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Seed priming with osmolytes chemical to enhance germination and vigour in blackgram (*Vigna mungo*. L)

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Abstract
Seed priming is one of the most important advancements in seed germination and emergence, as well as seed tolerance to adverse environmental conditions. Seeds of Black gram var. VBN 11 were treated with various osmolytes *viz*., Mannitol, Sorbitol, Chitosan and Salicylic acid @ 100 ppm, biochar 100g kg^{-1}, Water and untreated seed as control. The results revealed that all osmolytic treatments differed significantly from the control and the seeds primed with Mannitol 1% for 3 h were recorded highest germination percentage (94%) and vigorous seedling growth.

Keywords: Black gram, priming and seed quality

1. Introduction
Pulses grow mostly in marginal and rainfed areas leading to low productivity. The major constraint in increasing the productivity levels of pulses in drylands are inadequate soil moisture and poor fertility of the soil. Black gram (*Vigna mungo* L.) is one of the India's most important legume crop. Black gram is a drought resistant crop, hence ideal for dryland farming and is often used as an intercrop with other crops. In addition, it helps to maintain fertility of soil by enhancing soil physical qualities and fixing atmospheric nitrogen.
Seed priming is a controlled hydration technique in which seeds are immersed in water or low osmotic potential until germination related metabolic activities begin but radical protrusion (Mc Donald, 2000 and Farooq *et al.*, 2007). Seed priming is a pre-sowing treatment that has the potential to enhance post-harvest seed quality and enable the release of dormancy, which will increase ultimate germination and increase speed of germination and uniform growth. The strategy involves regulating seed hydration and stimulating various metabolic processes without enabling radical protrusion (Heydecker, 1973; Bradford, 1986 and Taylor *et al*., 1998). It also encourages the development of secondary metabolites, which increases the plant ability to withstand drought. The physiological mechanisms to increase crop production under drought stress are induced by seed priming methods.
Among the different priming treatments, using osmolytes is one of the pre-sowing treatments which encourages the development of secondary metabolites and increases the ability to withstand drought. Osmolytes are compatible osmoprotectant solutes that enhance the cell potential to maintain water without hampering the normal metabolism. The main purpose of these metabolites is to regulate osmotic adjustment and also used as an osmoticant to make solutions with low water potential (Heydecker, 1973) and improve the seedling under abiotic stress tolerance. With this background, a study was undertakenin black gram var. VBN 11 with the objective to evaluate the effect of various seed osmolytes on physiological seed quality parameters in black gram.

2. Materials and Methods
The research was carried out in the Department of Seed Science and Technology, Agricultural College and Research Institute, TNAU, Madurai. The graded black gram seeds were primed with the chemicals *viz.*, T_1- Control, T_2-Water, T_3- Mannitol 1%, T_4-Sorbitol 1%, T_5- Chitosan 1%, T_6- Salicylic acid 100 ppm and T_7- Biochar 100g kg^{-1}. The seeds were soaked in different chemicals 1:1 ratio (Seed: Solution) for 3 h after soaking, the seeds were dried to their original moisture content and tested for its seed quality.
The experiment was carried out using a completely randomized block design (CRD) with two replications. The treated seeds were tested in roll towel paper method at the temperature of 25± 2 ^{0}C and 95±3% RH with the germination period of 7 days (ISTA, 1990), speed of germination

(Maguire 1962) [14], shoot length (cm), root length (cm), dry matter production (g/seedlings^{-10}), and vigour index (Abdul-Baki and Anderson 1973), electrical conductivity (dSm^{-1}) Presley (1958) [21] and dehydrogenase activity (Kittock and law 1968) [12].

2.1 Statistical analysis
The data were evaluated using the 'F' test of significance, as described by Panse and Sukhatme (1985) [18]. Before analysis, the % data were converted to angular (arc-sine) values whenever necessary. At 5% probability level,the critical differences (CD) were determined.

3. Result and Discussion
Seeds primed with different chemicals significantly increased the germination percentage and vigour of the seedlings compared to control. Among the different chemical treatments, Mannitol 1% outperformed on seed quality parameters compared to other chemicals. Seed priming induces rapid imbibition of seeds with a limited amount of water to start the pre-requisite metabolic events for pre-germination without radical protrusion. Due to the rapid uptake of water, the induction of germination and the emergence of seedlings occur earlier compared to control seeds (Stanley *et al*., 2016) [25].

Mannitol 1% registered maximum germination percentage (94%) and speed of germination (15.37) followed by chitosan which recorded (90%) germination and speed of germination (13.88) when compared to the control resulted (76%) germination and (9.35) speed of germination (Table 1). This could be due to the modification of physiological and biochemical nature of the seeds that lead to increased drought resistance (Henckel, 1964) [8]. The percentage of germination is an excellent indicator for the survival and growth potential of seed. Due to the higher germination percentage and speed of germination may be attributed to enhanced colloidal hydration, higher viscosity and flexibility of protoplasm, an increase in bound water content, a decreased water deficit, and increased metabolic activity (Maitraa *et al.*, 1999) [15]. Seed soaked with chitosan increased the germination percentage of cabbage, cucumber, and chilli (Chandrkrachang *et al.*, 2002) [4], Sui *et al.* (2002) [26] in rape-seed, Zhou *et al.* (2002) in peanut, Shao *et al.* (2005) in maize, Sui *et al.* (2002) [26] in rape-seed, Cho *et al.* (2008) [5] in sunflower and Manjunatha *et al.* (2008) [16] in pearl millet. According to Zeng *et al.* 2012, Chitosan produces a semi permeable layer on the seed retaining seed moisture which stimulates the seed germination.

Table 1: Effect of Seed priming chemicals on seed quality parameters in black gram var. VBN 11

Treatments	Speed of Germination	Germination %	Vigour Index	Dry matter production (g seedlings^{-10})
T_1- Control	9.35	76	2316	0.189
T_2 – Hydro priming	11.11	80	2507	0.245
T_3- Mannitol 1%	15.37	94	3404	0.273
T_4- Sorbitol 1%	9.58	76	2535	0.251
T_5- Chitosan 1%	13.88	90	3267	0.266
T_6- Salicylic acid 100 ppm	12.17	84	2870	0.242
T_7- Biochar 100g/ kg	11.36	80	2673	0.255
Mean	11.83	66.04	2796	0.246
S. Ed	0.23	1.41	56.04	0.004
CD (0.05)	0.50	3.03	120.20	0.008

Mannitol recorded higher root length (16.35 cm), shoot length (19.86 cm) followed by chitosan with the root length (15.76 cm), shoot length (20.54 cm) when compared to control seeds with root length (14.24 cm), shoot length (16.23 cm) in contrast with other osmolytes treatments (Fig 1). Maize seeds osmoprimed with Mannitol exhibited early germination, accelerated initiation of root and shoot development, increased vigour, and much longer seedling length than non-primed seeds when treated with osmoprimed mannitol solutions (Mohammadi *et al.*, 2008) [17]. Early emergence may be the cause of the higher shoot length in mannitol-treated seeds as a result of increased DNA, RNA, and protein synthesis during priming (Bray *et al.*, 1989) [3]. Higher seedling length might be due to increase in number of adventitious roots (Ma *et al.*, 2011) [13]. Chitosan also increases root growth and induces specific enzymes such as chitinases, pectinases and glucanases (Hien, 2004) [9].

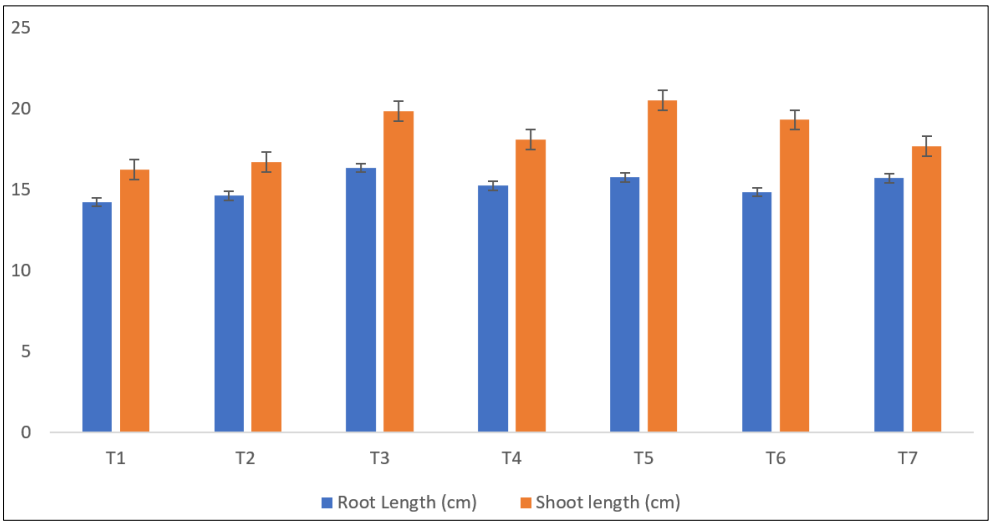

Fig 1: Performance of Seedling length (cm) of black gram under different priming chemicals

Mannitol (T_3) recorded more dry matter production (0.273 g seedlings^{-10}) followed by chitosan (0.266 g seedlings^{-10}) when compared to untreated seeds (0.189 g seedlings^{-10}) (Table 1) due to the simultaneous effect of a repair mechanism induced by primed and synchronised earlier germination, which causes seedlings to enter the autotrophic state much earlier, producing more photo assimilates from source to sink and thus increasing dry matter production Shah's (2007). Mannitol results in higher vigour (3404) followed by chitosan (3267) when compared to control (2316) (Table 1). Higher dry matter production enhances higher vigour index with increased germination percentage, root length and shoot length (Cokkizgin *et al.*, 2019 and Balaji (2019) [2] in millets. It also enhances the emergence index, synchronized emergence, and seedling vigour due to genetic repair mechanisms that take place during the priming process (Parera and Cantliffe, 1994) [19]. According to Sadeghi *et al.* (2011) [22], osmotic priming improves seed germination and vigour which was typically influenced by the mobilization of food reserves, activation and resynthesis of certain enzymes, DNA and RNA.

Seed vigour and viability were associated with biochemical factors such as electrical conductivity and dehydrogenase activity. Higher the germination percentage and dry matter production results lower the electrolyte leakage in 1% Mannitol and 1% Chitosan (0.19 dsm^{-1}) and (0.19 dsm^{-1}) respectively (Fig. 2) due to the quenching of free radicals, which restores membrane integrity (Powell and Matthews, 1977) [20]. The dehydrogenase activity was higher in 1% Mannitol (0.614) followed by 1% Chitosan (0.591) this may be possibly by the result of low moisture and low catabolic processes which was reported in black gram (Kavitha, 2002) [11] and green gram (Shenbaganathan, 2001) [24].

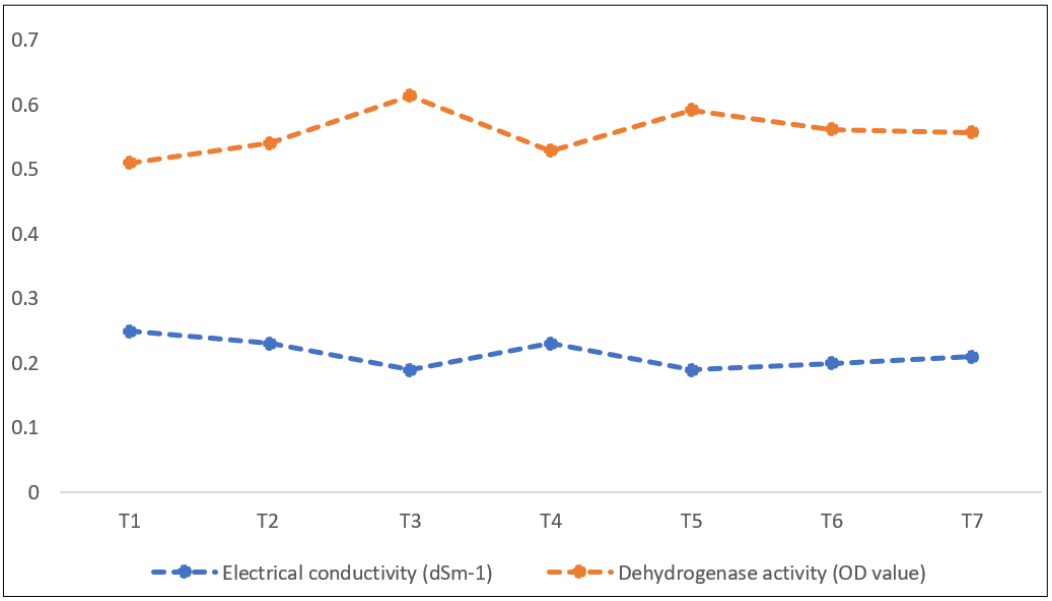

Fig 2: Difference in biochemical parameters of black gram under different priming chemicals

4. Conclusion

It can be concluded that seed priming with osmolytes of 1% Mannitol for 3 h performed well and increased seed quality and biochemical parameters of black gram and it could be recommended as pre sowing seed treatments for black gram.

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