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ORIGINAL ARTICLE

Effect of osmotic stress on seed germination and seedling characters of Mung bean [*Vigna radiata* (L.) Wildzek]

Reshmi G. R.

[1] *Department of Botany, Union Christian College, Aluva-683102, Ernakulam, India*

*E-Mail: ***grrechugr@gmail.com***

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The Screening of osmotic stress was undertaken to investigate the effect of water stress and salinity stress on the seeds of mung bean (*Vigna radiata* (L.) Wilczek) and its responses to drought tolerance at seedling stage. Water stress was simulated by non-ionic water-soluble polymer polyethylene glycol of molecular weight 6000 and salinity stress was induced with NaCl. The experiment demonstrated that osmotic stress caused by NaCl and PEG has a negative impact on the germination rate and seedling growth of *Vigna radiata*. Both NaCl and PEG inhibited germination and seedling growth in mung bean, but the effects of NaCl compared to PEG was less on germination and seedling growth.

Key words: NaCl, Osmotic stress, PEG, Seed germination

Seed germination is measured the most critical stage in seedling formation for determining successful crop production (Almansouri *et al.*, 2001; Finch Savage and Bassel, 2016). Crop establishment depends on an interaction between the seedbed environment and seed quality (Khajeh Hosseini *et al.*, 2003). Many factors adversely affect seed germination including seed sensitivity to drought stress (Sadeghian and Yavari, 2004) and salt tolerance (Almas *et al.*, 2013).

Water and salinity stresses are two of the most common environmental factors that regulate plant growth and limit plant production. Polyethylene glycol molecules with a molecular weight 6000 (PEG 6000) are inert, non-ionic and virtually impermeable chains that have frequently been used to induce water stress and maintain a uniform water potential throughout the experimental period (Hohl and Schopfer, 1991; Lu and Neumann, 1998). Molecules of PEG 6000 are small enough to influence the osmotic potential, but large enough to not be absorbed by plants (Carpita *et al.*, 1979; Saint-Clair, 1976). Because PEG does not enter the apoplast, water is withdrawn from the cell. Therefore, PEG solution mimic dry soil more closely than solutions of low molecular osmotic, which infiltrate the cell wall with solutes.

Sodim chloride create salinity stress on germinating seeds. Salinity affects the germination of seeds by creating an external osmotic potential that prevents water uptake by the toxic effects of sodium and chloride ions on the germinating seeds (Khajeh-Hosseini *et al.*, 2003). Water availability and movement into the seeds are very important to promote germination, initiate root growth, and initiate shoot elongation (Bewley and Black, 1994). Only highly negative water potentials, especially during early germination, may influence seed water absorption, making germination not possible. The relation of various seedling growth parameters to yield components and yield under drought and saline conditions are very important for the development of salt tolerant cultivars for production under drought and saline conditions. Drought or any other abiotic stresses results in reduction of yield and plant growth. They limit the photo synthesis and consequently, limited availability of photo synthetic assimilates and energy to the plant. It is imperative for plants to use this limited supply of nutrients to their maximal advantage to survive under stress.

Mung bean [*Vigna radiata* (L.) Wilczek] is an important leguminous crop and is being used in annual crop rotations on increasingly larger areas of depleted soils in many regions. Mung bean is an important pulse crop of global economic importance and is the best of all pulses on nutritional point of view. Also, it has a certain function of detoxification, increasing appetite, and lowering blood pressure, cancer and other health effects (Yin *et al.*, 2015; Bouchenak and Lamri-Senhadji, 2013). This legume is characterized by a relatively high content of proteins rich in leucine, phenylalanine, lysine, valine, isoleucine and certain vitamins. The successful breeding for drought tolerance is availability of reliable methods for screening of desirable genotypes (Feller, 2016).

Water potential studies enabled the identification of variety suitable for growing under moisture stress situations. Varieties that are found to germinate under reduced water potential do not usually fail to germinate and establish into seedlings. Different developmental stages of this crop are sensitive to drought and salinity stress. In order to select mung bean genotypes that can endure salt and drought stresses, the objective of this study was to evaluate the effects of PEG6000 and NaCl induced treatments during germination on mung bean. Objective of the present work was to study the effect of drought and salinity (osmotic) stress on the germination percentage and seedling characters of mung bean.

MATERIALS AND METHODS

Plant materials and growth conditions

This study was conducted in the laboratories of the Botany Department, Union Christian College, Aluva. Certified and healthy seeds of Green Gram (*Vigna radiata*) were purchased from Mannuthy Agricultural department, Government of Kerala. The chemicals polyethylene glycol (PEG) 6000 and sodium chloride (NaCl) are used for inducing osmotic stress and are laboratory grade standard.

Germination study

Healthy green gram of uniform size was selected and soaked in distilled water for 5-10 minutes for surface sterilization. The seeds were germinated in petri dishes on two layers of kitchen tissue paper. Twenty-five seeds were placed at equidistant in each sterilised petri dish. Six Petri plates were prepared for a set of study in which first Petri plate kept as control by adding distilled water. Details of the treatments were shown in table 1. The filter paper was moistened with 20 mL of each concentration regularly and for control 20 ml of distilled water was used. Four replicates of each concentration were taken for study. The seeds were allowed to germinate at room temperature in dark condition. The percentage of germination was studied after 24, 48,72 hrs and growth of seedling was evaluated after 3 days treatment. Germination was recorded every day for 6 days. Seeds were considered to have germinated when both the plumule and radicle had extended more than 2 mm. The final germination rate, shoot lengths and root lengths were recorded on the 3th day. Mean value was calculated for all the measurements.

Germination percentage were determined following the standard formula of Kumar *et al.* (2012). Below shows some germination parameters selected for study and its formula.

Final Germination percentage (FGP) (%) = Number of germinated seeds × 100/ Number of sown seeds

Mean Germination Time (MGT) = MGT = $\sum (n \times d) / N$

n = number of seeds germinated on each day

d = number of days from the beginning of the test

N = total number of seeds germinated at the termination of the experiment

First Day of Germination (FDG) = Day on which the first germination event occurred

Last Day of Germination (LDG) = Day on which the last germination event occurred

Coefficient of Velocity of Germination (CVG) = N1 + N2 + · · · + Nx/100 × N1T1 + · · · + NxTx

N=No. of seeds germinated each day

T=No. of days from seeding corresponding to N

Germination Index (GI) = no. of germinated seed / days of first count + ... + no. of germinated seed / days of last count

RESULTS

All the observations were made on 24 hrs, 48 hrs and 72 hrs after the treatment. Fig 1 shows the germinated seeds on day 3. It was seen that the percentage of germination was 96% in control and the percentage was decreased in treatments. Germination percentage noted was 88%, 52%, 64%, 40% and 8% for the treatments 1, 2, 3, 4 and 5 respectively. Number of germinated seeds in each day and germination percentage were shown in table 2 and other germination parameters were shown in table 3. Comparison of MGT, FDG and LDG were shown in Fig 2 and germination index and FGP were shown in Fig 3.

Table 1: Showing different treatments selected for the study

Control	Distilled water
Treatment 1	-0.2MPa (50mM NaCl)
Treatment 2	-0.025 MPa (PEG)
Treatment 3	-1 MPa (200mM NaCl)
Treatment 4	-2.5 MPa (500mM NaCl) + -0.025 MPa PEG
Treatment 5	-1 MPa (200mM NaCl) + -0.1 MPa (PEG)

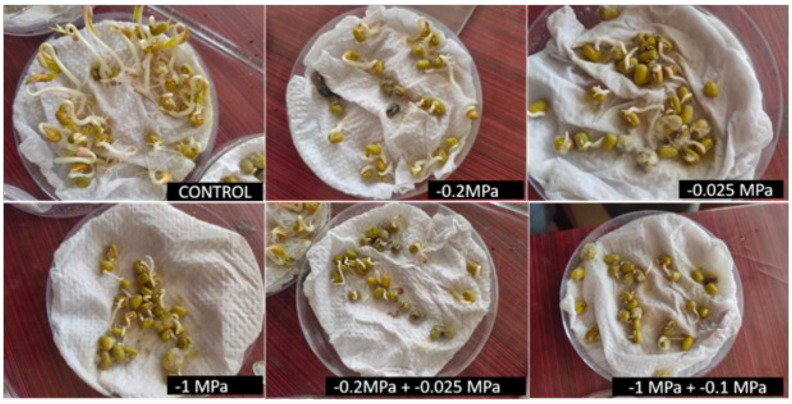

Figure. 1. Showing germination after 3 days on control and different treatments

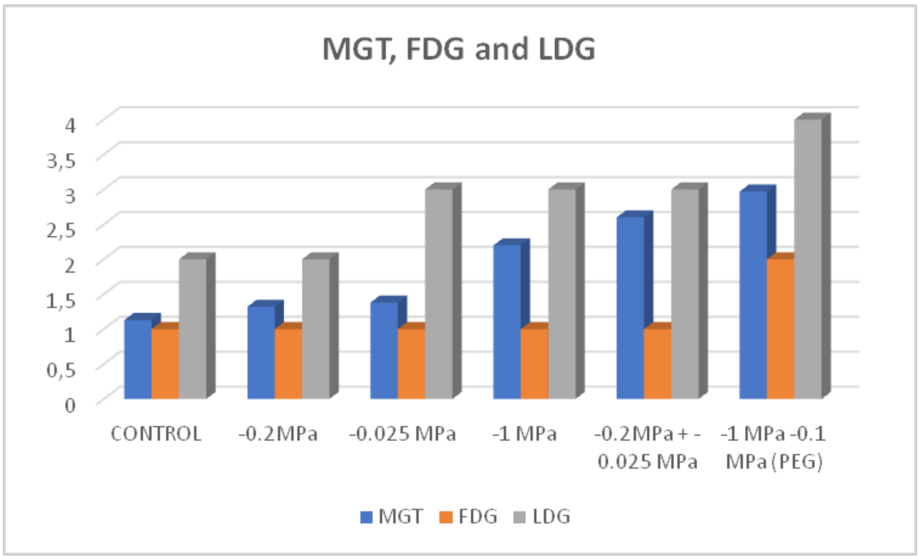

Figure 2. Showing MGT, FDG and LDG

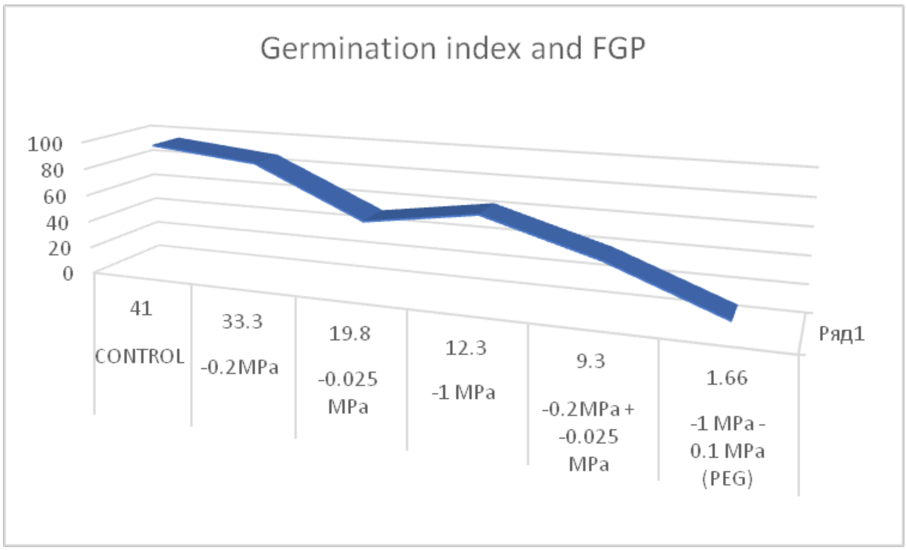

Figure 3. Line chart showing germination index and FGP

Table 2: Showing number of germinated seeds and germination percentage

Experiment		No. of germinated seeds			Germination percentage (%)		
		Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
Control	Distilled water	21±2.3	24±2.3	24±0.3	84	96	96
Treatment 1	-0.2MPa (50mM NaCl)	15±1.1	22±2.1	22±1.1	60	88	88
Treatment 2	-0.025 MPa (PEG)	10±1.2	11±1.4	13±1.3	40	44	52
Treatment 3	-1 MPa (200mM NaCl)	1±0.3	12±1.6	16±0.3	4	48	64
Treatment 4	--0.2MPa (50mM NaCl) + -0.025 MPa PEG	3±1.7	6±0.8	10±2.4	12	24	40
Treatment 5	-1 MPa (200mM NaCl) + -0.1 MPa (PEG)	0±1.3	2±0.6	2±1.5	0	8	8

Table 3: Shows different germination parameters

	FGP (%)	GI (%)	MGT (DAY)	FDG (DAY)	LDG	CVG
CONTROL	96	41	1.13	1st day	2nd day	0.00489
-0.2MPa	88	33.3	1.32	1st day	2nd day	0.00487
-0.025 MPa	52	19.8	1.38	1st day	3rd day	0.00478
-1 MPa	64	12.3	2.2	1st day	3rd day	0.00360
-0.2MPa + -0.025 MPa	40	9.3	2.6	1st day	3rd day	0.00322
-1 MPa -0.1 MPa (PEG)	8	1.66	2.97	2nd day	4th day	0.00287

Final Germination percentage = FDP; Mean Germination Time =MGT; First Day of Germination = FDG; Last Day of Germination = LDG; Coefficient of Velocity of Germination = CVG; Germination Index = GI

DISCUSSION

From the results, the effect of PEG was higher than NaCl. The drought stress induced by the PEG have much more effect than the stress induced by NaCl. In treatment 2 with -0.025 MPa (PEG) have 52% germination whereas treatment 1 with -0.2MPa (50mM NaCl) have 88% germination rate. In the case of combined effect of -0.2MPa (50mM NaCl) + -0.025 MPa PEG 40% germination percentage was noted and in treatment 5 with -1 MPa (200mM NaCl) + -0.1 MPa (PEG) shows only 8% germination. From the results we can understand that in treatment 4 having higher NaCl concentration and same concentration of PEG as in treatment 2. The treatment 2 have germination percentage of 52. But the treatment 4 have the 40% germination. From this we can assume that the higher concentration of NaCl not have much impact on the germination of seed in *Vigna mungo*. In treatment 5 have higher concentration of NaCl and high concentration of -0.1 MPa (PEG) and showed only 8% of germination. From this can assume that as the percentage of PEG increases the germination decreases. The NaCl also impart the germination rate but it doesn’t possess as much effect as PEG.

The higher the FGP value, the greater the germination of a seed population (Scott *et al.* 1984). In our present study higher FGP was noted in control and it reduces with treatments. It indicates that control have greater germination of seed population and as the treatment progresses the germination reduces. The lower the MGT, the faster a population of seeds has germinated (Orchard, 1977). The present study shows increase in the MGT as the treatment progresses. FDG values indicate a faster initiation of germination (Kader, 1998) and it is reduced in combined treatment compared to other treatments. Lower LDG values indicate a faster ending of germination (Kader, 1998) and in our study combined treatment shows higher value and it indicates slower ending of germination. The CVG gives an indication of the rapidity of germination.

It increases when the number of germinated seeds increases and the time required for germination decreases. Theoretically, the highest CVG would occur if all seeds germinated on the first day (Jones and Sanders, 1987). In the GI, maximum weight is given to the seeds germinated on the first day and less to those germinated later on. The lowest weight would be for seeds germinated on the last day. Therefore, the GI emphasizes on both the percentage of germination and its speed. A higher GI value denotes a higher percentage and rate of germination. In our study combined effect of higher osmotic potential of PEG and NaCl has lowest percentage of GI.

The results showed that as the concentration of NaCl or PEG increased, the germination rate decreased, and the time taken for the seed to germinate increased. Salt stress occurs when there is an excessive amount of salt in the soil around plant roots. This can cause drought-like conditions for plants, as the high salt levels can draw water away from the plant roots and into the surrounding soil. In addition, salt stress can damage the internal structures of plant cells, including the chloroplasts that are responsible for photosynthesis. The study also showed that the seedling growth was also affected by both stress factors, with a reduction in root and shoot length observed.

Drought stress was imposed through two concentrations of osmotic potentials of 0 (as control), –0.2MPa using PEG and salinity stress was induced using three concentrations of osmotic potentials of 0 (as control), –0.025, –0.1, using NaCl. Combined osmotic potentials of both PEG and NaCl were also studied. It was noted that combined application of osmotic potential of higher osmotic potential of NaCl and PEG have lower germination percentage compared to others. Seed germination and seedling growth are important phases in the life cycle of plant, which determines the successful establishment of seedlings and their subsequent growth (Zhang *et al.*, 2016). Seed germination and seedling growth are controlled by the interaction of ecological conditions and physiological factors (Dai *et al.*, 2009).

Reduced water potentials induced by both NaCl and PEG decreased germination and seedling growth of all the mung bean varieties in this study. Similar responses have been reported in rice (Alam *et al.*, 2002), pepper (Demir and Mavi, 2008), lentil, and mugwort (*Artemisia vulgaris* L.) (Almas *et al.*, 2013). These results revealed that the consequences of the decreased water potential gradients between the seeds and the surrounding media which adversely affected germination and subsequent seedling growth. In addition, Alam *et al.* (2002) showed that elevated concentrations of NaCl and PEG prevented water uptake into seeds, thereby inhibiting germination. In this study, PEG was observed to be more inhibitory to seed germination of the mung bean varieties compared to the NaCl treatments.

Each species has a particular range of ecological requirement needed for germination. Although osmotic stress affects all stages of the life cycle, seed germination and seedling growth stages are more susceptible to most of the species. Osmotic stress is one of the main limiting factors that inhibits seed germination and seedling growth (Sidari *et al.*, 2008).

Susceptibility to osmotic stress has been often related to oxidative damage which eventually harms the cellular mechanisms and leads to cell death, since one of the results of exposure to osmotic is the production of reactive oxygen species (ROS), such as superoxide, hydrogen peroxide, and hydroxyl radicals (Su *et al.*, 2016). High levels of ROS can damage cellular lipids, DNA, proteins, and carbohydrates; but the strong germination of seeds could only be obtained if ROS production is properly controlled (Wang *et al.*, 2009). To eliminate these toxic species, plants develop antioxidant defense systems, consisting of several antioxidant enzymes such as superoxide dismutase (SOD), peroxidase (POX), catalase (CAT), ascorbate peroxidase (APX), and non-enzyme antioxidants such as glutathione (GSH).

Osmotic stress is one of the main causes in various soil and water disorders in agricultural field crops, especially the seed germination and seedling growth. Plants of sensitive species to environments with high salt concentrations in the substrate during the initial processes of germination may have limited development and chances of survival (Di Tommaso, 2004). Osmotic stress can have a significant impact on the germination and seedling growth of Mung bean. When seeds are exposed to osmotic stress, such as high salt concentrations or drought conditions, the water potential of the seed is reduced, making it harder for water to enter and initiate germination. This can delay or decrease the overall germination rate of the seeds. Additionally, once the seed has germinated and begun to grow, osmotic stress can affect the seedling characters of Mung bean, such as shoot and root growth, chlorophyll content, and enzyme activity. In particular, high levels of salt in the soil can inhibit root growth and cause damage to the shoot system, ultimately leading to reduced plant growth and productivity. However, some studies have suggested that Mung bean may have some degree of tolerance to osmotic stress, and that certain treatments (such as pre-soaking seeds in water or using osmoprotectants) may be effective in mitigating the negative effects of stress on germination and growth. Information on the effect of stress on seed germination is important for ecophysiology, assisting in assessing the tolerance limits and adaptive capacity of species.

CONCLUSION

The experiment was carried out by *Vigna radiata* seeds with different concentrations of NaCl or PEG solutions, and then monitoring the germination rate, seedling growth and other growth parameters. NaCl and PEG causes salt stress and draught stress to the germinating seeds. NaCl affects the ion balance within the plant cell, leading to an osmotic imbalance and water loss from the plant cells. The PEG treatment, on the other hand, affects the water potential outside the cell, which impacts the uptake of water by the seeds. The results of a study on salt stress on *Vigna radiata* with PEG and NaCl showed that both stress factors caused a reduction in seed germination rate and other growth parameters. However, the effect of was PEG more significant than that of NaCl.

The experiment demonstrated that osmotic stress caused by NaCl and PEG has a negative impact on the germination rate and seedling growth of *Vigna radiata*. Both NaCl and PEG inhibited germination and seedling growth in mung bean, but the effects of NaCl compared to PEG was less on germination and seedling growth. The combined treatment shows less percentage of germination. It was concluded that inhibition in germination at combined water potential of NaCl and PEG was mainly due to an osmotic effect rather than salt toxicity.

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CONFLICT OF INTERESTS

The authors declared no conflicts of interests or publication of this article.

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