., 51: 914-916.
14. Wu, Q.S. and R.X. Xia, 2006. *Arbuscular mycorrhizal* fungi influence growth, osmotic adjustment and photosynthesis of citrus under well-watered and water stress conditions. J. Plant Physiol., 163: 417-425.
15. DuBois, M., K.A. Gilles, J.K. Hamilton, P.A. Rebers and F. Smith, 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem., 28: 350-356.
16. Ahmad, S., F. Anwar, A.I. Hussain, M. Ashraf and A.R. Awan, 2007. Does soil salinity affect yield and composition of cottonseed oil? J. Am. Oil Chem. Soc., 8: 845-851.
17. Piwowarczyk, B., I. Kaminska and W. Rybinski, 2014. Influence of PEG generated osmotic stress on shoot regeneration and some biochemical parameters in Lathyrus culture. Czech J. Genet. Plant Breed., 50: 77-83.
18. Murillo-Amador, B., R. Lopez-Aguilar, C. Kaya, J. Larrinaga-Mayoral and A. Flores-Hernandez, 2002. Comparative effects of NaCl and polyethylene glycol on germination, emergence and seedling growth of cowpea. J. Agron. Crop Sci., 188: 235-247.
19. Okcu, G., M.D. Kaya and M. Atak, 2005. Effects of salt and drought stresses on germination and seedling growth of pea (*Pisum sativum* L.). Turk. J. Agric. For., 20: 237-242.
20. Kaya, M.D., G. Okcu, M. Atak, Y. Cikili and O. Kolsarici, 2006. Seed treatments to overcome salt and drought stress during germination in sunflower (*Helianthus annuus* L.). Eur. J. Agron., 24: 291-295.
21. Jiang, J., M. Su, Y. Chen, N. Gao and C. Jiao *et al*., 2013. Correlation of drought resistance in grass pea (*Lathyrus sativus*) with reactive oxygen species scavenging and osmotic adjustment. Biologia, 68: 231-240.
22. Vicente, O., M. Boscaiu, M.A. Naranjo, E. Estrelles, J.M. Belles and P. Soriano, 2004. Responses to salt stress in the halophyte: Plantago crassifolia. J. Arid Environ., 58: 463-481.
23. Franco, J.A., S. Banon, M.J. Vicente, J. Miralles and J.J. Martinez-Sanchez, 2011. Root development in horticultural plants grown under abiotic stress conditions-A review. J. Hortic. Sci. Biotechnol., 86: 543-556.
24. Mohammadkhani, N. and R. Heidari, 2008. Water stress induced stomatal closure in two maize cultivars. Res. Biol. Sci., 3: 750-754.
25. Al Hakimi, A., P. Monneveux and G. Galiba, 1995. Soluble sugars, proline and relative water content (RCW) as traits for improving drought tolerance and divergent selection for RCW from *Triticum polonicum* into *Triticum durum*. J. Genet. Breed., 49: 237-244.
26. Yilmaz, H. and A. Kina, 2008. The influence of NaCl salinity on some vegetative and chemical changes of strawberries (*Fragaria×ananssa* L.). Afr. J. Biotechnol., 7: 3299-3305.
27. Turner, S.J. and A.M. Schwarz, 2006. Biomass development and photosynthetic potential of intertidal *Zostera capricorni* in New Zealand estuaries. Aquat. Bot., 85: 53-64.
28. Desclaux, D., T.T. Huynh and P. Roumet, 2000. Identification of soybean plant characteristics that indicate the timing of drought stress. Crop Sci., 40: 716-722.
29. Westgate, M.E., 1994. Water status and development of the maize endosperm and embryo during drought. Crop Sci., 34: 76-83.
30. Mirzaei, A., R. Naseri and R. Soleimani, 2011. Response of different growth stages of wheat to moisture tension in a semiarid land. World Applied Sci. J., 12: 83-89.
31. Parker, W.C. and S.G. Pallardy, 1985. Drought-induced leaf abscission and whole-plant drought tolerance of seedlings of seven black walnut families. Can. J. For. Res., 15: 818-821.
32. Pandey, P.K., J.W. Maranville and A. Admou, 2001. Tropical wheat response to irrigation and nitrogen in a Sahelian environment. I. Grain yield, yield components and water use efficiency. Eur. J. Agron., 15: 93-105.
33. Kage, H., M. Kochler and H. Stutzel, 2004. Root growth and dry matter partitioning of cauliflower under drought stress conditions: Measurement and simulation. Eur. J. Agron., 20: 379-394.
34. Zeid, I.M. and Z.A. Shedeed, 2006. Response of alfalfa to putrescine treatment under drought stress. Biol. Plant, 50: 635-640.