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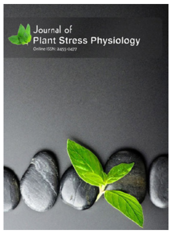
Research Article



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Impacts of drought and herbicide stress on seed germination and early seedling growth in a legume crop mung bean (*Vigna radiata* L.)

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ABSTRACT

Abiotic stress such as drought and agrochemicals leads the drastic reductions in legume yields, hence, in order to mitigate the loss of yields due to abiotic stresses, production of stress tolerance genotypes of the legumes could be a rewarding approach. Therefore, the aim of the present study was to evaluate the drought and herbicide stress effects under *ex-vitro* conditions on seed germination and early seedling growth in a legume crop mung bean (*Vigna radiata* L.). To begin with, drought stress was induced by employing various concentrations of mannitol (50mM, 100mM, 250mM, 500mM, 750mM, and 1000mM) and polyethylene glycol (PEG-5%, 10%, 15%, 20%, and 25%) while during herbicide stress experiments, dicamba (3,6-dichloro-2-methoxybenzoic acid) and picloram (4-Amino-3,5,6-trichloro-2-pyridine carboxylic acid) were considered in various concentrations (5mg/L, 25mg/L, 50mg/L, and 100mg/L) of each. Moreover, data were collected as partial and full seed germination after 3- and 7-days of stress treatments respectively. After 7-days of mannitol stress treatments, results reveals that even the high concentration of mannitol (500mM) could be proved as weak osmotic stressor for seed germination (35%±0.14) in comparison to control (91%±0.74) while further increase in mannitol concentration (750mM) was proved to be lethal, toxic and inhibits seed germination completely. Furthermore, in comparison to mannitol, PEG turns out as strong osmotic stressors and (15%) of PEG was proved to very lethal for seed germination. Even during early seedling growth, increased concentrations of both mannitol and PEG were found to be inhibitory. Among two herbicides, the result shows that both herbicides (dicamba and picloram) were proved to be completely toxic and lethal even at very low concentration (5mg/L) and induced abnormal seed germination and inhibited completely seedling growth. However, inhibitory response of picloram herbicide stress on seed germination was found to be more pronounced and severe than dicamba herbicide. Hence, present study reveals that in comparison to mannitol, PEG turns out as strong osmotic stressor while picloram proves to be relatively more toxic herbicide than dicamba for seed germination. Additionally, drought stress induced seedlings on transfer to soil exhibit inhibited growth under continuous irrigation with either mannitol or PEG solutions.

KEYWORDS: Seed germination; Mung bean; Osmotic stress; Herbicide stress

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INTRODUCTION

Pulses have been treated as the most cultivated and consumed legumes and some of them such as cowpea (*Vigna unguiculata*), green gram (*Vigna radiata*), black gram (*Vigna mungo*), pigeon pea (*Cajanus cajan*), lentil (*Lens culinaris*), and grass pea (*Lathyrus sativus*) are also being grown as alternative staple crops particularly in marginal and harsh areas of the world (Deikman *et al*., 2012; Ara´ujo *et al*., 2015). Pulses are known as the major source of proteins in the vegetarian diet of Indian population. Additionally, pulses are the preferred crops in Indian agriculture by contributing to the soil with nutrients for the next cycle crops (Mittler, 2006).

Unfortunately, various abiotic stresses are known to cause huge damage to legume crops and reduce yields all over the world. In general, drought, salinity, water logging, extreme temperatures and heavy metals are known to impose serious abiotic stress constraints to plant growth and development leading to drastic reduction in yield for major crop plants (Bray *et al*., 2000).

However, in recent past, it has been experienced that salinity and drought stresses minimize the food and fodder production worldwide and continuous increase in dry climatic conditions globally causes frequent occurrence of drought even on fertile and agriculture lands (Hu & Schmidhalter, 2005; Lieu *et al*., 2015). In general, these stresses have been considered as the primary causes of legume crops failures in India. Significantly,

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pulses are known to have narrow genetic base and thus in order to incorporate desirable characters against abiotic stresses, use of alternative technology or transgenic production for improving legume crops has been also suggested (Singh *et al.*, 2016).

Drought stress has been found to affect adversely the seed germination, plant growth and development (Ashraf *et al.*, 2002; Almaghrabi & Abdelomoneim, 2012; Vibhuti Shahi *et al.*, 2015) and eventually cause heavy reduction in crop yields. Production of crops that could be resistance to severe drought would be always significant; however, increasing crop yield in drought-prone areas always requires the optimization of the physiological processes involved in plant response to soil water crisis.

Additionally, in modern agriculture system, herbicides and pesticides are commonly being used to increase in crop productivity in terms of both yield and quality but their excessive and improper use cause a big burden for the desired crops and environment both. Moreover, it is believed that some of these toxic chemical compounds persist in plant and its products (Lyndon & Darlington, 1998; Jame *et al.*, 1999) and further in turn cause harmful effects on both animal and human health (Castro *et al.*, 2005).

It is further reported that herbicides cause negative impacts on photosynthesis in growing crops and moreover, use of excess herbicides shows a considerable reduction in photosynthetic pigment synthesis, carbon assimilation and efficiency of pigment system II (PSII) (Nabiha *et al.*, 2014). However, screening of herbicide tolerant genotypes in cultivated crops has been poorly investigated including pulse crops in general and mung bean in particular. During evaluation of the initial development of bean seedlings, low doses of glyphosate were found to inhibit radicle and hypocotyls formation (Oliveira *et al.*, 2013). Of late, the presence of glyphosate herbicide doses was found to reduce common bean seed germination and vigor (Ávila *et al.*, 2020).

Furthermore, germination and seedling development is known as very important requirement for early establishment of plants under stress condition and therefore, selecting varieties for rapid and uniform germination under abiotic stress conditions can contribute towards early seedling establishment. Hence, based on the importance of legume crops and gradual reduction in pulse yields due to various abiotic stresses, present study was undertaken to evaluate the physiological and morphological responses of mung bean (*Vigna radiata* L.) on seed germination and early seedling growth under water and herbicide stress conditions.

MATERIALS AND METHODS

Seed Collection and Sterilization

Seeds of mung bean (*Vigna radiata* L.) a wild cultivar, were collected from PASIC, Puducherry (India). Healthy and uniform seeds of mung bean were selected and washed thoroughly with teepol-20 and further were surface sterilized with ethanol (70%) for a minute followed by $HgCl_2$ (0.1%) treatments for 7-8 minutes.

Stress Treatments

Drought stress

Various concentrations of mannitol solutions (50mM, 100mM, 250mM, 500mM, 750mM, and 1000mM) and polyethylene glycol (PEG-6000) solutions (5%, 10%, 15%, 20%, and 25%) were employed to treat the seeds in order to induce water or osmotic stress.

Herbicide Stress

To understand the sensitivity response of herbicides on mung bean seed germination and early seedling growth; dicamba (3, 6-dichloro-2-methoxybenzoic acid) and pictogram (4-amino-3, 5, 6-trichloro-2-pyridine carboxylic acid) were employed in various concentrations (5 mg/L, 25 mg/L, 50 mg/L, and 100 mg/L) for each herbicide.

Further, sterilized seeds were washed 3-4 times with distilled water and were soaked in the respective concentrations of stress inducing solutions (mannitol, PEG, dicamba, and picloram) for 3hrs. The soaked seeds were further transferred in sterile petridishes (9.0cm diameter) lined with two sterile filter papers with 5.0ml of distilled water as control experiment or the respective test solutions for drought (mannitol and PEG) and herbicide (dicamba and picloram) stress experiments.

Furthermore, 10-15 seeds per petridish were considered for each treatment and two replicates in each treatment were performed. Germination tests were conducted under dark condition at normal room temperature (25-30^{0}C). A seed was considered germinated when radicle was 2 mm long. The germination percentage was determined counting the number of germinated seeds on the 3rdday for partial seed germination and 7th day of the treatments for the complete seed germination.

Transplantation of Seedlings to Soil

To begin with 7-days old germinated seedlings growing in sterile petridishes (9.0cm diameter) lined with two sterile filter papers supplemented with respective stress (mannitol and PEG) solutions were transferred to disposable glass contained with autoclaved soil. Further, transplanted seedlings were kept on irrigation with respective concentrations of stress (mannitol/PEG) solutions and observations were recorded.

Statistical Analysis

Statistical data were performed after first count (3rd day after treatments) and final count (7th day after treatments). Moreover, germination percentage (GP) and germination rate (GR) was calculated by the following formulae (Ruan *et al.*, 2002); GP = Number of total germinated seeds/Total number of seeds tested ×100; GR= Number of Germinated seeds/3rd Day of Count+ Number of Germinated seeds/7th Day of Count.

During this study, all the treatments were repeated two times and data are expressed as mean germination (%) and shoot-root lengths (cm) with standard error (S.E.). Moreover, the statistical analyses were generated by applying SPSS statistical software package.

RESULTS

Effects of Drought Stress

Seed germination and growth of mung bean seedlings were significantly affected by drought stress induced by various concentrations of mannitol and PEG. Moreover, variations in green gram seed responses were based on concentrations of stressors and also on durations of treatments.

Effect of mannitol-stress on seed germination

In control experiment, seeds were found to exhibit the indication of germination next day of the treatments but the complete emergence of radicle and plumule could be seen at the end of 3rd day of treatment. Moreover, after 3rd day of treatments, in control experiment and lower concentration of mannitol treated solutions (50mM and 100mM); seeds were found to exhibit quick germination with full emergence of radicle and plumule while with higher mannitol concentrations (250mM and 500mM), seeds could show relatively slow and inhibited germination. However, at very high concentrations of mannitol (750mM and 1000mM), initiation of seed germination was completely lacking up to 3rd day of treatments.

At the end of 7th day of treatments, seeds normally germinated and developed into the healthy seedlings (Figure 1A) and moreover, the seeds that were growing with lower concentrations of mannitol (50mM and 100 mM) solutions were found to show similar responses and seedlings could be found similar to control treatments (Figure 1B). However, further increase in concentration of mannitol (250mM) could show slight inhibitions in seed germination and seedling growth (Figure 1 C).

Significantly, seeds that were treated with further high concentration (500mM) of mannitol solution were seen to exhibit strong reduction and inhibitions in seed germination and seedling growth (Figure 1 D). Moreover, these poorly grown seedlings were failed to grow further into mature seedlings even after 10-days of treatments and finally died. Remarkably, further increase in mannitol concentrations (750mM and 1000mM) were proved to be toxic and seed germination was completely inhibited even after 10-days of mannitol treatments.

Effect of PEG-stress on seed germination

Similar to mannitol response, seed germination initiation could be observed within 3rd day of PEG treatments particularly with the lower concentrations (5% and 10%) of PEG solutions, while seeds treated with further high concentration (15%) of PEG solutions were failed to show germination initiation; however, emergence of radicle only could be seen rarely.

After 7th day of PEG treatments, seeds growing in very low concentration of PEG (5%) solution were found to be almost similar to the control seedlings (Figure 1 E & A) respectively indicating the non-inhibitory role of PEG to induce water stress inhibition on seed germination while further increase in PEG concentration (10%) was found to be slightly inhibitory in terms of seedlings elongation (Figure 1 F). However, strong inhibition on seed germination due to PEG induced water stress could be visible in the seeds that were treated with further high concentration (15%) of PEG solutions (Figure 1 G).

Rate of seed germination under mannitol- stress

During mannitol stress treatments, mean percentage of seed germination was found to decline with the increase in mannitol concentrations in the treatment solutions and therefore, at lower concentrations of mannitol (50mM and 100mM), the germination mean was recorded to be (74%±0.71 and 69%±0.35) respectively in comparison to the control experiment (91%±0.74) after 7-days of treatments (Table 1). Further, increase in mannitol concentrations (250mM and 500mM) were proved to cause relatively more inhibitions, therefore, the rate of seed germination was recorded as (50%±0.21and 35%±0.14) respectively while with very high concentrations of mannitol (750 mM and 1000 mM), seed germination was completely inhibited.

Rate of seed germination under PEG-stress

After 7th day of treatments, mean percentage of seed germination could be recorded as (81% ±0.35) and (79%±0.28) in case of seeds that were treated with lower concentrations (5% and 10%) of PEG solutions respectively in comparison to control experiment (91%±0.74) (Table 2). This result indicates a very slight reduction in mean percentage of seed germination due to osmotic stress inhibition caused by the lower concentrations of PEG (5% and 10%).In contrast, higher concentrations (15% and above) of PEG were proved to inhibit seed germination strongly.

Effects of Herbicide Stress

During present study, responses of mung bean during seed germination and growth of seedlings were significantly affected by various concentrations and durations of dicamba and picloram herbicides.

Effect of herbicide dicamba- stress on seed germination

Similar to control experiments, the seeds that were treated with lower concentrations of dicamba solutions (5mg/L and 25mg/L) were found to show the beginning of germination at the end of 3rd day of treatments but these germinated seeds were further found to be abnormal in terms of inhibition in hypocotyl elongation and gradually appeared to be swollen. However, in contrast seeds that were growing with high concentrations of dicamba (50mg/L and 100mg/L) solutions could show merely the proliferation of cotyledons and emergence of radicles.

Figure 1: (A-L)–*Vigna radiata* L., Effects of drought (mannitol and PEG) and herbicide (dicamba and picloram) stress on seed germination in mung bean after 7-days of treatments. (A) Control (B) Mannitol-100mM (C) Mannitol-250mM (D) Mannitol-500mM (E) PEG-5% (F) PEG-10% (G) PEG-15% (H) Dicamba-5mg/L (I) Dicamba-25mg/L (J) Dicamba-50mg/L (K) Picloram-5mg/L (L) Picloram-25mg/L

Table 1: Effect of various concentrations of mannitol induced drought stress on seed germination and early seedling growth in mung bean (*Vigna radiata* L.)

S. No.	Concentration of Mannitol (mM)	3rd day	7th day	Shoot Length (cm)	Root Length (cm)
		Mean Germination (%) ± S.E.	Mean Germination (%) ± S.E.	Mean ± S.E.	Mean ± S.E.
1	Control	69±0.35	91±0.74	5.55±0.47	4.28±0.37
2	50	35±0.21	74±0.71	4.5±0.81	3.17±0.71
3	100	33±0.14	69±0.35	3.84±0.45	2.41±0.76
4	250	15±0.07	50±0.21	3.56±0.51	1.97±0.62
5	500	2±0	35±0.14	0	0.28±0.88
6	750	0	0	0	0
7	1000	0	0	0	0

At the end of 7th day of treatments, normal seed germination was not seen in the dicamba treated seeds and instead abnormal swelling in hypocotyl was apparent in poorly germinated seeds. Results further indicate that herbicide dicamba even at a very low concentration (5mg/L) proves to be toxic and inhibits the normal pattern of seed germination (Figure 1H). Remarkably, the deformation or morphological abnormalities in germinating seeds were found to be more prominent and severe in seeds that were treated with higher concentrations (25mg/L and 50mg/L) of dicamba (Figure 1I & J)solutions respectively. Moreover, even after 10-days of dicamba herbicide treatments, these abnormally germinated seeds were failed to grow into normal seedlings and finally necrosed.

Effect of herbicide picloram- stress on seed germination

Similar to dicamba herbicide response on mung bean seed germination, lower concentrations (5mg/L and 25mg/L) of picloram herbicide treatments also were found to show beginning of seed germination but immediately exhibited inhibitions in elongation of radicle and plumule even within 3rd day of treatments. However, with further high concentration of picloram (50mg/L) treatment, few seeds could exhibit the proliferation of cotyledons and emergence of radicles while majority of the treated seeds were found to be non-responsive. Moreover, strong inhibition in seed germination was seen in the seeds that were treated with the very high concentration (100mg/L) of picloram solution and seeds were completely failed to show even the symptoms of germination initiation.

At the end of 7th day of treatments, poorly germinated seeds were found to exhibit the inhibitions in elongation of radicle and plumule leading to abnormal appearance in the morphology with all the tested concentrations of picloram solutions. Moreover, morphological abnormalities in germinating seeds were also visible in seeds that were treated with lower concentrations (5mg/L and 25mg/L) of picloram herbicide (Figure 1 K & L). Additionally, with higher concentrations (50mg/L and 100mg/L) of picloram treatments, inhibitions in germinating seeds were found to be more prominent.

Significantly, these partially and abnormally germinating seeds were failed to grow into normal seedlings and finally necrosed after 10-days of picloram treatments. Results indicate that similar to dicamba herbicide, picloram also exhibits inhibitory response even at a very low concentration (5mg/L), however, picloram shows more toxicity than dicamba and alters the metabolic processes critically that are required for seed germination.

Rate of seed germination under herbicide stress

In case of control treatment, germination percentage was found to be (69%±0.35 and 91% ±0.74) and, moreover, seedlings after 3rd day and 7th day of the treatments respectively whereas, it was found to be zero with both dicamba and picloram solutions with the very low concentration (5mg/L). Moreover, both herbicides were found to exhibit very strong inhibitions in seed germination and altered the seeds to germinate with abnormal morphology (Tables 3 & 4).

Effect of mannitol stress on early seedling growth

Growth and height of the seedlings were significantly affected by water stress caused by mannitol and PEG. In case of mannitol treatments, seedling growth was found to be similar to the control seedlings particularly at low (50mM) concentration of mannitol (Figure 2 A) and shoot-root length ratio was recorded as (4.5cm ±0.81/3.17cm ±0.71) in comparison to control (5.55cm ±0.47/4.28cm ±0.37) (Table 1).

However, the seedlings that were growing with mannitol (100 mM) solutions were found to be slightly shorter and less elongated (3.84cm ±0.45/2.41cm ±0.76) than the control seedlings (Figure 2 B). With further high concentration of mannitol (250mM), seedlings lengths as shoot-root ratio (3.56cm ±0.51/1.97cm ±0.62) were recorded and seedlings were appeared to be very slow and strongly inhibited (Figure 2 C). Significantly, poorly germinated seedlings without shoot (0.0cm/0.28cm ±0.88) growing with mannitol (500 mM) solutions were failed to develop into mature seedlings.

Effect of PEG stress on early seedling growth

In contrast to mannitol treatments, PEG treatment (5%) exhibited normal seedling growth (3.84cm ±0.45/2.34cm ±0.29) at the end of 7th day of treatments and treated seedlings were appeared to be the similar to the control (5.55cm ±0.47/4.28cm ±0.37) seedlings (Table 2, Figure 2 D). While further increase in PEG concentration (10%), was found to be slightly inhibitory for seedling growth and seedlings lengths as shoot-root ratio (3.11cm ±0.71/2.65cm ±0.7) were found to be little shorter (Figure 2 E). However, seeds that were treated with high concentration (15%) of PEG solution could show merely the emergence of radicle and were failed to grow further into seedlings (Figure 2 F).

Effects of drought stress during early seedling growth in soil

Hence, in comparison to control seedlings, there were gradual inhibitions in seedlings elongations growing with mannitol solutions. Further, 7-day-old seedlings were transferred to soil

Table 2: Effect of various concentrations of polyethylene glycol (PEG) induced drought stress on seed germination and early seedling growth in mung bean (*Vigna radiata* L.)

S. No.	Concentration of PEG (%)	3rd day	7th day	Shoot Length (cm)	Root Length (cm)
		Mean Germination (%) ± S.E.	Mean Germination (%) ± S.E.	Mean ± S.E	Mean ± S.E.
1	Control	69±0.35	91±0.74	5.55±0.47	4.28±0.37
2	5	42±0.14	81±0.35	3.84±0.45	2.34±0.29
3	10	33±0.07	79±0.28	3.11±0.71	2.65±0.7
4	15	0	0	0	0
5	20	0	0	0	0
6	25	0	0	0	0

Table 3: Effect of various concentrations of dicamba herbicide induced stress on seed germination and early seedling growth in mung bean (*Vigna radiata* L.)

S. No.	Concentration of Dicamba (mg/L)	3rd day	7th day	Shoot Length (cm)	Root Length (cm)
		Mean Germination (%) ± S.E.	Mean Germination (%) ± S.E.	Mean ± S.E	Mean ± S.E.
1	Control	69±0.35	91±0.74	5.55±0.47	4.28±0.37
2	5	0	0	0	0
3	25	0	0	0	0
4	50	0	0	0	0
5	100	0	0	0	0

and were given treatments with respective concentrations of mannitol solutions. Significantly, gradual inhibitions during early seedling growths could be observed in seedlings that were growing with increased concentrations of mannitol (50mM, 100mM and 250mM) solutions (Figure 3 A, B & C) respectively in soil.

During PEG treatments also, gradual inhibitions in shoot and root elongations were seen in the seedlings that were treated with the increased PEG concentration solutions. To begin with, 7-day-old seedlings treated with PEG solutions (5% and 10%) were transferred to soils and further seedlings were irrigated with respective PEG solutions (Figure 3 D & E) respectively. Moreover, after 10-days of treatments, early seedling growths were found to be strongly inhibited in case of PEG (10%) treated seedlings (Figure 3 E).

DISCUSSION

Abiotic stresses like drought, water logging, high salinity, heavy metals, excess heat, frost, and ultraviolet-B light irradiance (UV-B) have been extensively known in plants (Mittler, 2006; Barnabás *et al*., 2008; Bhargava *et al*.,2008; Munns & Tester, 2008; Bhargava *et al*., 2012; Hasanuzzaman *et al*., 2013; Pereira, 2016; Suzuki *et al*., 2014; Hinojosa *et al*., 2018). Additionally, it is also realized that the uncontrolled use of fertilizers, pesticides and weedicides in the agricultural lands affect crop productivity and also convert cultivable lands to unfertile and barren.

Effects of Drought Stress

It is documented that drought problems for mung beans are already under the worsening state with the rapid expansion of water stressed areas of the world by 2030 (Postel, 2000). Moreover, mung bean yield is more dependent on an adequate supply of water than on any other single environmental factor (Kramer & Boyer, 1997). Additionally, mung bean cultivation has been known to undergo significant drought period particularly in low country dry zone which is the major constrain of growth and pod filling (Ranawake *et al*., 2011).

Further, it is argued that dehydration avoidance and desiccation tolerance could be the major mechanisms by which plants resist drought stress (Beebe *et al*., 2013). Moreover, it is suggested that dehydration avoidance involves the amelioration of drought effects by reducing either the water deficit or the crop's exposure to it, and also increases the plant's potentials to maintain its water status during soil water shortage conditions. Further, early maturity could be the one of the most common and partial

Table 4: Effect of various concentrations of picloram herbicide induced stress on seed germination and early seedling growth in mung bean (*Vigna radiaata* L.)

S. No.	Concentration of Picloram (mg/L)	3rd day	7th day	Shoot Length (cm)	Root Length (cm)
		Mean Germination (%) ± S.E.	Mean Germination (%) ± S.E.	Mean ± S.E.	Mean ± S.E.
1	Control	69±0.35	91±0.74	5.55±0.47	4.28±0.37
2	5	0	0	0	0
3	25	0	0	0	0
4	50	0	0	0	0
5	100	0	0	0	0

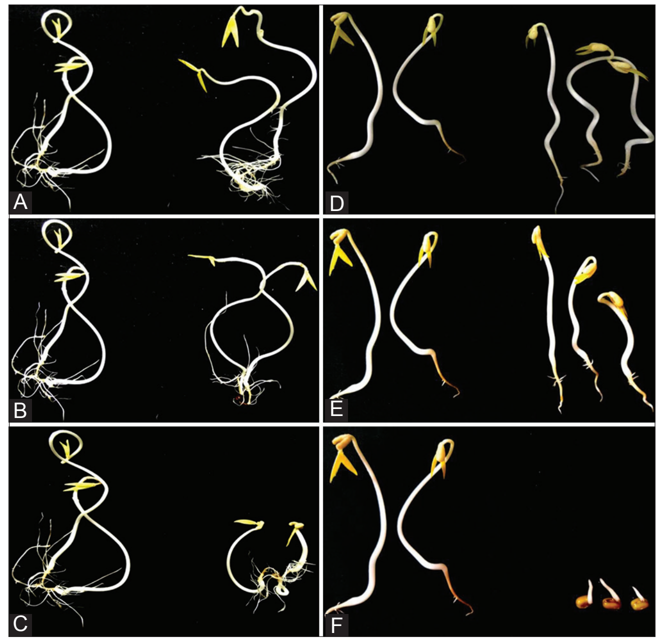

Figure 2: (A-F)–*Vigna radiata* L., Effects of drought (mannitol and PEG) stress on seedling growth in mung bean after 7-days of treatments. (A) Control+Mannitol-50mM (B) Control+Mannitol-100mM (C) Control+Mannitol-250mM (D) Control+PEG-5% (E) Control+PEG-10% (F) Control+PEG-15%.

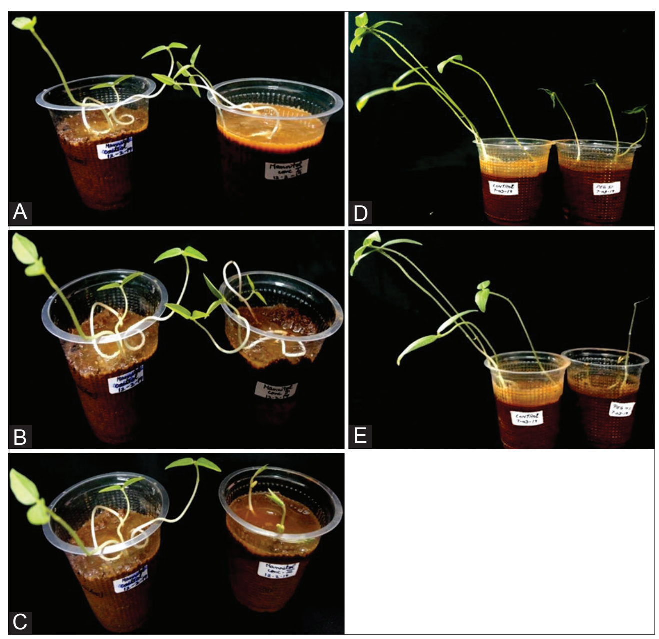

Figure 3: (A-E)–*Vigna radiata* L., Drought (mannitol and PEG) stress treated mung bean seedlings are growing in soil supplemented with respective stress solutions after 10-days of treatments. (A) Control+Mannitol-50mM (B) Control+Mannitol-100mM and (C) Control+Mannitol-250mM (D) Control+ PEG-5% (E) Control+ PEG-10%

escape mechanisms to minimize the exposures of crops to terminal drought (White & Singh, 1991).

It was further documented that photosynthesis together with cell growth, is among the primary processes to be affected by drought (Chaves, 1991). Additionally, impact of water deficit on carbon metabolism results in changes in the pool of sugars used for signaling cellular processes or substrates for biopolymers like cellulose, starch and proteins (Chaves *et al*., 2009; Liu *et al*., 2013). Similarly, the scarcity of water may exert an adverse effect upon seed germination and embryo growth rate in the field; however, several sorghum cultivars have been reported that are adapted well to semi-arid areas (Patane *et al*., 2013).

Moreover, in soybean seed as a model legume, several studies have been performed under greenhouse controlled conditions to understand the physiological mechanisms activated by drought on flowering, pod abortion, pod expansion, seed yield and individual seed weight (Desclaux & Roumet, 1996; Desclaux *et al*., 2000; Liu *et al*., 2003, 2004).

During present study, mannitol at lower concentrations (50mM and 100mM) were recorded as insufficient to cause water stress, thus seed germination and seedling growth was slightly affected adversely in comparison to the control seedlings. Moreover, mannitol even with high dose concentration (500mM), was found to be significantly ineffective to inhibit seed germination indicating that mannitol is either not competent enough to induce osmotic stress during seed germination or the cultivar is significantly tolerant to osmotic stress. However, results reveal that very high concentration (750mM) of mannitol proves to be fully lethal and shows toxicity for mung bean seed germination.

Additionally, a non-penetrating osmotic agent polyethylene glycol (PEG) is known to reduce the water potential of the medium and moreover has been used extensively to stimulate drought stress in plants (Smith *et al*., 1986). During present study, in contrast of mannitol, polyethylene glycol (PEG) proves to be strong stressor and PEG (15%) could be proved strongly effective to show the water stress effects and inhibits seed germination completely while in contrast, lower concentrations (5% and 10%) were proved to be inefficient to cause water stress and inhibitions in seed germination.

In an earlier study on *in vitro* regeneration, mannitol is known as neither supports *in vitro* tissue growth nor is it metabolized by higher plants and moreover, in comparison to PEG, mannitol is found as ineffective to stimulate somatic embryogenesis. Present study is also in conformity with the statements that mannitol causes a less stress effects on the germination and seedling growth in cowpea than PEG (Murillo-Amador *et al*., 2002). However, in contrast, mannitol was found to cause severe damage during seed germination by inducing water stress in sugar beet (Sadeghian & Yavari, 2004).

Recently, in V. *radiata*, germination percentage was also found to decrease in all tested hybrids along with the increase in PEG concentrations (5% and 10%) induced drought stress (Saima *et al*., 2018). Moreover, it is suggested that inorganic ions are not inhibitory compared to mannitol and polyethylene glycol (PEG) in several halophytes (Ungar, 1978) and seeds are mainly affected by osmotic stress rather than specific ion toxicities (Zhang *et al*., 2010).

In the present investigation, drought stress greatly affects seed germination, but the response intensity and adverse effect of drought stress depend on the concentrations of mannitol and PEG. Amongst the stressor varieties, the high concentration of mannitol (750 mM) proves to be completely toxic while PEG shows the toxicity level at 15% and above. Moreover, when petri dish grown seedlings were transplanted to soil and irrigated with respective concentrations of mannitol as well as PEG solutions, inhibitory response on seedling growth was observed and such responses were found to be dependent on concentrations of osmotic stressors solutions.

Effects of Herbicides Stress

In modern agriculture system, chemical weed control has been undertaken as preferred approach to conventional methods and is an integral part of the current crop production system (Patel *et al*., 2006). In practice, weeds are being controlled effectively by using selective herbicides. In case of severe weed attack, the use of proper herbicide turns out to be inevitable for the control of weeds and therefore, chemical weed control method is realized as preferred approach over other weed control methods because it is quicker, more effective and relatively cheaper (Shah *et al*.,1989).

However, on the other hand, the continuous use of herbicides may cause weed resistance to herbicides and weed shift problem (Zhang, 2003). Further, it is suggested that chemical weed control causes a significant negative effects on crop plant height and grain yield resulting a lower number of seeds to germinate and determine a plant density lower than the planned one (Avola *et al*., 2008). In general, the herbicide 2,4-D is used for broadleaf weed control in agricultural and non-agricultural conditions and glyphosate is commonly used as a non-selective herbicide to control all weeds before crop sowing. However, glyphosate is also known as very poorly effective on some weeds, such as the *Ipomoea* species (Culpepper *et al*., 2001).

Furthermore, it was observed that *Urena lobata* seedlings were failed to survive when sprayed with bispyribac-sodium while total weed control could be possible if 2,4-D was used either with glyphosate or thiobencarb. These herbicides generally reduce the shoot and root biomass by 99% –100% and result to total weed control while some other herbicides fenoxaprop-p-ethyl with ethoxysulfuron give poor weed control. Significantly, glyphosate application to control *U. lobata* is possible only when there is no rice crop in the field (Awan *et al*., 2014).

Moreover, in order to increase the legume yields, farmers have been found to use high dose of various agrochemicals like herbicides, insecticides, pesticides and other fertilizers during cultivation of legume crops in the field. Hence, during this study, experiments were undertaken to understand the lethal effects of herbicide stress like dicamba and picloram during seed germination and seedling growth in mung bean legume crop.

Dicamba is known to kill annual and perennial broadleaf weeds and it is commercially used weed control chemical for grain crops and turf areas. Moreover, in combination with a phenoxy herbicide or with other herbicides, dicamba can be used for weed control in crop land and other non-crop areas. Dicamba is toxic to conifer species but in general is less toxic to grasses (Arnold *et al*., 2011). However, picloram is a systemic herbicide used for general woody plant control and a wide range of broad-leaved weeds but most grasses are known to be resistant.

During present study, uses of two herbicides (dicamba and picloram) have been proved equally very toxic for seed germination. Moreover, even the low concentration (5mg/L) of these two herbicides was proved to be lethal and normal germination was strongly inhibited. Significantly, partially germinated seeds turn out to be morphologically very abnormal and gradually die under the treatments of dicamba and picloram herbicides both. However, picloram herbicide proves to be more toxic and lethal than dicamba when both herbicides were employed alone at the equivalent concentrations.

Similar inhibitory responses in terms of morphological changes in plants have been also recorded when herbicides were employed either alone or in combination (Kumar & Singh, 2010). Furthermore, a significant reduction in shoot length was also seen with the application of herbicides such as isoproturon and 2,4-D (Khan *et al*., 2004, 2018).

Further, it is seen that when herbicides are applied to the soil then these herbicides not only affect the weed seed germination but also inhibit the crop growth as well (Barker, 2007). Similarly, when legumes were exposed to several herbicides then process of nodulation and nitrogen fixation in these crops were found to drastically reduce (Drew *et al*., 2007). Additionally in *Vigna radiata*, a remarkable reduction in protein content was recorded with the application of Atrazine herbicide (Alvi *et al*., 2003).

In another study, the phytotoxic effect of herbicides on chickpea plant height demonstrates that pre-emergence herbicides (pendimethalin and s-metolachlor) are more effective in controlling the plant height as compared to the post-emergence herbicides (clodinafop-propargyl, fenoxaprop-p-ethyl and di-methyl urea). Moreover, it is suggested that remarkable reduction in the growth of plant might be due to reduction in photosynthates production or slower the translocation of photosynthates to the tops (Qasim, 2005).

Furthermore, such depressive effects of herbicides on crops suggest that the occurrence of low plant height in herbicides treated plants is based on the facts that herbicides greatly reduce the weed attack but affect the plants by reducing the plant height and other growth parameters (Barker, 2007; Khan *et al*., 2018).

CONCLUSION

This study in mung bean legume crop concludes that mannitol at very high concentration (750mM) exhibits toxicity and inhibits complete seed germination due to water stress induction while PEG shows the toxicity symptoms at low concentration (15%). Similarly, both herbicides (dicamba and picloram) were proved to be very toxic for the seed germination even at very low concentration (5 mg/L). However, the inhibitory stress response caused by herbicide picloram on seed germination was found to be more pronounced and lethal than dicamba herbicide. During early seedling growth, both mannitol and PEG prove to be inhibitory with their increased concentrations and moreover, reduced seedling lengths were observed. Additionally, on transplantation of drought stress-treated seedlings in soil were also found to exhibit inhibitions in seedling growth with the increase in concentrations of osmotic stressors (mannitol or PEG).

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