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Understanding the Response of Water and Hormonal Stress on Seed Germination and Early Seedling Growth in Kodo Millet (*Paspalum scrobiculatum* L.)

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The objective of the present study was to understand and evaluate the effects of water and hormonal stresses on seed germination and early seedling growth in kodo millet crop (*Paspalum scrobiculatum* L.) and observations were recorded for partial seed germination and full seed germination after 6-days and 12-days of stress treatments. During water stress experiments, various concentrations of mannitol (50mM, 100mM, 250mM, 500mM, 750mM, and 1000mM) and polyethylene glycol (PEG- 5%, 10%, 15%, 20%, and 25%) respectively were employed. Results achieved during water stress treatments reveal that mannitol concentrations (250mM and 500mM) were proved to be very significant and causing promotions in seed germination and seedling growths instead of osmotic stress inhibition and therefore, after 12-days of treatments, the mean germination percentage were recorded as (100%±1.41) and (93±1.06) respectively in comparison to control (88%±0.84). However, further increased mannitol concentrations (750mM and above) were found to be lethal and seed germination (%) was found to be zero. Additionally, PEG treatments (5% and 10%) were found to cause gradual inhibitions in germination percentage (79%±0.63 and 71%±0.35) respectively. However, PEG concentrations (15% and above) were turned out to be toxic for seed germination. Furthermore, experiments were also designed to find out the responses of hormonal stresses during seed germination and early seedling growth in kodo millet and hence, abscisic acid (ABA) and gibberellic acid (GA_3) in various concentrations (5mg/L, 25mg/L, 50mg/L, and 100mg/L) of each were employed. Moreover, ABA even at low concentration (5mg/ L) was proved to be very toxic and causes strong inhibitions in seed germination while in contrast, GA_3 at high concentration (100mg/L) turns out to be significantly inhibitory for seed germination (47%±0.77) as compared to control (88%±0.84). Interestingly, GA_3 at all tested concentrations were proved to be effective to cause significant promotions in seedling elongations.

Key words: Abiotic stress, Abscisic acid, Gibberellic acid, Mannitol, Polyethylene glycol, Kodo Millet

Kodo millet (*Paspalum scrobiculatum* L.) is cultivated as food and fodder crops across the globe in general and dry areas of temperate, sub-tropical and tropical regions of the world in particular (Dwivedi *et al.*, 2012; Lata *et al*., 2013). It is documented that kodo millet is inherently drought tolerant and can be grown in a variety of poor soil types from gravelly to clay (de Wet *et al*., 1983; M'Ribu and Hilu 1996). Today, it is being cultivated as important food crop globally and therefore, contributes to the regional food security especially for the dry and marginal lands where major cereal crops generally fail to grow (Sao *et al*., 2017).

Significantly, kodo millet grain contains a wide range of high-quality proteins (Geervani and Eggum 1989; Kulkarni and Naik 2000), and in comparison to other millets, it has high anti-oxidant activity (Hegde and Chandra 2005; Hegde *et al*., 2005; Chandrasekara and Shahidi 2011). Additionally, like finger millet, kodo millet is also rich in fiber and hence may be consumed as alternative food source for diabetic patients (Geervani and Eggum 1989; Sao *et al*., 2017).

In general, abiotic stresses due to continuous climate change hamper plant growth and productivity. Among various environmental stresses, drought stress has become a severe problem worldwide due to its dramatic effects on plant physiology and performance (Janmohammadi *et al*., 2008). Moreover, it is suggested that several components are possibly involved in stress regulatory networks and these components may function synergistically or antagonistically to each other, thus promote or compromise plant resistance to various occurring abiotic stresses (Glazebrook 2005; Kissoudis *et al*., 2014).

Seed germination is known as a crucial stage in plant life that plays important roles in seedling establishment and subsequent growth (Bewley 1997) and furthermore, process of germination is being regulated by multiple endogenous factors, such as plant hormones, and external environmental conditions, including temperature and light (Qu *et al*., 2008; Weitbrecht *et al*., 2011; Cho *et al*., 2012; Miransari and Smith 2014; Liu *et al*., 2018).

Moreover, it is documented that the effects of drought stress on the germination of seedling depend on the availability of water and further the impact of low water stress is closely related to leaf formation and secondary root development (Gregory 1983; Shivhare and Lata 2017). However, plant responses to water deficit depend upon various factors such as duration and degree of stress, growth stage and time of stress exposure (Gupta and Sheoran 1983). Of late, seed germination and seedling growth under salinity stress caused by NaCl and sea water has been analyzed in kodo millet and further protein content was estimated during seed germination under NaCl stress (Vikrant *et al*., 2020).

Additionally, ABA and GA_3 are well known plant growth regulators involved in seed germination. Further, GA_3 is generally known to promote seed germination events while ABA causes inhibitory effects on germination and related changes through multiple regulatory mechanisms, including transcriptional control and synthesis of specific enzymes (Fincher 1989). Moreover, increase in biochemical activities such as enhanced levels of antioxidants, reactive oxygen species and their scavenging enzymes, and synthesis of osmolytes and other stress-related proteins have been identified in response to abiotic stresses in teff (Smirnoff and Colombe 1988), foxtail millet (Lata *et al*., 2011) and little millet (Ajithkumar and Panneerselvam 2014).

Hence, present study was undertaken to understand the responses of drought stress induced by exogenous applications of mannitol and polyethylene glycol (PEG) and also to evaluate the physiological effects of stress responses caused by external employment of abscisic acid (ABA) and gibberellic acid (GA_3) on the seed germination and early seedling growth in kodo millet (*Paspalum scrobiculatum* L.).

MATERIALS AND METHODS

Seed Collection and Sterilization

To begin with, seeds of kodo millet (*Paspalum scrobiculatum* L.) wild cultivar were collected from PASIC, Puducherry (India). Healthy and uniform seeds of kodo millet 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 10 minutes. Finally, sterilized seeds were washed 3-4 times with distilled water to remove the traces of $HgCl_2$.

Stress Treatments

Water and hormonal stress inducing agents were employed in various concentrations and responses of these stresses were observed in terms of ability of kodo millet seed to germinate under stress conditions and further stress effects on early seedling growth.

Water Stress Treatment: During water stress treatments, sterilized seeds were treated with various concentrations of mannitol solutions (50mM, 100mM, 250mM, 500mM, 750mM, and 1000mM) and polyethylene glycol (PEG-6000) solutions (5%, 10%, 15%, 20%, and 25%).

Hormonal Stress Treatments: Sterilized seeds were incubated in various solutions (5mg/L, 25mg/L, 50mg/L and 100mg/L) of abscisic acid (ABA) and gibberellic acid (GA_3) each.

Furthermore, sterilized seeds were soaked in the respective stress solutions (mannitol, PEG, ABA and GA_3) for 3hrs. The soaked seeds were further transferred in sterile petridishes (9.0cm diameter) lined with two sterile filter papers with 5ml of distilled water as control experiment or the respective test solutions as mentioned above for water and hormonal stress experiments.

Further, soaked seeds were incubated (15-20 seeds/petridish) in petridish added with respective stress solutions and three replicates in each treatments were performed. Germination tests were conducted under dark condition at normal room temperature (25-30^{0}C). A seed was considered partially germinated when coleoptile was 2mm long and fully germinated when seeds were emerged with root and shoot. Further, the germination percentage was determined counting the number of germinated seeds on the 6th and 12th day of the treatments.

Statistical Analysis

Statistical data were performed after first count (6th day after treatments) and final count (12th 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

$$GR = \frac{\text{Number of Germinated seeds}}{6^{th}\text{ Day of Count}} + \frac{\text{Number of Germinated Seeds}}{12^{th}\text{ Day of Count}}$$

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

RESULTS

During present study, all the observations for water and hormonal stress treatments were recorded at the end of 6th day of the treatments for the partial or incomplete germination and 12th day of the treatments for the full and complete germination. Moreover, germination and seedling growth of kodo millet seeds were significantly affected by concentrations of treated stress solutions (mannitol/PEG/ABA/GA_3) and also on durations of the treatments.

Effect of Mannitol- Water Stress on Seed Germination

At the end of 6th day of treatments, control and lower concentrations of mannitol-treated seeds (50mM and 100mM), were found to begin germination and these concentrations of mannitol were proved to be ineffective to cause stress inhibitions in seed germination caused by osmotic stress. However, with further increase in mannitol concentrations (250mM and 500mM); seeds could exhibit relatively slow and slightly inhibited germination at the end of 6th day of treatments.

After 12th day of treatments, complete seedlings with well developed roots and shoots could be observed in control experiment (Fig. 1A) and also in the seeds that were treated with mannitol solutions (50mM, 100mM, 250mM and 500mM). Significantly, the length of seedlings was found to increase with the increase in mannitol concentrations (Fig. 1B-D). However, with very

high concentrations of mannitol solutions (750mM and 1000mM), seeds were failed to show germination response completely (Fig.1E). Results thus reveal that mannitol concentrations (up to 500mM) were proved to be ineffective to cause water stress rather induce seed germination and seedlings growths indicating the water stress resistant nature of this millet variety, however, higher concentrations (750mM and 1000mM) of mannitol could be found as toxic levels and cause inhibitions in the seed germination completely.

Effect of Polyethylene glycol (PEG) - Water Stress on Seed Germination

During another water stress causing agent PEG treatments, seeds that were treated with all the concentrations (5%, 10%, 15%, 20%, and 25%) were failed to show symptoms of seed germination at the end of 6th day of the treatments. However, at the end of 12th day of the treatments, PEG solutions (5% and 10%) treated seeds only could be able to show germination and later develop into healthy seedlings (Fig. 1F & G). In contrast, seeds that were treated with the further higher concentrations of PEG (15%, 20%, and 25%) were failed to show germination even after 12-days of treatments (Fig. 1H & I). Results indicate that PEG concentrations (15% and above) were proved to be toxic concentrations and seed germination was thus found to be strongly inhibited due to osmotic stress induced by PEG.

Rate of Seed Germination under Mannitol-Water Stress

During mannitol treatments, seed germination frequency was observed to increase with increasing mannitol concentrations at the end of 6-days of treatments. With lower concentrations of mannitol solutions (50mM and 100mM), the germination frequency (63%±1.75 and 59%±0.74) respectively was recorded in comparison to control experiment (52% ±1.99). However, further increased mannitol concentrations (250mM and 500mM) were found to exhibit remarkable inhibitions in germination frequency (47%±0.24 and 36%±1.0) respectively (Table 1). Significantly, at very high concentrations of mannitol (750mM and 1000mM), the frequency of seed germination was obtained as zero.

After 12th days of mannitol treatments, seed germination frequency (87%±0.35, 89%±0.07, 100% ±1.41, and 93%±1.06) was recorded with (50mM, 100mM, 250mM, and 500mM) of mannitol solutions respectively in comparison to the control (88%±0.84) experiment. However, very high concentration of mannitol (750mM) and above could be turned out fully toxic and germination frequency was recorded to be zero even after mannitol treatments.

Rate of Seed Germination under Polyethylene glycol (PEG) - Water Stress

In comparison to control experiment (52%±1.99), the rate of seed germination was found to be zero at all the tested solutions (5% to 25%) of PEG concentrations at the end of 6th day of treatments (Table 2). However, at the end of 12th day of treatments, seeds that were treated with lower concentrations (5% and 10%) of PEG solutions, complete seed germination could be seen (79%±0.63 and 71%±0.35) respectively in comparison to control treatment (88%±0.84). Significantly, with further increase in PEG concentrations (15% and above), the seed germination frequency was obtained as zero even after 15-days of PEG treatments (Table 2). Results reveal that in comparison to mannitol osmotic stressor, PEG was proved to be more lethal and worked as strong inhibitor for kodo millet seed germination.

Effect of Water Stress on Seedling Growth

In general, seedlings heights were significantly affected by water stress caused by mannitol and PEG. In terms of the survival tendency and growth of the seedlings, the maximum elongations in root and shoot lengths were observed in the seedlings that were growing with lower concentrations (100mM and 250mM) of mannitol (Fig. 2A & B) respectively while with increased high concentration (500mM) of mannitol treatment, the lengths of the seedlings were seen slightly reduced due to exposure of seeds to high osmotic stress (Fig. 2 C).

In case of PEG-induced stress treatments, the development of complete seedlings could be observed at the end of 12-days of PEG (5%) treatments and significantly, these seedlings were appeared to be more elongated even than the control seedlings (Fig. 2D).

However, with the further increase in PEG concentration (10%), there was significant reduction in both shoot and root lengths of the seedlings (Fig. 2E). Additionally, when the mannitol-treated seedlings were transferred to disposable plastic cup soil and were supplemented with the respective mannitol solutions; slight inhibitions were observed in the growth of mannitol-treated seedlings (Fig. 2F).

Effect of Abscisic Acid (ABA) – Hormonal Stress on Seed Germination

In comparison to control treatment, sterilized seeds that were treated with ABA solutions (5mg/L, 25mg/L, 50mg/L and 100mg/L) were found to exhibit merely the proliferation of mature embryos from the seeds and failed to show further symptoms of partial seed germination at the end of 6th day of treatments indicating the inhibitory responses of ABA for seed germination. Significantly, even after 12-days of treatments, these ABA solutions (5mg/L, 25mg/L, and 50mg/L) treated seeds were also failed completely to germinate (Fig. 3 B, C & D) respectively in comparison to the control experiment (Fig. 3A). Therefore, result reveals that ABA proves to be strong inhibitor even at very low concentration (5mg/L) and causes the complete inhibitions in seed germination.

Effect of Gibberellic Acid (GA_3) - Stress on Seed Germination

In comparison to control experiment, seeds treated with high GA_3 concentrations (50mg/L and 100mg/L), were found to exhibit slight inhibitions in seed germination at the end of 6th day of treatments. However, after 12th days of treatments, seeds treated with lower concentrations of GA_3 (5mg/L and 25mg/L) could show the maximum support for full seed germination and these seedlings were also observed to be more elongated (Fig. 3 E & F) respectively in comparison to the control seedlings (Fig. 3A). Interestingly, elongations in these seedlings were seen in terms of shoot length while inhibition was noticed in root length. Further, seeds that were treated with very high concentration of GA_3 solution (100mg/L), could exhibit slightly inhibited germination (Fig. 3 H) while seeds that were treated with 50mg/L of GA_3, seedlings were seen to be more elongated in terms of shoot and root lengths both (Fig. 3 G). Results therefore indicate that all the tested solutions of GA_3 (5mg/L, 25mg/L, 50mg/L and 100mg/L) could prove as promotory for seedling development instead of seed germination.

Rate of Seed Germination under Abscisic Acid (ABA) - Stress

In control experiments, mean seed germination percentage (52%±1.99 and 88%±0.84) was recorded after 6thday and 12thday of treatments respectively. However, seeds that were treated with ABA solutions with all the tested concentrations were failed to show even partial germination at the end of 12-days of treatments and therefore the rate of seed germination either partial or full was recorded as zero (Table 3). This result indicates that ABA proves to be fully inhibitory for kodo millet seed germination even at very low concentration (5mg/L).

Rate of Seed Germination under Gibberellic Acid (GA_3) - Stress

After 12th day of gibberellic acid stress treatments, the maximum mean percentage of seed germination (87%±1.41) was obtained in case of seeds that were treated with GA_3 solution (25mg/L) which was almost equal to the control (88%±0.84) treatments. Hence, this concentration of gibberellic acid stress was found to be fully ineffective to inhibit seed germination caused by hormonal stress. However, other concentrations of GAs were found to be slightly effective to cause stress inhibitions during seed germination and significantly drastic reductions in mean percentage of seed germination were recorded as (54%±0.71 and 41% ±0.77) with the further increase in concentrations (50mg/L and 100mg/L) of GA_3 solutions respectively (Table 4).

Effect of Gibberellic Acid- (GA_3) - Hormonal Stress on Seedling Growth

The overall growth of GA_3 treated millet seedlings were significantly affected by the concentrations of GA_3 solutions and the maximum length of seedlings were observed in seeds that were growing with lower concentrations (5mg/L and 25mg/L) of GA_3 solutions and moreover, these seedlings were apparent more elongated (Fig. 3 I & J) respectively even than the

control seedlings. Interestingly, seedlings that were found to grow with 25mg/L of GA_3 solutions were failed to exhibit elongations in root while other seedlings that could grow with very high concentration (100mg/L) of GA_3 solutions were observed to be slightly inhibited (Fig. 3L) but more elongated even than control seedlings. However, seedlings that were seen to grow with high concentration GA_3 solution (50mg/L) were found to exhibit promotions in both shoot and root elongation (Fig. 3 K).

Hence, results indicate that ABA induced hormonal stress was observed strongly inhibitory for kodo millet seed germination while in contrast, GA_3 caused hormonal stress could proved slightly inhibitory for seed germination but strongly supportive for seedling elongations and growth.

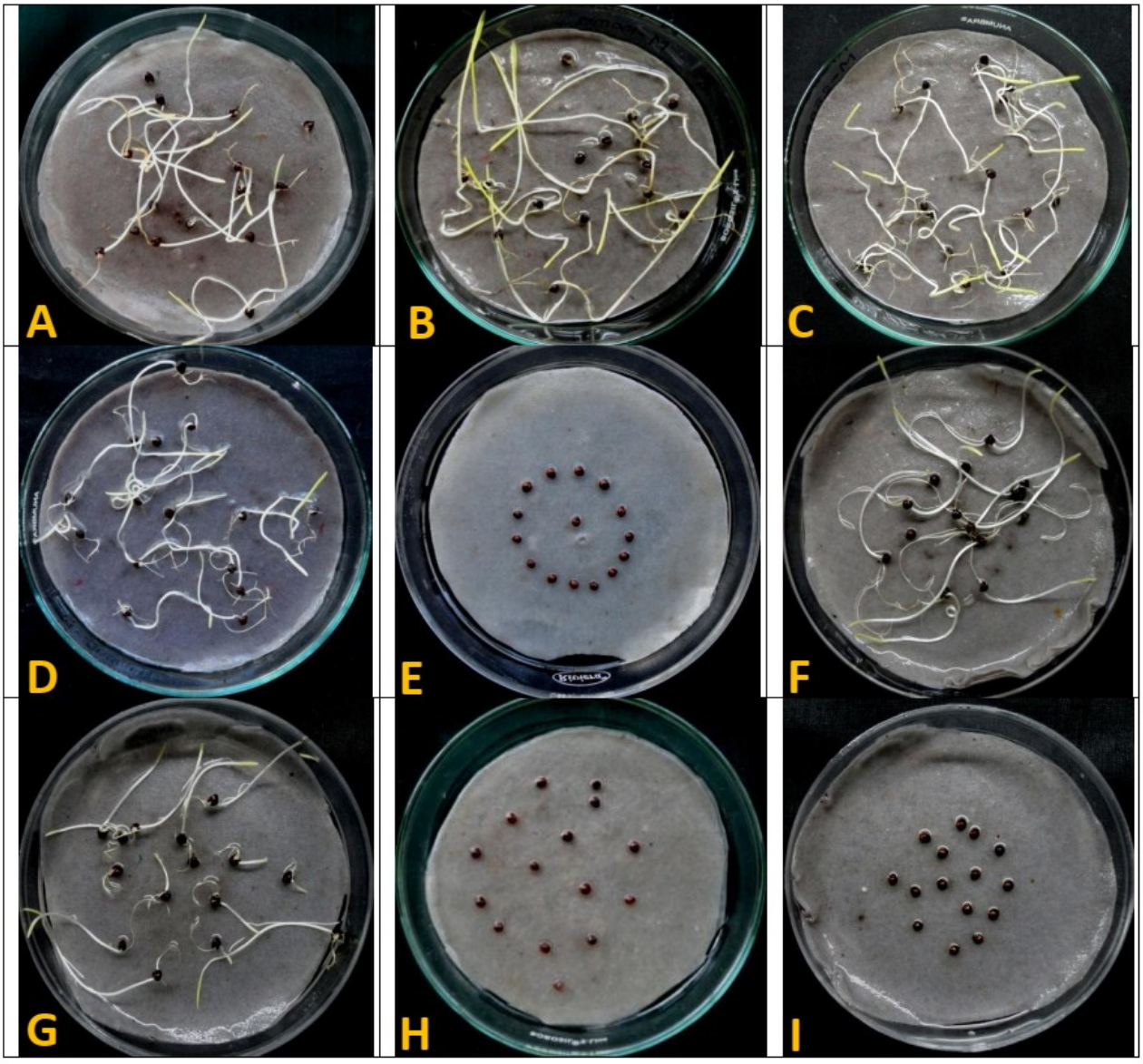

Figure 1. ***Paspalum scrobiculatum*** **L., showing the effects of water stress on seed germination;**
(A) Control **(B)** Mannitol-100mM **(C)** Mannitol-250mM **(D)** Mannitol-500mM **(E)** Mannitol-750mM **(F)** Polyethylene glycol (PEG)-5% **(G)** PEG-10% **(H)** PEG-15% **(I)** PEG-25% (after 12-days of treatments).

Table 1. *Paspalum scrobiculatum* L., showing the effects of Mannitol water stress on seed germination.

S. No.	6th Day		12thDay
	Concentration of Mannitol (mM)	**Mean Germination (%) ± S.E.**	**Mean Germination (%) ± S.E.**
1	0 (Control)	52±1.99	88±0.84
2	50	63±1.75	87±0.35
3	100	59±0.74	89±0.07
4	250	47±0.24	100±1.41
5	500	36±1.0	93±1.06
6	750	0	0
7	1000	0	0

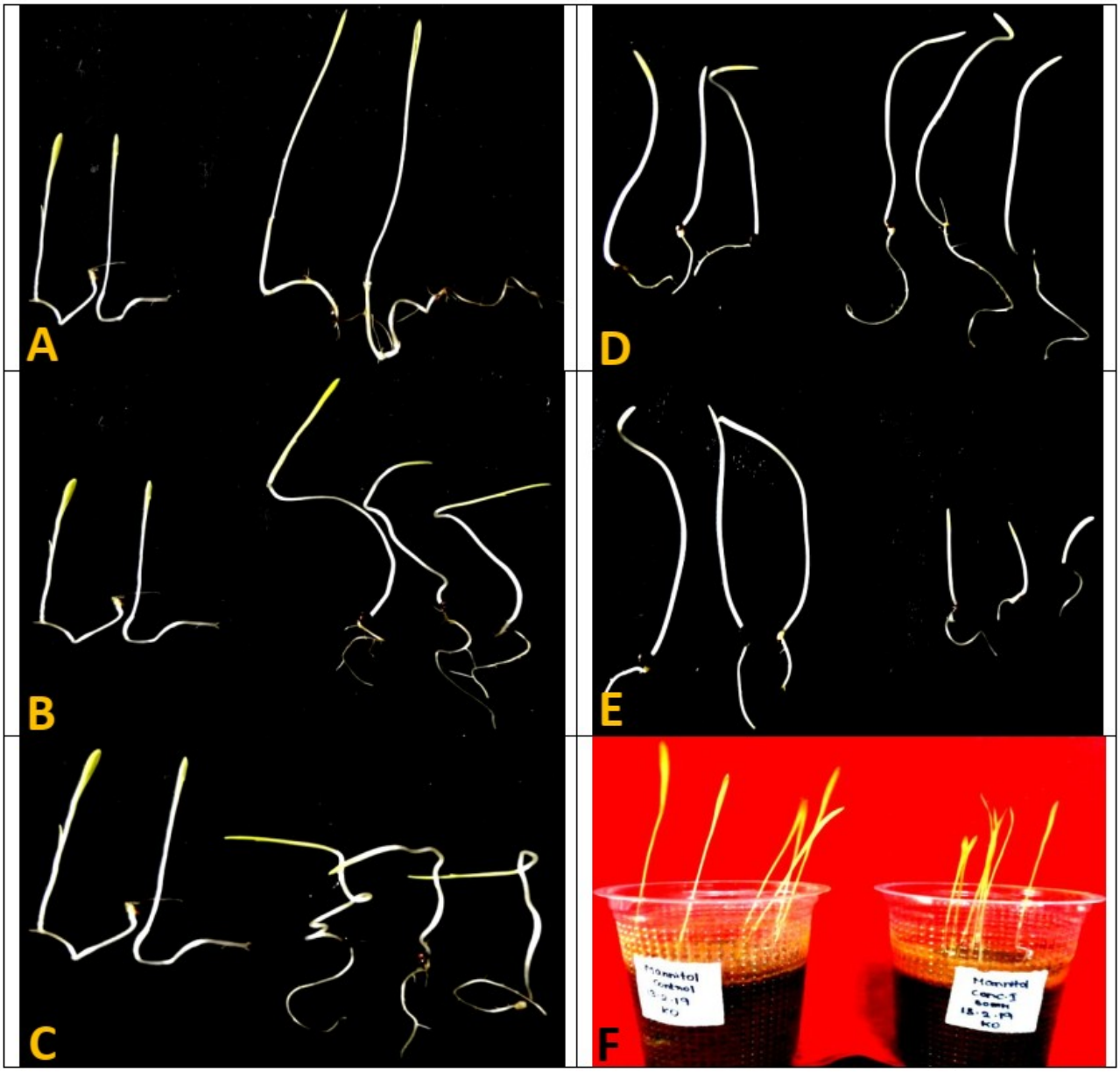

Figure 2. *Paspalum scrobiculatum* L., showing the effects of water stress during early seedling growth;
(A) Control+Mannitol (100mM) **(B)** Control+Mannitol (250mM) **(C)** Control+ Mannitol (500mM) **(D)** Control+ PEG (5%) **(E)** Control+ PEG (10%)-Seedlings after 12-days of treatments **(F)** Control+ Mannitol (50mM)-Seedlings in disposable plastic cup after 15-days of treatments.

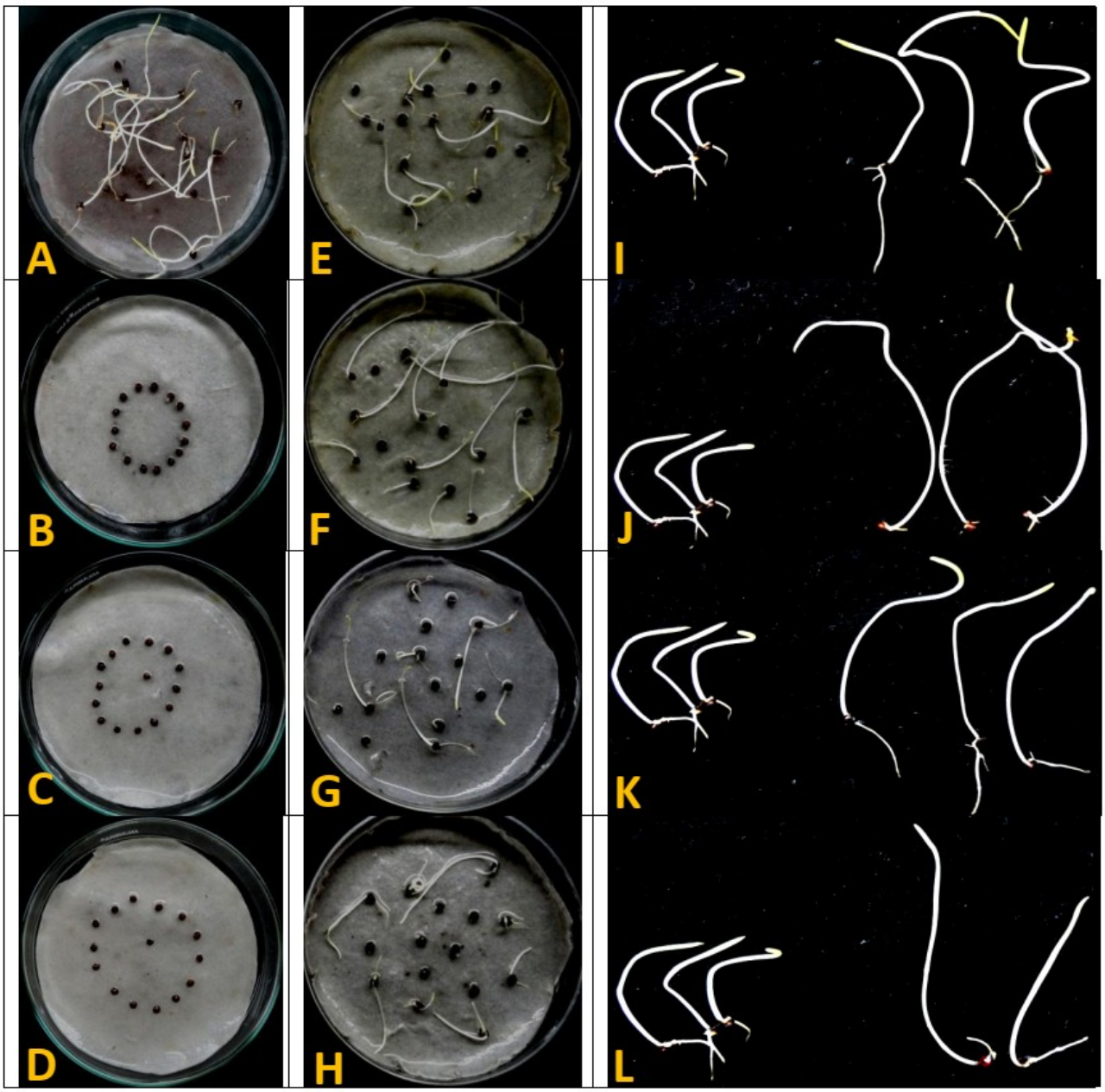

Figure 3. *Paspalum scrobiculatum* L., showing the effects of hormonal stress on seed germination and early seedling growth;
(A) Control **(B)** ABA (5mg/L) **(C)** ABA (25mg/L) **(D)** ABA (50mg/L) **(E)** GA_3 (5mg/L) **(F)** GA_3 (25mg/L) **(G)** GA_3 (50mg/L) **(H)** GA_3 (100mg/L) after 12-days of treatments.
(I) Control+GA_3 (5mg/L) **(J)** Control+ GA_3 (25mg/L) (**K)** Control+GA_3 (50mg/L) **(L)** Control+ GA_3 (100mg/L)-Seedlings after 12-days of treatments.

Table 2. *Paspalum scrobiculatum* L., showing the effects of Polyethylene Glycol (PEG) water stress on seed germination.

S. No.	6th day		12th day
	Concentration of PEG (%)	Mean Germination (%) ± S.E.	Mean Germination (%) ± S.E.
1	0	52±1.99	88±0.84
2	5	0	79±0.63
3	10	0	71±0.35
4	15	0	0
5	20	0	0
6	25	0	0

Table 3. *Paspalum scrobiculatum* L., showing the effects of Abscisic acid (ABA) stress on seed germination.

S. No.	6th day		12th day
	Concentration of ABA (mg/L)	Mean Germination (%) ± S.E.	Mean Germination (%) ± S.E.
1	0	52±1.99	88±0.84
2	5	0	0
3	25	0	0
4	50	0	0
5	100	0	0

Table 4. *Paspalum scrobiculatum* L., showing the effects of Gibberellic acid (GA_3) stress on seed germination.

S. No.	6th day		12th day
	Concentration of GA_3 (mg/L)	Mean Germination (%) ± S.E.	Mean Germination (%) ± S.E.
1	0	52±1.99	88±0.84
2	5	51±0.35	86±0.77
3	25	54±1.41	87±1.41
4	50	50±0.71	54±0.71
5	100	37±0.35	47±0.77

DISCUSSION

Kodo millet is being cultivated as food crops in major parts of India and has been treated as the main staple food (Bandyopadhyay *et al*., 2017). However, abiotic stresses have emerged recently as the major cause of crop failure leading to drastic reduction in average yield for major crops and thus, today abiotic stress is being treated as a big challenge for the sustainability of the agricultural industry (Mahajan and Tuteja 2005). Further, it is documented that abiotic stresses can directly or indirectly affect the physiological status of an organism by altering its metabolism, growth and development (Chutia and Borah 2012; Vibhuti Shahi *et al*., 2015) and cause adverse affect to agricultural productivity (Bartles and Sunkar 2005).

Water Stress Effects on Seed Germination and Early Seedling Growth

Drought or water stress is known to adversely affect the seed germination, plant growth and development (Almaghrabi and Abdelomoneim 2012) and seedling growth (Ashraf *et al*., 2002). In general, water crisis has been found to cause an negative effect upon seed germination and embryo growth rate in the field, however, several sorghum cultivars have been identified that are well adapted to semi-arid areas (Patane *et al*., 2012). Although water consumptions and other physiological characteristics of sweet sorghum indicate that this species can successfully adapt to drought conditions (Tari *et al*., 2012).

Polyethylene glycol (PEG), a non-penetrating osmotic agent that lowers the water potential of the medium, has been used extensively to stimulate drought stress in plants (Smith *et al*., 1986). During present study, mannitol stress treatments even with high concentrations (250mM and 500mM) were found to be ineffective to cause osmotic stress inhibitions in kodo millet seed germination, rather cause significant enhancements in tendency of seed germination (100% and 93%) respectively in comparison to control treatment (88%±0.84). In contrast, previous study reports that seed germination was severely reduced by water stress caused by mannitol in sugar beet (Sadeghian and Yavari 2004). However, this study also reveals that toxicity of mannitol for millet seed germination was recorded with very high concentrations (750mM and above).

Additionally, previous study indicates that the application of PEG at various concentrations affect germination percentage, root and shoot length and root/shoot ratio (Govindaraj *et al*., 2010). However, during this study, PEG concentration (15% and above) were proved to be strongly inhibitory and cause the severe water stress resulting in complete inhibitions in seed germination. Significantly, in case of halophytes, inorganic ions are not found to be inhibitory compared to mannitol and polyethylene glycol (PEG) and seeds were

mainly affected by osmotic stress rather than specific ion toxicities (Ungar 1978; Zhang *et al*., 2010).

Moreover, it is documented that during *in vitro* regeneration, mannitol neither supports tissue growth nor it is metabolized by higher plants and in comparison to PEG, mannitol was proved to be ineffective to stimulate somatic embryogenesis (Vikrant 2015). Also in pearl millet, screening of germplasm could be possible based on using PEG-6000 during germination and early seedling growth stages (Bidinger *et al*., 2007; Mahalakshmi *et al*., 1987). Furthermore, severe moisture stress during seedling stage was found as the major cause of low yield of pearl millet in the semi-arid regions (Carberry *et al*., 1985; Soman and Peacock 1985; Soman *et al*., 1987; Shivhare and Lata 2017).

Furthermore, it is also experimentally proved that osmotic adjustment can reduce growth sensitivity to water stress or allow growth to proceed at a slow rate under water stress by maintaining turgor (Cutler *et al*., 1980). Normally, salt and water stresses are known to affect the physiology and biochemistry of plant cells under *in vitro* and *in vivo* conditions. These stresses have been reported to enhance acid phosphatase activity in pea and wheat (Barrett-Lennard *et al*., 1982). Moreover, a water deficit has also been shown to affect acid phosphatase in wheat (Thakur and Thakur 1993).

It is documented that acid phosphatases are known to act under salt and water stress by maintaining a certain level of inorganic phosphate which can be co-transported with H^+ along a gradient of proton motive force (Olmos and Hellin 1997). However, in contrast, literatures reveal that phosphatases activities are independent of phosphate levels (Barrettt-Lennard *et al*., 1982; Szabo-Negy *et al*., 1992). It is established that when soil water level comes down, plant growth usually decreases suggesting that the growth inhibition may be metabolically regulated as an adaptation mechanism causing to restrict the development of transpiring leaf area in the water-stressed plants (Sharp 1996).

Hormonal Stress effects on Seed Germination and Early Seedling Growth

During present investigation, seeds were exposed under hormonal stresses (ABA and GA_3) and observations were monitored in terms of germination rate and early seedling growth of *P. scrobiculatum* L. Exogenous ABA treatment even with low concentration (5mg/L) caused a complete restriction in kodo seed germination and was proved to be fully inhibitory. Moreover, similar studies based on inhibitory responses of ABA during seed germination are also available (Gill *et al*., 2003; Garciarrubio *et al*., 2003; Sharma *et al.*, 2004).

Additionally, it is suggested that decrease in seed germination rate under ABA treatment may be due to metabolic alternations and ABA is also found to be involved in inhibiting the seed germination by restricting the availability of energy and metabolites (Garciarrubio *et al*., 2003). Furthermore, exogenous application of ABA has been shown to inhibit mature embryo germination by modulating the endogenous level of ABA (Dewar *et al*., 1998), and moreover, when ABA synthesis is inhibited by fluridone then precocious germination could be possible to occur (Sharma *et al*., 2004).

Further, embryonic growth was found to be suppressed by ABA treatments and similar observations on decrease in water level under stress conditions have been seen in wheat (Siddique *et al*., 2000) and in alfalfa (Pennypacker *et al*., 1990). Moreover, it was suggested that contribution of growth at lower water potential is a result of turgor maintenance, whereas the inhibition of growth is not entirely dependent on turgor (BassiriRad and Coldwell 1992). Previous results also reveal that imposition of ABA treatments cause a significant reduction in dry matter of embryos (Sharma *et al*., 2004).

Moreover, another plant growth hormone, gibberellic acid (GA_3) is known to induce embryo growth and stimulate the germination process. Moreover, GA_3 is well-documented regulator of germination and associated enzymes with generally having promoting effects (Fincher 1989). Thus, it is argued that at onset of germination, ABA and GA_3 appear to act in a fully antagonistic manner (Jacobsen and Beach 1985; Fincher 1989). In contrast to the inhibitory responses of ABA stress, GA_3 stress treatments during present study prove to be ineffective to cause stress inhibitions at

lower concentrations (5mg/L and 25mg/L), however, with the further increase in GA_3 concentration, rate of germination decreases. Significantly, GA_3 treated seedlings have been found to be more elongated than control seedlings indicating the involvement of GA_3 during early time of seedling growth.

Similar to present results, another study reveals that if the seeds are subjected to environmental stresses then germination, growth, respiration and other physiological processes are being affected (Gill and Singh 1985). Significantly, slight alteration in anyone of these processes can affect other metabolic activities, particularly the enzymes of phosphate metabolism that is known to play an important role during seed germination and seedling development (Fincher, 1989).

CONCLUSION

During this study in kodo millet crop, mannitol at the high concentrations (250mM and 500mM) proves to be supportive rather than inhibitory for the seed germination and seedling elongation while further increase of mannitol (750mM) stress turns out to be lethal for seed germination. In contrast, PEG is found to be strong inhibitor and shows the toxicity level at 15%. Furthermore, in terms of hormonal stress, ABA stress inhibits seed germination completely at all tested concentrations while the stimulatory effects of GA_3 with all the tested concentrations could be recorded in terms of seedling elongations instead of kodo millet seed germination. However, very high concentrations (50mg/L and 100mg/L) of GA_3 stress cause strong inhibitions in seed germination.

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CONFLICTS OF INTEREST

The authors declare that they have no potential conflicts of interest.

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