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Impact of drought stress on germination and seedling growth of three forage legumes species (Fabaceae)

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Abstract

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Drought causes major economic and ecological problems, such as reduced production of crops and fodder species. In the present study, using morpho-physiological approaches, we have characterised the response of three of the most widely grown forage legume species, cultivated in Algeria under water deficit conditions, with a goal of determining the species that is most tolerant to this constraint.

The effect of drought stress induced by three concentrations of polyethylene glycol (PEG 6000), 5%, 10% and 15% corresponding respectively to three osmotic potentials -0.06 MPa, -0.17 MPa and -0.32 MPa on germination percentage (GP%), mean germination time (MGT), germination kinetics and seedling growth (assessed by seedling dry weight) were studied in vitro for vetch (*Vicia sativa* L.), faba bean (*Vicia faba* L. *minor*) and forage pea (*Pisum sativum arvense* L.).

The results show the influence of treatment and species factors on the three parameters studied. Our results showed that germination was not affected in *V. sativa*, but was significantly reduced from 10% PEG in *V. faba* and 15% in *P. sativum*. Seedling growth was significantly reduced at 5% PEG in *V. sativa* and *P. sativum*, whereas in *V. faba* it was only negatively affected at 15% PEG.

Keywords: PEG 6000; *Vicia sativa* L.; *Vicia faba* L. *minor*; *Pisum sativum arvense* L. germination percentage; seedling growth

Introduction

Most of the world's food and feed supply comes from cereals and pulses (Cordain, 1999). Pulses are the second most important crop in terms of arable land and food production, representing 27% of global crop production, contributing 33% of protein needs and over 35% of global crop oil production (Graham & Vance, 2003; Klein et al., 2014). In addition to their importance for human nutrition, they are of particular importance for preserving the fertility of agricultural soils by fixing atmospheric nitrogen and improving the solubilisation of phosphorus in the soil (Schneider & Huyghe, 2015).

Forage is considered to be an essential factor in the development of livestock production, the shortage of which is a restricting factor (Hamrit, 1995). Given the diversity of its environments, Algeria is an abundant source of plants, with fodder legumes alone represented by 33 genus and about 293 species (Issolah & Beloued, 2005). However, the forage area, which is estimated at 1.1 million hectares, is insufficient (MADR, 2014). In addition, the non-availability of green fodder for long periods of the year and climatic constraints has a negative impact on livestock yields (Lobell et al., 2013). Abiotic stresses such as drought, salinity and extreme temperatures pose significant threats to agricultural production (Narusaka et al., 2003; La Peña & Hughes, 2007).

Drought is widely recognised as the major factor limiting agricultural production worldwide, particularly in the Mediterranean basin (Clavel et al., 2005; Hessini et al., 2009). Yield reductions attributed to drought stress surpass those caused by other factors together (Sánchez-Rodríguez et al., 2010). Water stress is the most important factor that adversely affects seed germination and early seedling growth (Almansouri et al., 2001; Ansari et al., 2013). The work of Catalan et al. (1994) has shown that germination is the most susceptible phase to drought. Water deficit negatively affects seed germination, reducing or completely inhibiting seedling emergence (Kaya et al., 2006) due to the drop in water content, which leads to a reduction in water uptake (Farooq et al., 2009).

Due to climatic warming, the drought will increase and will have to exert greater pressure on the sustainability of agriculture than in the past (Papworth et al., 2015; Saleem et al., 2018). The Mediterranean region is currently considered to be a global warming "hotspot", with temperatures above the global average in the 21st century (Adloff et al., 2015).

Algeria is suffering from a severe fodder deficit (Abbas et al., 2006), due to drought on the one hand, and a reduction in fodder areas and the use of non-adapted cultivars on the other (Nedjraoui, 2003). The most appropriate solution at present is to grow cultivars adapted to drought conditions (Reddy et al., 2012). Research into the responses of forage plants to drought stress has been conducted principally on model plants such as alfalfa (Inès et al., 2021), wheat and barley (Sassi et al., 2012; Araújo et al., 2015). According to Reddy et al. (2012), forage legumes can reveal specific mechanisms implicated in resistance/tolerance to abiotic stresses.

The objective of the present study was to evaluate, *in vitro*, the effect of drought stress on the germination and growth of three forage legume seedlings, *Vicia sativa* L., *Vicia faba* L. *minor*, and *Pisum sativum arvense* L. Drought stress was induced by PEG 6000, considered to be a powerful simulator of water stress under laboratory conditions (Kaur et al., 1998; Benidire et al., 2015; Petrović et al., 2021; Tamindžić et al., 2021). Indeed, *in vitro* techniques for evaluating plant responses to stress minimise the impact of changes in the external environment (Benidire et al., 2015; Petrović et al., 2021). A positive correlation between drought resistance of genotypes in the field and in the laboratory has been reported (Kosturkova et al., 2014).

Materials and Methods

Vegetal material

The seeds of the legume varieties used in this study were delivered by the Technical Institute of Field Crops (ITGC) located in Algiers (Algeria) in 2021. We have selected three leguminous varieties that are the most cultivated in Algeria: the Serva 174 variety of vetch *Vicia sativa* L., the Sidi aiche variety of broad bean *Vicia faba* L. *minor* and the Sefrou pea variety *Pisum sativum arvense* L. (Table 1).

Application of drought stress

Drought stress is induced by polyethylene glycol 6000. Three osmotic potentials of -0.6, -1.7 and -3.2 bar were simulated by three concentrations of PEG 6000: 5%, 10% and 15% according to the method of Michel & Kaufmann (1973) (Table 2).

Table 2. Concentrations of PEG 6000 and osmotic potential values applied

Concentrations of PEG 6000, %	Quantity of PEG, $g.L^{-1}$	Osmotic potential, MPa	Osmotic pressure, bars
Control	0	0	0
5%	50	-0.06	-0.6
10%	100	-0.17	-1.7
15%	150	-0.32	-3.2

The seeds of the three varieties were disinfected in 1% sodium hypochlorite for 5 min, then carefully rinsed with distilled water three times to remove all traces of sterilizing agent before germination (Piwowarczyk et al., 2014). The seeds were placed in 9 cm diameter Petri dishes lined with two discs of filter paper Whatman n°1 moistened with a volume of 15 ml per dish of distilled water for the control or PEG 6000 solution at 5%, 10% or 15%. We used 20 seeds of Sefrou (*P. sativum*)

Table 1. Geographical and climatic data for seeds collection stations

Species/ varieties	Collection site	Longitude	Latitude	Altitude	Bioclimate stage	Pluviometry	Temperatures (°C) m	t	M	Origin of countries (References)
Vicia sativa L. Serva 174	Saïda	0°09′00″E	34°50′00″N	868 m	Semi-aride	365.4 mm	11.0	20.3	25.1	Agricome international (Abdelguerfi & Laouar, 2013)
Vicia faba L. *minor* Sidi aiche	Guelma	7°26′00″E	36°28′00″N	290 m	Subhumide	654 mm	10.4	16.2	22.7	Algeria (Abdelguerfi & Laouar, 2013)
Pisum sativum arvense L. Sefrou	Sidi-Bel-Abbès	0°38′29″O	35°11′38″N	483 m	Semi-aride	337.4 mm	10.8	16.3	22.9	Morocco (Ouafi et al., 2016)

and Serva 174 (*V. sativa*) varieties and 15 seeds of Sidi Aiche (*V. faba*) variety per Petri dish and four dishes per treatment (control, 5%, 10% and 15%). The dishes were then placed in an oven in the dark in the incubator at a temperature of 20±2°C. Each Petri dish was moistened with 15 ml of distilled water or PEG 6000 solutions 3 times a week.

According to Michel & Kaufmann (1973), the equation linking the different parameters is as follows:

$$\Psi h = -(1.18\times10^{-2})\,C - (1.18\times10^{-4})\,C^2 + (2.67\times10^{-4})\,CT + (8.39\times10^{-7})\,C^2T$$

Ψh: Osmotic potential, bar;
T: Incubation temperature, °C;
C: Concentration of PEG6000, $g.L^{-1}$.

Parameters measured

Germination percentage (GP)

The number of germinated seeds is recorded daily for 15 days. The seed is considered germinated when the radicle reaches 5 mm in length (Côme, 1970; Kaya et al., 2006). The germination percentage is calculated using the formula of Mazliak (1982):

$$GP\ (\%) = n/N \times 100$$

N: total number of seeds germinated;
n: number of seeds germinated.

Mean germination time (MGT)

The MGT corresponds to the time taken for seeds to germinate (Côme, 1970). It is calculated by the following formula (Kandil et al., 2012):

$$MGT = \sum dn / \sum n$$

n: number of seeds that were germinated on the day (d);
dn: number of days counted from the beginning of germination.

Germination kinetics

This enables the physiological significance of the germination behaviour of the species studied to be understood, as well as all the events from the absorption of water by the seed to the elongation of the embryonic axis and the emergence of the radical through the integuments (Côme, 1970).

Seedling growth

After germination, the seedlings continued to grow under controlled laboratory conditions at a temperature of 20°C±2 and a relative humidity between 70% and 80%.

The effect of drought stress on the growth of seedlings of the three species was assessed after 20 days of germination by determining the dry weights (g) of five seedlings per dish, giving a total of 20 seedlings per treatment (Control, 5%, 10% and 15% PEG) after oven drying at 70°C for 48h (until constant weight was obtained) (Böhm, 1979).

Statistical analysis

The data were analysed using R software (*version 3.6.2 2019*) for an analysis of variance (Two-Way ANOVA). The analysis was completed using the Newman & Keuls post hoc test when a significant difference was found at the 5% error threshold. Non-parametric Kruskal-Wallis test was used if there was no normal distribution.

Results

Drought stress effect on germination percentage (GP)

The germination process was affected by both factors (treatment and species), showing that the three species studied did not have the same response to the stress applied by the different concentrations of PEG 6000. Indeed, analysis of variance revealed very highly significant differences ($P < 0.001$) between varieties and levels of stress applied.

In control seeds, germination percentage were statistically higher in *V. faba* (100%) and *P. sativum* (97.5%) than in *V. sativa* (85%) (Figure 1, Table 3). The treatments of PEG osmotic stress do not have the same influence on germination in the three species. The germination of *V. sativa* was not affected by different concentrations tested. The most sensitive species is *V. faba* because its germination is affected by 10% and 15% PEG with reduction rates of 20% and 35% respectively (Table 4). The germination percentage of *P. sativum* (Figure 1, Table 4) only decreased significantly at 15% PEG with a 15.19% reduction rate.

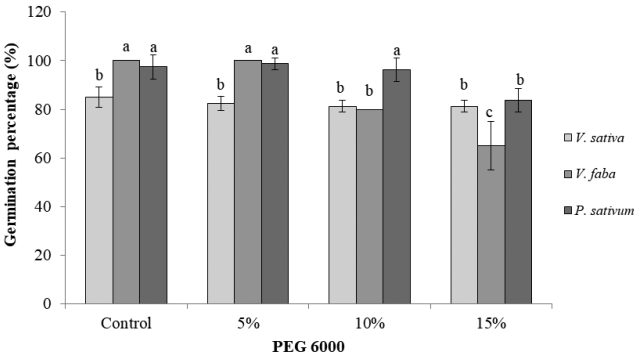

Fig. 1. Drought stress effects on the germination percentage (%) of *V. sativa*, *V. faba* and *P. sativum*. The letters (a,b,c ...) show very highly significant differences according to ANOVA ($P < 0.001$), supplemented with Newman & Keuls test

Table 3. Effects of different PEG concentrations on GP, MGT and seedling growth of *V. sativa, V. faba* and *P. sativum*

Species /Varieties	PEG 6000, %	Germination percentage, %	Mean germination time	Seedling dry weight, g
Vicia sativa L. (Serva 174)	Control	85 ± 4.082 b	3.134±0.121 d	0.061 ± 0.012 g
	5%	82.5 ± 2.887 b	4.091±0.146 bc	0.058 ± 0.007 gh
	10%	81.25 ± 2.5 b	4.149±0.282 bc	0.051 ± 0.009 h
	15%	81.25 ± 2.5 b	4.645±0.097 b	0.035 ± 0.004 i
Vicia faba L. *minor* (Sidi aiche)	Control	100 ± 0 a	3.3±0.176 d	0.401 ± 0.031 a
	5%	100 ± 0 a	4.133 ±0.356 bc	0.392 ± 0.019 a
	10%	80 ± 0 b	4.604 ± 0.415 b	0.397 ± 0.013 a
	15%	65 ± 10 c	5.576± 0.868 a	0.286 ± 0.012 b
Pisum sativum arvense L. (Sefrou)	Control	97.5 ± 5 a	1.931±0.256 f	0.189 ± 0.008 c
	5%	98.75 ± 2.5 a	2.515±0.182 e	0.150 ± 0.009 a
	10%	96.25 ± 4.787 a	3.158±0.322 d	0.116 ± 0.007 e
	15%	83.75 ± 4.787 b	3.623±0.116 cd	0.104 ± 0.012 f

Analysis of variance followed by determination of homogeneous groups (a. b. c...) using the Newman & Keuls test at a threshold of α = 5%. Values followed by the same letter are not significantly different, but those with different letters are at P < 0.001***

Table 4. Reduction rate of GP and seedling growth by different concentrations of PEG in *Vicia sativa* L., *Vicia faba* L. *minor* and *Pisum sativum arvense* L.

Species /Varieties	PEG 6000, %	Reduction rates of germination percentage, %	Reduction rates of seedling dry weight, %
Vicia sativa L. (Serva 174)	5%	(ns)	4.92(s)
	10%	(ns)	16.40(s)
	15%	(ns)	42.63(s)
Vicia faba L. *minor* (Sidi aiche)	5%	(ns)	(ns)
	10%	20.00(s)	(ns)
	15%	35.00(s)	28.68(s)
Pisum sativum arvense L. (Sefrou)	5%	(ns)	20.64(s)
	10%	(ns)	38.63(s)
	15%	15.19(s)	44.98(s)

(s): statistically significant compared with its control /(ns) not statistically significant compared with its control

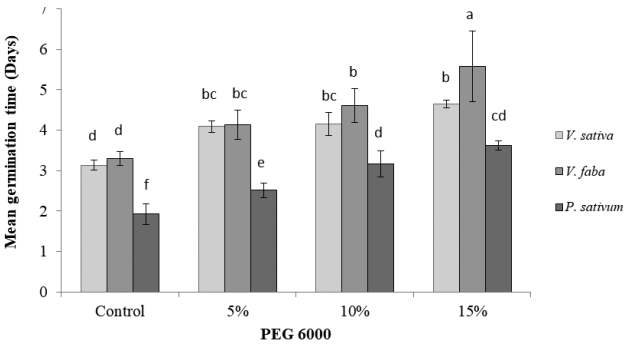

Fig. 2. Drought stress effects on the MGT of *V. sativa*, *V. faba* and *P. sativum*. The letters (a,b,c;..) show that the mean germination times differ very significantly (P < 0.001), according to one ANOVA completed with the Newman & Keuls test

Drought stress effect on mean germination time (MGT)

Mean germination time (MGT) was also affected by both treatment and species factors, whose difference was very highly significant (P < 0.001). The control seeds showed the shortest germination times. The lowest MGT was obtained for *P. sativum* (1.9 days) and the MGTs of *V. sativa* and *V. faba* were close (3.4 and 3.1 days respectively) (Table 3). An increase in MGT correlated with the concentration of PEG 6000 was observed in all three species. However, *P. sativum* showed the best germination times at the different PEG concentrations tested, while *V. faba* recorded the longest MGT (Figure 2).

Drought stress effect on germination kinetics

We found that germination always took place in three phases for all three species studied, controls and those treated with different concentrations of PEG 6000: a latent phase, an exponential phase and a stationary phase (Figure 3).

The lag phase corresponds to the time required for adequate seed imbibition. This time is shorter in all controls; it is one day in *P. sativum* and *V. faba* and two days for *V. sativa*. This phase is not affected by different concentrations of PEG 6000 tested in *V. sativa*, as it lasts for one day at all times. In *V. faba*, this latent phase was extended to two days, but only at 15% PEG. In *P. sativum*, however, this phase is affected from 5% of PEG.

The exponential phase corresponds to the time when the germination percentage increases to reach a maximum value at which germination stops. In all three species, the exponential phase was shorter in the controls and spread out as the PEG 6000 concentration increased. In the controls, germination reached its maximum after three days in *P. sativum*, 4

days in *V. sativa* and 5 days in *V. faba*. The longest duration of this exponential phase was always obtained at 15% PEG 6000 in the three species, with *V. sativa* recording the longest phase at 15% PEG (Figure 3).

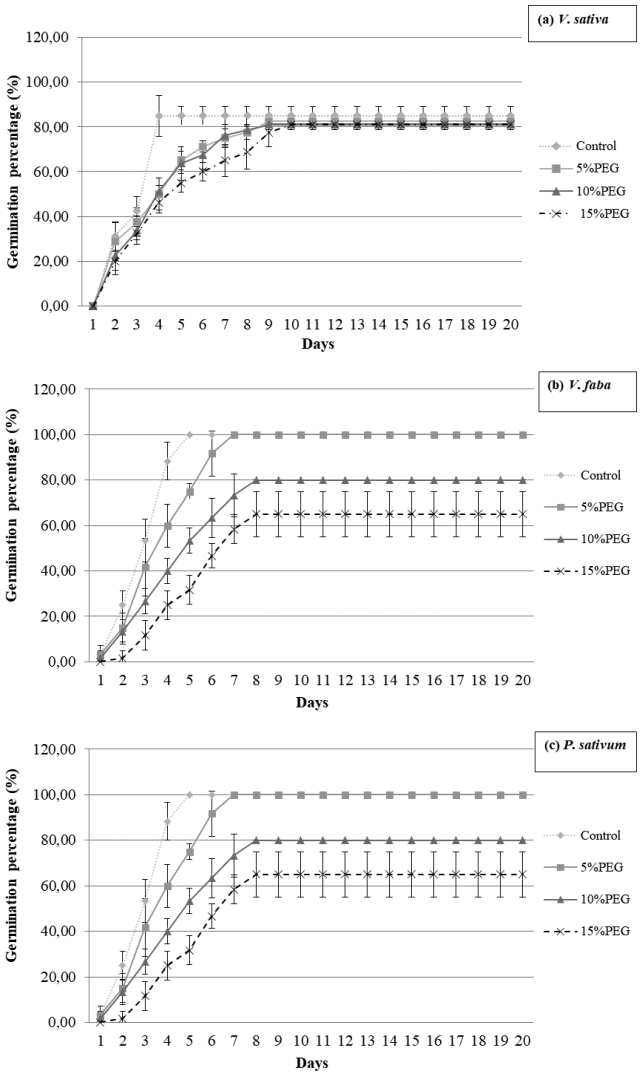

Fig. 3. Drought stress effect on germination kinetics of (a) *V. sativa*, (b) *V. faba* and (c) *P. sativum* seeds. The values represent the mean of 5 replicates (5 Petri dishes)

Drought stress impact on seedling growth

The seedling growth as well shows the influence of species and treatment in a very highly significant way ($P < 0.001$). The control seedlings showed the best growth, but this was much greater in *V. faba* than in the other two species. In fact, the dry weight of *V. faba* seedlings (0.401g) was more than 6 times greater than that of *V. sativa* (0.061g) and more than twice that of *P. sativum* (0.189g) (Table 3).

The increase in PEG 6000 concentrations, and hence the decrease in osmotic potential, resulted in a highly significant ($P < 0.001$) reduction in growth, essentially for *V. sativa* and *P. sativum*, and this decrease was negatively correlated with the PEG 6000 concentration. In *V. faba*, only the 15% PEG concentration (-0.32 MPa) caused a significant reduction in growth, with a reduction rate of 28.68%. However, this reduction rate is lower than those recorded for *V. sativa* (42.63%) and *P. sativum* (44.98%) at the same PEG concentration (15%) (Figure 4, Table 4).

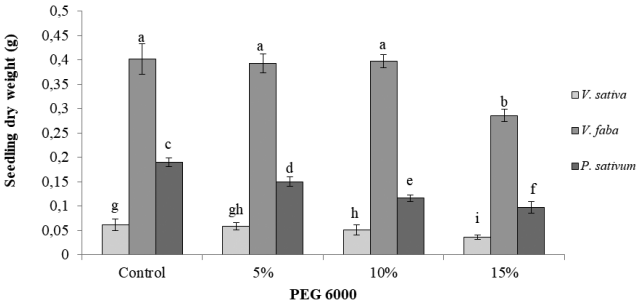

Fig. 4. Drought stress effects on the dry weight of *V. sativa*, *V. faba* and *P. sativum* seedlings. The letters (a,b,c ...) show that growth differed very significantly according to ANOVA ($P < 0.001$), completed with the Newman & Keuls test

Discussion

The aim of the present study was to evaluate the effects of different osmotic pressures induced by different concentrations of polyethylene glycol 6000 on seed germination capacity and seedling growth of three forage legume species under controlled conditions. Statistical analysis of our results showed that germination percentage (GP), mean germination time (MGT) and seedling growth were influenced by PEG 6000 and species ($P < 0.001$).

The *V. sativa* species showed no reduction in germination at the different concentrations tested, whereas the germination of *V. faba* and *P. sativum* was reduced at 10% and 15% PEG, respectively.

Germination capacity in the face of drought stress varies according to several factors, including the intensity of the stress and the species (Murillo-Amador et al., 2002; Benjelloun et al., 2013). According to Catalan et al. (1994), the highest degree of sensitivity of plants to drought is at the germination stage. Moreover, drought tolerance during the

germination phase is an important criterion for assessing the ability of species to tolerate a water deficit during their first phase of development (Sy et al., 2001; Benjelloun et al., 2013). However, inter- and intraspecific variability has been observed in the face of drought stress (Lüscher et al., 2022).

A moderate water deficit (5% PEG) generally has no effect on germination in many species such as *Vigna unguiculata* L. (Murillo-Amador et al., 2002); *Prosopis strombulifera* (Sosa et al., 2005); *Vicia faba* (El-Tayeb, 2006). According to Petrović et al. (2021), in 7 varieties of *P. sativum*, germination percentages decrease even under low osmic pressures of -0.1 MPA and -0.2 MPA equivalent to 6% and 12% PEG respectively. However, Tamindžić et al. (2021) showed that PEG 6000 inhibited germination by 10% in three varieties (C1, C2, C3) and by 20% in one variety (C4), demonstrating the existence of intraspecific variability within this species.

The greatest reduction in germination percentage was observed in *Vicia faba* L. *minor* (Sidi aiche) with 65% to 15% PEG. A similar result was obtained previously in this species by El-Tayeb (2006).

This inhibition of germination is attributable in particular to a deficit in tissue hydration following high osmotic pressure (Dodd & Donavon, 1999; Almansouri et al., 2001; Okçu et al., 2005), leading to lower infiltration/diffusion of water throughout the integument and therefore a reduction in water uptake by the seeds (Bahrami et al., 2012; Channaoui et al., 2019), affecting the radicle elongation process (Hegarty & Ross, 1978), which inhibits hydration of the seed's nutrient reserves and consequently germination process (Bewley & Black, 1994; Dirik, 2000). Indeed, according to Pratap & Kumar Sharma (2010) and Rana et al. (2017), the degradation and inactivation of hydrolytic enzymes and other enzymes required for germination (α-amylase ß-amylase, phosphatase... etc) could be the result of severe drought stress. This inhibition of germination can also be attributed to poor diffusivity of O_2 through the integument (Braccini et al., 1996) and to an alteration in seed enzymes and hormones (Botía et al., 1998). However, osmotic potential can be maintained by low- or medium-intensity water stress, by osmotic adjustment and by accumulation of numerous molecules such as soluble sugars. Sucrose is often accumulated preferentially in dehydrated tissues (Dejardin et al., 1999).

Although the MGT differed significantly between the three species studied (shorter for *P. sativum* and longer for *V. sativa* and *V. faba*), increasing the osmotic pressure of the imbibition medium with PEG 6000 caused a delay in germination by increasing the MGT in all three species. Pantola et al. (2017) and Okçu et al. (2005) reported that drought stress retarded germination. The results obtained are similar to those obtained by Okçu et al. (2005) on *Pisum sativum* L., Castroluna et al. (2014) on *Medicago sativa* and Medjebeur et al. (2018) on *Hedysarum Flexuosum*. In agreement with our results, Petrović et al. (2021) obtained an increase in MGT from 6% PEG 6000 for *P. sativum*.

For *V. faba*, the delay in germination caused by higher PEG concentrations can be explained by the longer time required for seed imbibition (Jaouadi et al., 2010). This delay is also thought to be due to a disruption in the enzymatic functions responsible for triggering germination (Naddem et al., 2019).

Our results show that *V. sativa* and *P. sativum* have a greater ability to germinate under a drought deficit (5%, 10%, 15% PEG), but according to McGinnies (1960), species that are tolerant to drought stress during germination are not always adapted to drought at the adult stage.

Assessing the effect of water stress on growth by determining dry biomass is considered to be a reliable and effective method for screening species tolerance to water stress (Ahmad et al., 2009).

The effect of applied drought stress depends on the PEG concentration and the legume species. Dry biomass was significantly reduced from 5% PEG in *Pisum sativum arvense* L. and *Vicia sativa* L. These results are similar to those obtained in many legume species such as *Medicago sativa* L. (Farooq et al., 2008; Inès et al., 2021), *Pisum sativum* L. (Pereira et al., 2020), *Vigna unguiculata* L. (Murillo-Amador et al., 2002), *Vigna radiata* (Saima et al., 2018), *Phaseolus lunatus* (Nascimento et al., 2017) and *Vicia faba* (El-Tayeb & Hassanein, 2000; El-Tayeb, 2006).

The decrease in dry biomass of *P. sativum* and *V. faba* is attributed to a decrease in shoot and root growth (Farooq et al., 2009). However, according to Okçu et al. (2005), El-Tayeb & Hassanein (2000), El-Tayeb (2006), Petrović et al. (2016), Pereira et al. (2020), Petrović et al. (2021) and Tamindžić et al. (2021), this poor growth is due to a decrease in shoot growth and not root growth. In many species exposed to drought stress, this results in a major change in plant architecture, just to reduce the water budget at the cost of yield loss (Schuppler et al., 1998).

The reduction in seedling growth in the face of drought stress is attributed to the reduction in seed metabolism due to the reduced availability of water needed to digest reserves and translocate metabolised products (Bewley & Black, 1994; Petrović et al., 2021; Kalefetoğlu et al., 2009), to the reduction or non-transfer of nutrients from seed storage tissues to the embryo and to a reduction in hormone and enzyme secretion and ionic balance (Botía et al.,1998; Pratap & Kumar Sharma, 2010).

This reduction in plant growth under drought stress can also be attributed to many other factors such as reduced cell

turgor and division and/or cell enlargement (Terry et al., 1971; Tardieu et al., 2006; Farooq et al., 2008), reduced net photosynthesis (Hassanein, 1985), production of toxic reactive oxygen species (Polle, 1996) and induction of changes in gene expression (Hare et al., 1996). Impaired mitosis, reduced cell elongation and expansion at root level result in reduced plant height, leaf area and crop growth under drought conditions (Kaya et al., 2006; Hussain et al., 2008).

Conclusion

The study of the effect of PEG 6000 at 5%, 10% and 15% corresponding to -0.06 MPa, -0.17 MPa and -0.32 MPa respectively on germination (GP, MGT and kinetics) and seedling growth shows that there is a difference in performance between the three forage legume species studied. *V. sativa* and *P. sativum* appear to be the least sensitive to applied drought stress in the germination phase but the result of *V. faba* was more interesting in seedling growth compared to other species. Further studies in pots and in the field are needed to confirm this hypothesis. Once the tolerant character has been proved, these varieties could be recommended for use in agriculture to provide high yields in environments where water shortages are becoming increasingly frequent in the current context of climate change.

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