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Full Length Research Paper

Response of Tunisian autochthonous pearl millet (*Pennisetum glaucum* (L.) R. Br.) to drought stress induced by polyethylene glycol (PEG) 6000

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Seeds of pearl millet (*Pennisetum glaucum* (L) R. Br.) from six provenances of Tunisia were subjected to germination and shoot and root length tests on filter paper treated with polyethylene glycol 6000 (PEG 6000) solutions made up to provide osmotic potentials of 0, -1 and -2 MPa. Mean germination percent for all provenances decreased about 73% in -2 MPa compared to control (0 MPa) treatment. Osmotic potential of -1.0 MPa improves the rate of germination but not significantly compared to the control. There were significant differences among the relative germination percent of the provenances in different treatments. Decreases in the external osmotic potential induced decreased shoot growth while a slight increase in root length associate with the -1 MPa treatments was observed for some ecotypes. This reflects an adaptive response involving an increase in root length to reach deeper water in the soil. It was also notified that the elongation of the radicle is more sensitive to the osmotic constraint than the coleoptile.

Key words: Drought stress, polyethylene glycol (PEG), ecotypes, *Pennisetum glaucum,* shoot, root, germination and genetic diversity.

INTRODUCTION

The first imperative of a satisfactory culture consists of its suitable establishment and the success of the germination phase determines that of all the period vegetative. In fact, when establishment levels are low, higher seedling rates are required, yield is reduced, and replanting may be necessary, all of which reduce financial returns. Furthermore, the answer of seeds to drought could also be an indicator of the tolerance of plants for the later stages of development. Therefore, there have been attempts at germinating under variable stress conditions to identify the populations which adapt to dryness. Seedling tests under variable osmotic stress may lead to the selection of genotypes adapted at different geographical regions.

The tolerance of many species to drought during germination stage constitutes an advantage because it leads to the establishment of these species in zones where others, sensitive to the drought, cannot colonize, and permit the fast occupation of the space. One of the greatest challenges in drought is to sow a seed type that has the capacity to produce abundant biomass and cover in a short period of time (Van den Berg, 2002). Pearl millet is one of those grass which has strong development of underground organs and tends to have efficient adaptive mechanisms to cope with drought (Winkel et al., 1992, 1997; Bezançon et al., 1997).

The study of the influence of the drought using osmotic solutions is one of the methods in the study of resistance during the germinal phase. Gautreau (1966) found that the germination of groundnut sprayed with a solution of sucrose was similar to drought of this plant in the natural conditions. Furthermore, Saint-Clair (1976) demonstrated that resistance of sorghum to drought is related to its capacity to germinate at 10.88 bars. In a comparative study on the effect of the PEG on the germination of pearl millet and sorghum, Saint-Clair (1980) demonstrated that the germination of *Pennisitum glaucum* is much less affected than that of sorghum because the depressive effect of the osmotic potential of 10.88 bars is less than 18.5 bars which affect pearl millet. Several literatures have indicated the superiority of the germination capacity

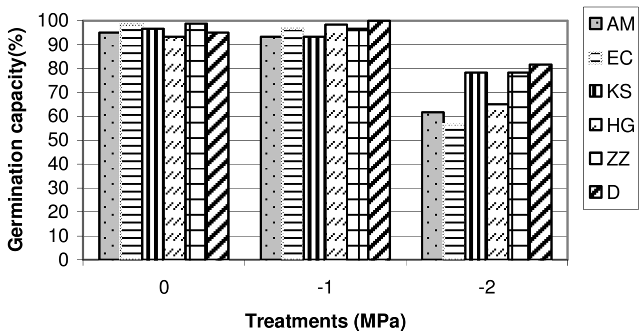

Figure 1. Germination capacity of six ecotypes of pearl millet during drought stress induced by polyethylene glycol (PEG) 6000.

Table 1. Decrease of shoot length during drought stress induced by polyethylene glycol (PEG) 6000.

Treatment (MPa)	Reduction of shoot length (% of control)
control	100.0 a
-1.0	44.0 b
-2.0	84.5 c

Different letters indicate statistically significant differences for shoot length ($P < 0.05$).

of pearl millet compared to that of sorghum as well as its remarkable resistance in the drought (Van Den Abeele and Vandenput, 1956; Gaudy, 1957; White and al., 1959; Martin and Leonard, 1967).

The objective of this work was to assess the variability among genetically diverse pearl millet ecotypes (*P. glaucum* (L.) R. Br.) for germination during drought stress induced by PEG 6000.

MATERIALS AND METHODS

Osmotic solutions are used to impose water stress reproducibly under *in vitro* conditions (Pandey and Agarwal, 1998). Polyethylene glycol molecules with a $M_r \geq 6000$ (PEG 6000) are inert, non ionic and virtually impermeable chains that have frequently been used to induce water stress and maintain a uniform water potential throughout the experimental period (Hohl and Peter, 1991; Lu and Neumann, 1998). Molecules of PEG 6000 are small enough to influence the osmotic potential, but large enough to not be absorbed by plants (Carpita et al., 1979; Saint-Clair, 1980). Because PEG does not enter the apoplast, water is withdrawn from the cell. Therefore, PEG solution mimic dry soil more closely than solutions of low M_r osmotica, which infiltrate the cell wall with solutes (Veslues et al., 1998).

Results from initial germination capacity tests conducted by several authors (Roussel, 1978; Saint-Clair, 1980; Popignis, 1985; Albuquerque and al., 2004) indicated that temperature of 25 to 30°C is optimal for seed germination of *P. glaucum*.

Water stress was applied through incubation in two concentrations of PEG 6000 that provide solutions with water potentials equal to – 10 and – 20 MPa. PEG 6000 was dissolved in distilled water and placed in a shaker bed (25°C) for 16 h (Van den Berg and Zeng, 2006). Fifty randomly chosen seeds of each ecotype were placed on filter paper in a Petri dish, 9 cm in diameter, and 4 Petri dishes per group. One ml of distilled water was supplied to each Petri dish daily. After seven days of incubation, the shoot length of germinated seeds was measured. The same procedure was applied for root length.

The experiment was designed as a completely randomized design with two factors. The first factor was the ecotypes and the second the drought stress treatments. Those ecotypes are autochthonous and were collected from different ecological regions from Tunisia. Three pearl millet populations have tall stature (KS, EC and ZZ) and the others have short stature (HG, AM and D). Data were analyzed with ANOVA, and means were separated by an LSD using $P < 0.05$.

RESULTS

Effect of PEG on Germination

The various concentrations of PEG had a significant effect on the germination of the seeds. For all ecotypes, germination decreases with a decrease in osmotic potential (Figure 1). The final germination capacity varies between 100% for the control and 73% for the most concentrated in PEG. However, osmotic potential of -1.0 MPa improves the rate of germination although not significantly compared to the control.

There is also a significant difference in the germination of the six ecotypes. On average, the highest germination capacity was obtained from D and ZZ (92%) and the lowest from ecotypes EC and AM (approximately 83%). Moreover, EC and AM were the most affected by an osmotic potential of -2.0 MPa, with reduction of the germination capacity of approximately 50% in EC.

Effect of PEG on shoot length

For all ecotypes, the shoot lengths of seedlings decreased with an increase in water stress (Table 1). Osmotic potential of -1.0 MPa decreases the length of the coleoptile by 44%, while -2.0 MPa reduces this dimension by 84.5%. There was also variation in shoot length and its response to drought. Ecotype D produced the shortest shoot (< 6 cm).

Effect of PEG on root length

A moderate osmotic stress (-1.0 MPa) improves the root length of all the ecotypes (15.8%). In contrast, root elongation for all ecotypes decreased (reduction more than 88%) with a decrease of osmotic potential. However, for ecotypes KS, ZZ and D, the improvement noted for the moderate stress is not significant compared to the control (Figure 2). There was also variation in root length and its response to osmotic stress. HG ecotype is distinguished from the other populations by its short radicle. The differences between the other ecotypes are not significant.

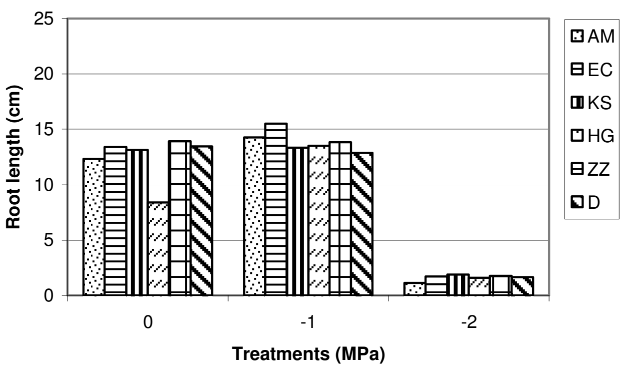

Figure 2. Root length of six ecotypes of pearl millet during drought stress induced by polyethylene glycol (PEG) 6000.

Table 2. Correlation between germination capacity and shoot and root length during drought stress induced by polyethylene glycol (PEG) 6000.

Parameter	Stress osmotique
Shoot length	0.61**
Root length	ns

Correlations

With osmotic stress, the rate of germination is correlated positively with the shoot length, which means that the longer the coleoptile, the better is its germination capacity (Table 2). No correlation was noted between germination and root length in the presence of osmotic stress

DISCUSSION

The exposure of pearl millet seeds to osmotic stress affects germination capacity. At osmotic potential of -2 MPa, the rate of germination of the millet is 73%, which is similar to the 75% for a potential of -21.7 bars obtained by Grouzis (1986). This value, according to this author, is a limit for which all species do not germinate and confirms the adaptive character of the species to dryness during germination phase. For all ecotypes, moderate osmotic potential -1 MPa improved germination capacity as previously indicated by Ashraf et al. (2003) for pearl millet.

There is also genotypic difference in sensitivity to drought at the germination stage. The ecotypes native to south of Tunisia (D and ZZ) showed rates of germination higher than the other ecotypes, indicating the importance of the origin of the seed for resistance to dryness. Saint-Clearly (1980) found that Indian ecotypes were more adapted to drought than those originating in wet conditions of Georgia (the USA).

Decreases in the external osmotic potential generally caused decreased morphological development of organs from the young grass seedling of the six ecotypes tested. Whereas shoot growth was always decreased by exposure to all the stress levels tested, there was a slight increase in root length associate with the -1 MPa treatments for HG, EC and AM. This reflects an adaptive response involving an increase in root length to reach deeper water in the soil. It was also observed that the elongation of the radicle is more sensitive to the osmotic constraint than the coleoptile. For the same osmotic potential, the reduction in the root length is 88% whereas that of the shoot length is 84.5%. Similar observation was reported by Hegarty and Ross (1978) for *Brassica oleracea* and *Lepidium sativum*. This reduction may be due to an impediment of cell division and cellular elongation leading to a kind of "tuberization" (Fraser and Al, 1990). The tuberization and the lignification of the root system allow the plant to enter a slowed-down state, while waiting for the conditions to become favourable again (Vartanian, 1973).

Conclusion

This study has shown that exposure of pearl millet seeds to osmotic stress affects germination capacity as well as root and shoot lengths in six ecotypes and has demonstrate the importance of the origin of the seed for resistance to drought. This species which could germinate even in the presence of a potential of -2 MPa will be important as "fodder" seeds being able to adapt to dry conditions. This character of adaptation is desired since it determines the number of plants per unit of area and makes it possible to extend this plant towards drier areas.

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