INTERNATIONAL JOURNAL OF PLANT, ANIMAL AND ENVIRONMENTAL SCIENCES

IJPAES

Volume-6, Issue-2, April-June-2016 Coden: IJPAJX-CAS-USA, Copyrights@2016 ISSN-2231-4490

Received: 5th Jan-2016 Revised: 18th Feb 2016 Accepted: 20th Mar 2016

Research article

EFFECT OF OSMOPRIMING ON SEED GERMINATION AND SEEDLING VIGOUR IN *MACROTYLOMA UNIFLORUM* (LAM.) VERD C.

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ABSTRACT: Seeds of *Macrotyloma uniflorum*, a drought tolerant taxon, were osmoprimed in various concentrations of Polyethylene glycol – 6000 (PEG -6000) ranges from 5% to 25% having water potential of -0.05MPa to -0.73MPa. Percent germination, mean germination time and seedling vigour of one month old were determined to analyze the effect of water stress caused by osmopriming at various concentrations of PEG. Percent germination and MGT of osmoprimed seeds at 5% of PEG were found to be in par with the control. Decline in the percent germination, MGT and seedling vigour was recorded as the concentration of PEG increased. Effect of induced drought by PEG treatments on percent germination, MGT, Germination index and SVI is discussed.

Key Words: PEG, osmopriming, MGT, percent germination, seedling vigour index

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INTRODUCTION

Germination represents a critical event in plant life cycle and its timing largely predetermined the chances of survival of a seedling up to maturity [1]. Seeds are particularly vulnerable to stress encountered between sowing and seedling establishment. In arid and semi arid regions, drought serves as limitation for seed germination and subsequent growth. Many studies on seed germination and seedling vigour in response to induced drought have been reported in the past two decades [2,3,4,5]. Osmopriming of seeds in various osmoticums is one of the popular methods followed to study the effect of drought on germination. The most commonly used osmoticums are PEG, sugars and glycerol [6].

Macrotyloma uniflorum, a well known drought resistant crop, grows mostly under dry land agriculture. Though the seeds are utilized mainly as cattle feed, they are also consumed as other food legumes in human dietary since they are excellent source of iron and molybdenum. Except a report by Chauhan *et al.*, [7] on germination and seedling growth under the influence of various growth regulators, this taxon is totally underexploited with respect to priming the seeds and its effect on germination. Hence the main objective of the study is to evaluate the effect of osmopriming the seeds with PEG at various concentrations on germination and seedling vigour.

MATERIALS AND METHODS

Seeds of *Macrotyloma uniflorum* (Lam.) Verdc, var. PHG-9 were procured from GKVK, University of Agricultural Sciences, Bangalore.

Germination studies

The experiments to study percent seed germination, MGT and germination index were conducted in petriplates measuring 90mm in diameter. Two pieces of Whatman No.1 filter papers were used for each petriplate pair as substrate. Healthy 20 seeds were chosen from the lot and were washed with tap water. Then they were soaked in different concentrations of PEG -6000 ranging from 5 to 25 % for overnight. PEG solutions were prepared by dissolving the required amount in 100ml of distilled water. The osmotic potentials of these solutions were varied from -0.05 to -0.73MPa. Osmoprimed 20 seeds were plated in pair of petriplate and were incubated under ambient light and temperature. Seeds soaked in distilled water serve as control. Germination was considered to have occurred when the radicals were emerged from the seeds.

Experimental units were arranged factorial in a completely randomized design with five replications. The number of germinated seeds was recorded every day for 15 days.

Mean Germination Time (MGT) was calculated following the formula MGT= $\sum Dn/\sum n$ [8], where n is the number of seeds, which were germinated on day D and D is the number of days counted from the beginning of germination.

The germination index (GI) was calculated as described by the association of official seed Analysts [9] following the formula

$$GI = \frac{No.of\ germinated seed}{days of 1st count} + \frac{No.of\ germinated seed}{days of final count}$$

Percent germination was calculated as per the following formula after 15 days of observations.

$$Percent\ of\ germination = \frac{No.of\ seeds\ germinated}{Total\ No.of\ seeds} \times 100$$

Seedling vigour index was calculated using the following formula [10].

SVI= R+ S x G

Where R = average root length, S = average shoot length and G = germination percentage

Statistical Analysis

The data thus obtained was subjected to statistical analysis, one way ANOVA. Significant F ratios between the group means were subjected to least significant difference (LSD). Probability (p) values <0.05 were considered significant [11].

RESULTS AND DISCUSSION

Seed germination and early seedling growth are critical stages for plant establishment and seeds are more sensitive to drought stress during these stages [12]. Not much information is available on the mechanism of osmotic tolerance in crop plants especially grain legumes which are considered to be the most sensitive to water and salt stress [13].

Success of PEG treatments in controlling germination process during the incubation and maintaining high percent germination appears to be highly species dependent [2]. The osmotic potential and priming duration had significant effect on germination percent, MGT and GI in the germination studies on the seeds of soybean [3]. Even in the present study, germination was significantly affected by the osmotic potential. As shown in Fig 1, an increase in PEG concentration had markedly decreased the percent germination. 75.67% of germination was recorded which was nearer to the control (75.82%) at the concentration of 5% PEG. The daily germination data showed that the seeds were germinated more quickly in the first two days under mild drought stress. However, the maximal germination was delayed in gradual increasing stress intensity. A significant decline in the percent germination was recorded at 15% of PEG indicating that PEG at 15% with an osmotic potential of -0.30MPa is a threshold value for the good germination of seeds of *M. uniflorum* which was in conformity with the results of Li *et al.,* [5] in *Eremosparton songoricum*, a drought resistant desert plant(Table 1). Threshold value for good germination in other desert plants was reported to be -1.2MPa [14] which was lower than the present result which indicates the higher water demand for germination in *M. uniflorum*. The higher moisture requirement reflects the limited ability of *M. uniflorum* to resist drought stress during the germination stage as reported for *E. songoricum* by Li *et al.,* [5].

Table1. Effect of Osmopriming on *Macrotyloma uniflorum* seeds

Treatment %	Osmotic potential (MPa)	Percentage of germination	Germination index	MGT	Seed vigour index
Control	0MPa	75.82±0.85[a]	13.5±0.44[a]	1.0±0.23[a]	25.01±0.21[a]
5	-0.05	75.67±0.75[a]	14.10±0.32[a]	1.0±0.44[a]	19.70±0.66[b]
10	-0.15	62.34±0.56[b]	16.22±0.58[b]	1.0±0.74[a]	18.91±0.32[b]
15	-0.30	25.87±0.47[c]	22.10±0.22[d]	3.0±0.68[b]	16.09±0.47[c]
20	-0.49	14.54±0.52[d]	18.66±0.65[c]	5.0±0.25[c]	12.54±0.54[d]
25	-0.73	9.34±0.41[e]	17.83±0.44[c]	5.0±0.77[c]	9.84±0.84[e]

The superscribed alphabets indicate the number of ranges indicating values of %, GI, SVI, MGT values having same alphabets did not differ significantly as determined by LSD ($p<0.05$)

Rosner and Harrington [2] have reported the decline in percent germination at the osmotic potentials of -0.18 and -0.37MPa in their studies on seed germination in *Shepherdia canadensis*. In several other crops like Italian rye grass, *Sorghum*, rice and *Lens culinaris* osmotic priming is however has increased the percent germination and higher than those of unprimed seeds [15,16]. The increased rate of germination by priming was attributed to the partial accomplishment of pre germinative metabolic activities [17,18, 4, 16, 19]. Osmopriming has been shown to activate processes related to germination, for instance by affecting the oxidative metabolism such as increasing superoxide dismutase and peroxidase [20] or by the activities of ATPase as well as acid phosphotase and RNA synthesis [21].

Mean germination time (Fig.2.) was also affected by osmotic potential. As the concentration of PEG increases, correspondingly MGT also enhanced. Maximum MGT of 5.0±0.77 was recorded at the osmotic potential of -0.73MPa (25%). However MGT of 1.0±0.23 was recorded in control. Sadeghi *et al.*, [3] have reported less MGT from seed osmoprimng treatments than control in soybean. They are of the opinion that the observed least MGT in osmoprimed seeds is due to the completion of pre germination metabolic activities during priming period. PEG treatments also had inconsistent effects on germination speed (MGT) in a few North American shrub species [2]. However MGT was dependent on the incubation period and concentration of PEG in these species.

Germination Index (GI) was significantly ($p<0.05$) affected by induced drought by PEG treatments (Fig. 3.). Highest GI was attained at -0.30MPa of osmotic potential, an indication for threshold value for good germination. Germination was initiated after 24 hours of incubation and reached maximum percent between 3rd and 4th day and then declines. However, in *Glycine max*, the highest germination index was reported at -1.2MPa and decreased as the osmotic potential reduced [3].

Seedling vigour index (SVI) also depends on the osmotic potential. As the osmotic potential increases, the seedling vigour index shows decline. However, highest SVI was recorded in control plants followed by -0.05MPa which was caused by treating the seeds with 5% PEG (Fig.4). However, SVI was more than control in *Glycine max* and highest was achieved from -1.2MPa and 12hrs seed priming duration treatment [3]. The highest SVI found in primed seeds was attributed to the repairs of damage to membrane caused by deterioration / seed erosion and improved seed quality by priming [22, 23, 3] have correlated the enhanced SVI with electrical conductivity (EC). Highest EC was related to control treatment, where as the lowest EC was attained at -1.2MPa among primed treatments. Basra *et al.*, [18] and Esmeili and Heidarzade [4] have suggested that increased seed germination and SVI in rice due to osmopriming is a result of enhanced synthesis of superoxide dismutase and peroxidase as reported by Jie *et al.*, [20] or by the activation of ATPase, acid phosphotase and RNA synthesis as observed by Fu *et al.*, [21]. Islam *et al.*, [19] also reported that seed priming with KCl had shown highest SVI compared to control in rice.

Hydropriming significantly improved germination and seedling growth under both stress and non stress conditions [24] in the inbred lines of maize. Janmohammadi *et al.*, [24] are of the opinion that hydropriming could alternate the effects of salinity and also drought stress on germination and seedling early growth.

The aforesaid results obtained in the present investigation, clearly demonstrate that the induced drought stress affects the percent germination, MGT, GI and SVI depending on the various osmotic potentials caused by different concentrations of PEG. Drought has a suppression effect on the seed germination in *E. songoricum* [5] whereas the slight stress (5%) has a promoting effect as observed in the present studies. Li *et al.* [5] are of the opinion that these characteristics are the indications of adaptive strategy to harsh environment and physiological response at the seed germination stage which are mainly dependent on the accumulation of osmolytes such as proline and soluble sugars.

It can be concluded that reduced germination under water stress may be an adaptive strategy to develop an osmotically enforced dormancy to prevent germination under stressful environment ensuring proper establishment of seedlings**.**

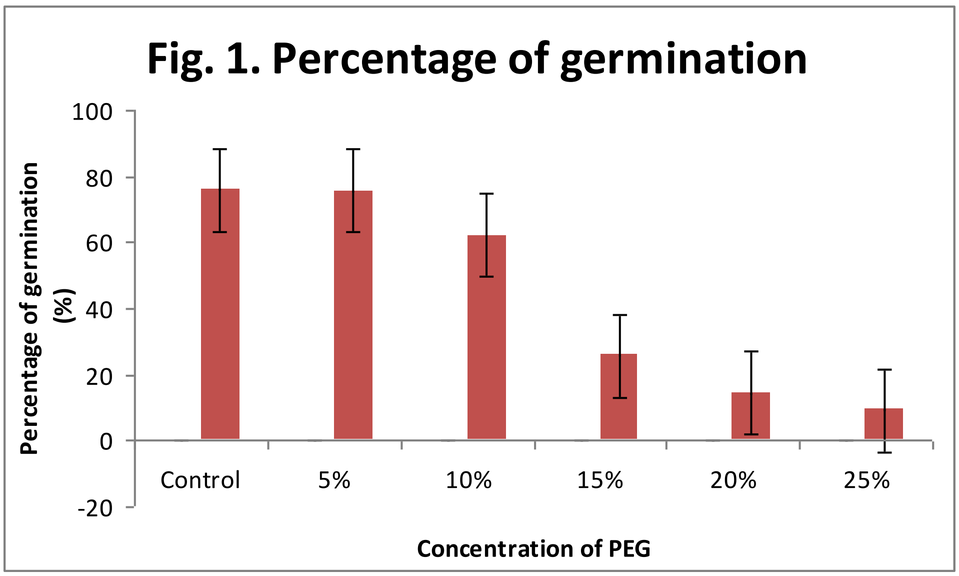

Fig-1: Percentage of germination

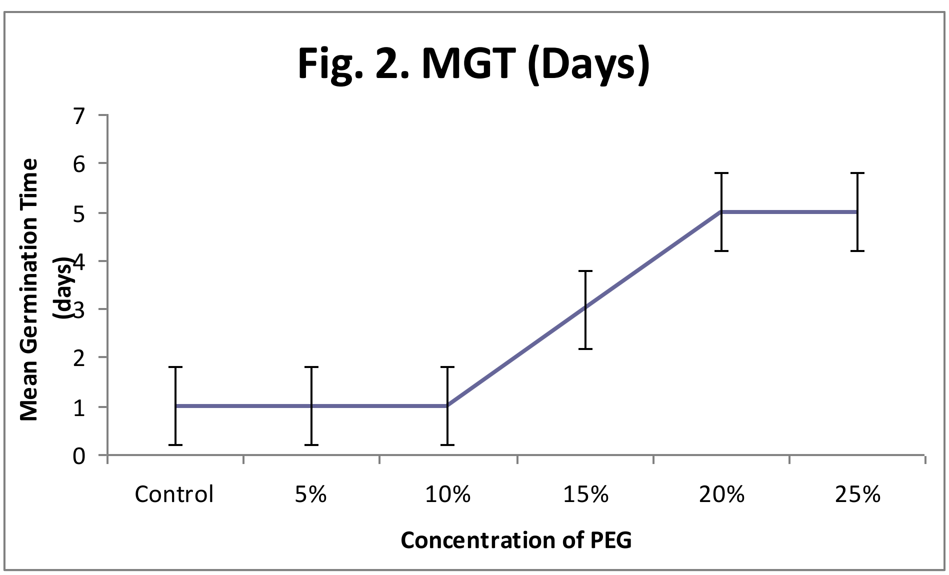

Fig-2: MGT (Days)

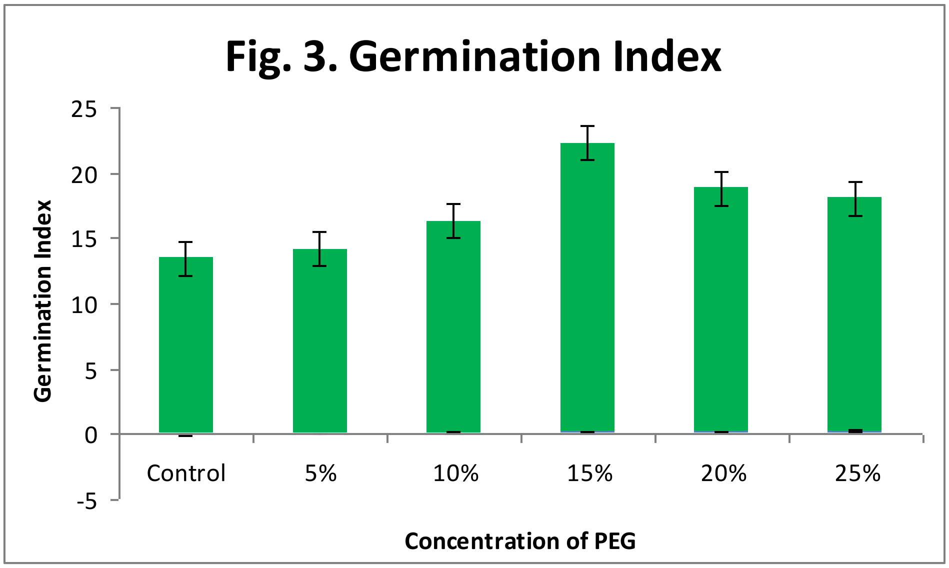

Fig-3: Germination index

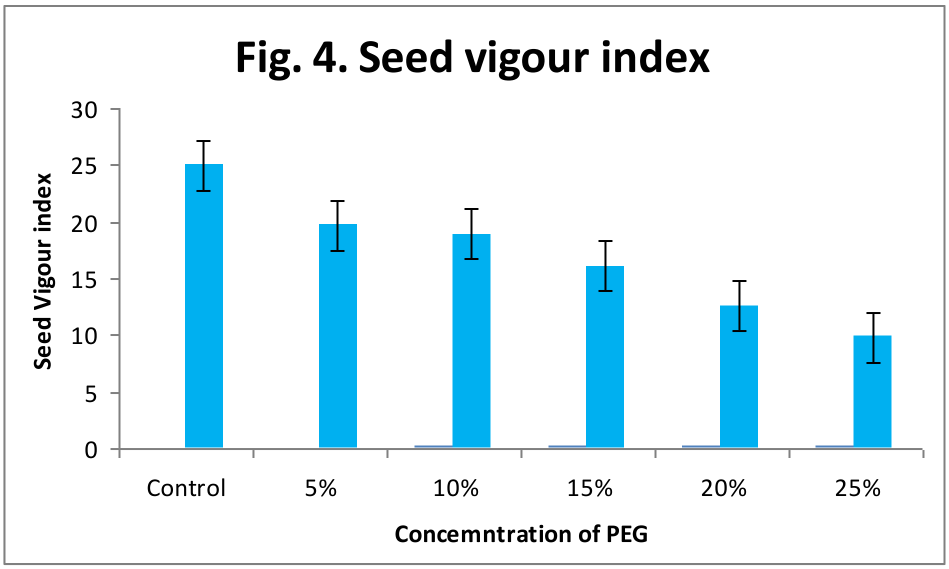

Fig-4: Seed vigour index

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INTERNATIONAL JOURNAL OF
PLANT, ANIMAL AND ENVIRONMENTAL SCIENCES

ISSN 2231-4490

International Journal of Plant, Animal and Environmental Sciences