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EFFECT OF DROUGHT STRESS BY USING PEG 6000 ON GERMINATION AND EARLY SEEDLING GROWTH OF *Brassica juncea* Var. Ensabi

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Abstract

To study on effect of PEG 6000 stress on germination and early seedling growth of B. juncea (L.) Czern. var. Ensabi an experiment was conducted in the laboratories of Institute of Biological Sciences, University of Malaya, Malaysia. Seed germination and final germination rate of B. juncea var. Ensabi significantly affected by PEG 6000 concentration. By increasing osmotic potential of PEG 6000, seed germination and final germination were decreased. In distilled water, percentage of seed germination was highest. The higher amount of PEG 6000 concentration in this research (10 and 12 MPa) completely inhibited seed germination. Signs of germination for control and low PEG 600 osmotic potential occurred between 24 and 48 hours after sowing but at higher PEG 6000 osmotic solution it was being later. Most complete germination occurred at 25°-30°C. In PEG 6000 solution, the hypocotyl lengths of seedlings decreased with an increase in water stress. Shoots elongation significantly decreased by concentration of 2-8 MPa whereas no hypocotyl elongation at concentration of 10 and 12 MPa and shoot elongation completely inhibited.

Keyword: *Ensabi, germination, temperature, PEG 6000 concentration, hypocotyls.*

INTRODUCTION

Abiotic stresses, such as drought, salinity, extreme temperatures, chemical toxicity and oxidative stress are serious threats to agriculture and result in the deterioration of the environment.

Seed germination is an important stage in the life history of plant, affecting seedling development and survival, and population dynamics. Stress and strain are fundamental physical concepts that can be applied to biological systems. Physical scientists define stress as a force per unit area applied to an object. Strain is a change in a dimension of an object developed in response to a stress (Hopkins and Huner, 2003).

The Indian mustard (*B. juncea* L.) is an important oil-yielding crop that has been shown to be more heat and drought tolerant than the other spices, with a range of contributing characters (Mendham and Salisbury, 1995).

Salinity and drought affect the plants in a similar way. Reduced water potential is a common consequence of both salinity and drought. Water stress acts by decreasing the percentage and rate of germination and seedling growth (Macar, 2008).

Drought plays an important role not only in determining germination rates, but also influences seedling development (Macar, 2008). This stress type is one of the most important environmental stresses affecting agricultural productivity worldwide and can result in considerable yield reductions (Mohammadkhani and Heidari, 2008). It is one of the main causes for crop yield reduction in the majority of agricultural and natural regions of the world. The effects of sodium nitroprusside treatment on induced drought stress were investigated (Gomaa, 2010).

The principal aim of present study was to survey the effects of drought stress induced on germination and early seedling stage of two cultivars of *B. juncea* (L.) Czern. var. Ensabi.

MATERIALS AND METHODS

Germination and early seedling growth (10 days) of *B. juncea* var. Ensabi was studied in an experiment using distilled water (control) and osmotic potentials (-0.20, -0.40, -0.60, -0.80, -1 and -1.2 MPa), which were prepared adding polyethylene glycol (PEG 6000) to distilled water according to Michel and Kaufman (1983) to have the osmotic potential in PEG. Mature, healthy and equal sized seeds of Ensabi were previously disinfected by immersion in a calcium hypochlorite solution, containing 5% active chlorine, for one minute. The seeds were then washed three times with sterilized distilled water.

Seed germination tests were carried out in sterilized 9 cm petri dishes (that had been autoclaved for four hours) with Whatman No.1 filter paper. Each dish was moistened with the appropriate osmotic solutions (PEG-6000 solutions, osmotic potentials of -0.20, -0.40, -0.60, -0.80, -1 and -1.2 MPa) or distilled water for 0 MPa as a control. Germination tests were carried out in a growth chamber (Shel Lab, Model 2015-2E) at 20^{o}C, 25^{o}C and 30^{o}C. Same appropriate solutions were added daily to each petri dish. Seeds were considered germinated when the radicle emerged with at least 2 mm long. The number of germinated seeds was recorded daily (germination rate), and the final germination percentage and rate were estimated.

The following parameters, previously reported by others such as Jefferson et al. (2003), were calculated for all four species:

A) Final germination (FG) %: The maximum average percentage of seeds that germinated during the experiment.

B) Mean period of final germination (MPFG) = $(\sum_{i=1}^{d} NiDi)/FG$

C) Rate of germination (RG) = $\sum_{i=1}^{d} \frac{Ni}{Di}$

D) Percentage inhibition or stimulation= (100-$\frac{FG\ in\ different\ solution(\%)}{FG\ in\ distilled\ water(\%)}$) Where,

N = daily increase in seedling number.

D = number of days from seed placement, the subscript i might be any integer value up through D.

At the end of eighth day, 5 seedlings were randomly selected and measured of the root, shoot and also seedling length of them. Additionally weight of oven dried (70^{o}C for 48 hours) of root and shoot of seedlings were measured. The experimental design with respect to three factors arranged in a completely randomized design with three replications of 25 seeds per replicate. The first factor (temperature) had three levels (20 ^{o}C, 25^{o}C and 30^{o}C), the second one had seven levels (0, -0.20, -0.40, -0.60, -0.80, -1 and -1.2 MPa).

RESULTS AND DISCUSSIONS

Results showed the final germination rate and percent germination of *B. juncea* var. Ensabi significantly affected by PEG 6000 (Table 1, Figure 1). By increasing osmotic potential of PEG 6000, percent, rate and final germination were decreased. In distilled water, percentage of seed germination was highest. The higher amount of PEG 6000 concentration in this research (10 and 12 MPa) completely inhibited seed germination.

The first physiological disorder, which takes place during germination, is the reduction in imbibitions of water by seeds which leads to a series of metabolic changes, including general reduction in hydrolysis and utilization of the seed reserve (Ahmad and Bano, 1992). Osmotic stress limit the mobilization of reserves in several species (Sidari et al., 2008).

Effects of osmotic (polyethylene glycol) stress on seed germination of 98 genotypes of *B. juncea* were investigated by Kuhad et al., 1989. This stress type significantly reduced germination percentage, dry matter weight, shoot and root length of seedlings. Signs of germination for control and low PEG 6000 osmotic potential occurred between 24 and 48 hours after sowing but at higher PEG 6000 osmotic solution it was being later (Figure 1).

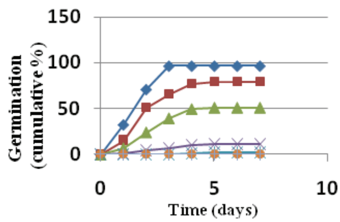

Figure 1. Cumulative mean percentage germination of *Brassica juncea* var. Ensabi seeds against time and decreasing external osmotic potentials of PEG 6000

Most complete germination occurred at 25^{o}C - 30^{o}C and these temperatures were the best temperature for germination percentage and 30^{o}C was the best temperature for velocity.

Germination percentages improved with an increase in temperature under different PEG 6000 osmotic solutions. In spite of Fallah Toosi and Baki (2007) reported temperature significantly affected seeds germination of *B. juncea* var. Ensabi, there was no significant statistical difference between different temperatures.

Seeds have the highest resistance to extreme environmental stresses, whereas germination is considered as the most sensitive stage and seedlings are most susceptible in the life cycle of a plant (Qu et al., 2007; Sidari et al., 2008). Therefore, successful establishment of a plant population is dependent on the adaptive aspects of seed germination and of early seedling growth (Qu et al., 2007).

Hypocotyl length was affected by PEG 6000 osmotic stress (Figure 2). The hypocotyl lengths of seedlings decreased with an increase in water stress. Shoots elongation significantly decreased by concentration of 2-8 MPa whereas no hypocotyl elongation at concentration of 10 and 12 MPa and shoot elongation completely inhibited (Figure 2).

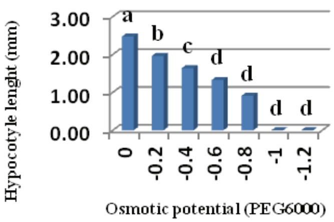

Figure 2. Mean shoot length in solutions of increasing PEG 6000 concentrations for *Brassica juncea* var. Ensabi. Different letters indicate statistically significant differences *(P* < 0.05) between different osmotic potentials (by Tukey's test)

Radicel elongation also significantly affected by PEG 6000 solutions (Figure 3). Results showed radicel growth was fast and by decrease PEG osmotic potential, radical elongation significantly increased. Low osmotic stress (-2 and -4 MPa) improves the root length of *B. juncea* var. Ensabi. These concentrations of PEG osmotic potential exhibited longer seedling roots and radicles were significantly longer to compare the control. Radical elongation declined by increasing concentration of the solution more than 4 MPa and completely inhibited at 10 and 12 MPa. It was not observed any significantly differences between control and 6 MPa PEG solutions (Figure 3).

These results agree with Murillo-Amador *et al.* (2002) in cowpea, Radhouane (2007) in pearl millet and Yagmur and Kaydan (2008) in triticale, they affirmed that a moderate and low osmotic stress (PEG) improves the root length of the seedlings.

Results showed growth of hypocotyl inhibited by increasing PEG 6000 solutions. It was no significantly differences between control and moderate of osmotic potential of PEG 6000 on shoot dry weight of *B. juncea* var. Ensabi (Figure 4).

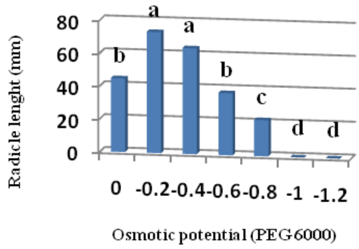

Figure 3. Mean root length in solutions of increasing PEG6000 concentrations for *Brassica juncea* var. Ensabi

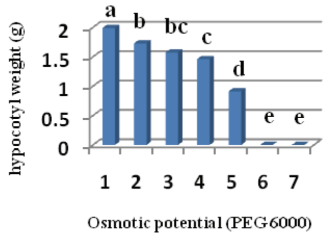

Figure 4. Mean hypocotyle weight in solutions of increasing PEG6000 concentrations for *Brassica juncea* var. Ensabi

Dry weight of Ensabi roots sharply decreased by increasing of PEG 6000 osmotic potentials. In spite of the length of roots that was significantly longer to compare with control in lower PEG solutions, the dry weight of roots in same solutions significantly lower to compare with control. This results indicated the roots that growth under drought were longer but they were very thin and delicate (Figure 5).

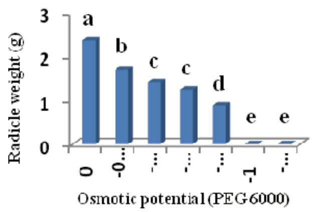

Figure 5. Mean radical weight in solutions of increasing PEG 6000 concentrations for *Brassica juncea* var. Ensabi

CONCLUSIONS

Drought is a major abiotic stress that plants encounter and can be responsible for the inhibition or delayed seed germination, poor seedling growth, and establishment.

The first physiological disorder, which takes place during germination, is the reduction in imbibitions of water by seeds which leads to a series of metabolic changes, including changed enzyme activities and general reduction in hydrolysis and utilization of the seed reserve (Ahmad and Bano, 1992). Upon imbibition, the quiescent dry seeds rapidly resume oxygen uptake and oxidative phosphorilation, processes required for supporting the high energy cost of germination (Baranova et al., 2006).

Osmotic stress examined in this work reduced the germination percentage of *B. juncea* var. Ensabi in respect to the concentration of PEG used. PEG, which is a non-penetrating agent, affects seed germination only by compromising water uptake. The marked differences in germination percentages observed with NaCl and PEG at the same osmotic potentials indicate specific ionic effects and point that germination is solely controlled by the osmotic potential.

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