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Influence of osmoprotectants seed priming on green gram Var. VBN 4 under drought stress

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Abstract
Green gram (*Vigna radiata* L.) typically grown under rainfed conditions, it is more susceptible to water stress than other legume crops. Drought affects the crop during different growth stages and decreases the productivity. The current study was designed to assess the optimum concentration of osmoprotectants for seed priming to alleviate the water stress in green gram. Seeds were primed with proline at the concentration of 300, 350 and 400 ppm and glycine betaine at the concentration of 100, 150 and 200 ppm along with hydro priming, whereas unprimed seeds which served as control. Water stress was induced by polyethylene glycol (PEG) 6000 at osmotic potential of -0.5 bar and -1.0 bar. The experimental results found that proline @ 350 ppm recorded the highest speed of germination (12.64), germination percentage of 83% and seedling vigour (14.94). Proline @ 350 ppm had the highest stress tolerance index 87.14% against the water stress than the hydro primed and unprimed seeds. Glycine betaine @ 150 ppm registered as the next best treatment. The unprimed seeds had registered the lowest seed quality parameters than the primed seeds.

Keywords: Green gram, priming, proline, glycine betaine, water stress

Introduction

Green gram is India's third most important pulse crop, covering 2.98 million hectares and contributes for 13.77% of total acreage, but it accounts for only 10.98% of total pulse production. In many parts of Tamil Nadu Green gram is grown as a rainfed crop. Drought is a notable abiotic stress that affects the plant physiologically and biochemically, as the crop cannot withstand moisture stress. Drought stress created a oxidative stress in the form of increased level of Reactive oxygen species (ROS) in the plant which are the major reasons for cause damage in cell membrane, DNA and some macromolecules (Raja *et al*., 2017) [13]. The germination of seeds under PEG stress is the most common germination experiment. Because it is a non-ionic polymer that is water soluble but it will not allow rapidly into plant cells, therefore used to induce drought stress in higher plants (Badiane *et al*., 2004) [4].

Seed priming is one strategy for enhancing seed vigour and crop growth under different stresses (Carvalho *et al*. 2011) [5]. Nascimento and Aragao (2004) reported that priming is a physiological process wherein seeds are soaked prior to planting, allowing for partial imbibition but restricting germination. Seed priming with compatible solutes such as glycine betaine and proline could be an effective approach for alleviating the negative impacts of drought and facilitating seedling emergence. Both of these are considered to have beneficial effects on enzyme and membrane integrity, as well as responsive functions in plants grown under adverse conditions (Ashraf and Foolad, 2005) [3]. Exogenously applied glycine betaine and proline to crop plants either prior or after stress exposure has been shown to increase internal levels of these chemicals and enhance plants growth and yield of crops. Hence the present investigation was carried out to study the effect of proline and glycine betaine priming at different concentrations to mitigate the negative impact of crops grown under drought stress.

Materials and Methods

The study was carried out at the Department of Seed Science and Technology, Agricultural College and Research Institute, Madurai in green gram seeds var. VBN 4. The seeds were collected from Krishi Vigyan Kendra, Sandhiyur. The experiment was conducted in completely randomized design with three replications. Water stress was induced by using polyethylene glycol (PEG) 6000 at -0.5 bar and -1.0 bar osmotic potential. Seeds were primed with proline and glycine betaine at different concentrations as follows: T_0- Control, T_1- Hydopriming, T_2- Proline @ 300 ppm, T_3- Proline @ 350 ppm, T_3- Proline @ 400 ppm, T_4-

Glycine betaine @ 100 ppm, T_5- glycine betaine @ 150 ppm, T_6- Glycine betaine @ 200 ppm. Seeds were evaluated for speed of germination (Maguire 1962) [11], germination percentage (ISTA 2013) [8], seedling length (cm), Dry matter production (gm), Vigour index (Abdul-Baki and Anderson 1973) [1] and Stress tolerance index were calculated by using formula proposed by (Dhopte and Livera-Muoz 1989) [6].

Statistical analysis

The data observed were analyzed statistically using SPSS software. Whenever necessary value in the percent data was transformed to arcsine transformation and 5% level critical difference was computed.

Result and Discussion

According to the present investigation, results revealed that water stress induced by PEG had a negative impact on the germination characteristics of control seeds when compared to hydro primed and osmoprotectant treated seeds, regardless of the concentration of the solutes. Among all the treatments seeds primed with proline @ 350ppm recorded the highest germination percentage of 90% and 76% at -0.5 and -1.0 bar osmotic potential respectively imposed by polyethylene glycol (Fig 1). Singh and Bhardwaj (2019) reported that proline seed treatment of wheat seedlings increases the germination parameters by enhancing ROS antioxidative pathways thereby the pretreatment of proline may improve drought stress tolerance. Glycine betaine @ 150 ppm registered 86% and 68% at osmotic potential of -0.5 bar and -1.0 bar respectively (Fig 1). Drought tolerance may be improved through seed priming with glycine betaine due to increased antioxidant activity, decreased reactive oxygen species accumulation, and decreased lipid peroxidation. Henrique *et al*., 2011 [12] found that higher concentrations of polyethylene glycol (PEG) 6000 in the solution lowered the water uptake by the seeds and reduces the germination percentage. Seed priming is a controlled hydration process in which seeds are exposed to limited water potentials, inhibiting germination but allowing pre-germination and metabolic changes to occur (Khan *et al*., 1992) [9]. Seed priming with osmoprotectants have shown better performance than unprimed seeds (Sindhu *et al*., 2021) [14]. Proline @ 350 ppm recorded the highest speed of germination (12.64), dry matter production (0.182) and vigour index (14.94) followed by glycine betaine @ 150 ppm registered as the next best treatment, whereas unprimed seeds recorded the lowest speed of germination (10.36), dry matter production (0.135) and vigour index (7.35) (Table 4). The enhanced speed of germination reflected on the dry matter production of the primed seeds which improves the vigour of the seedlings to the extent of 93% over control (Fig 2).

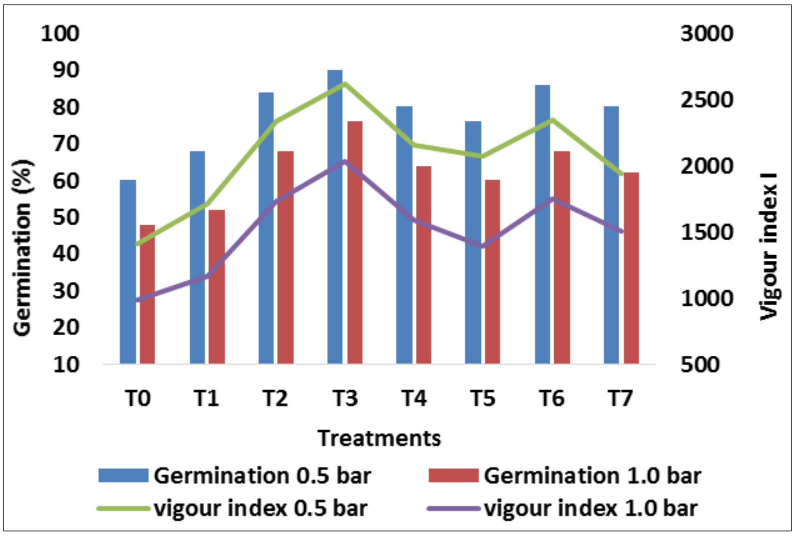

Fig 1: Effect of seed priming with osmoprotectants on germination Percentange and vigour index of green gram var. VBN 4.

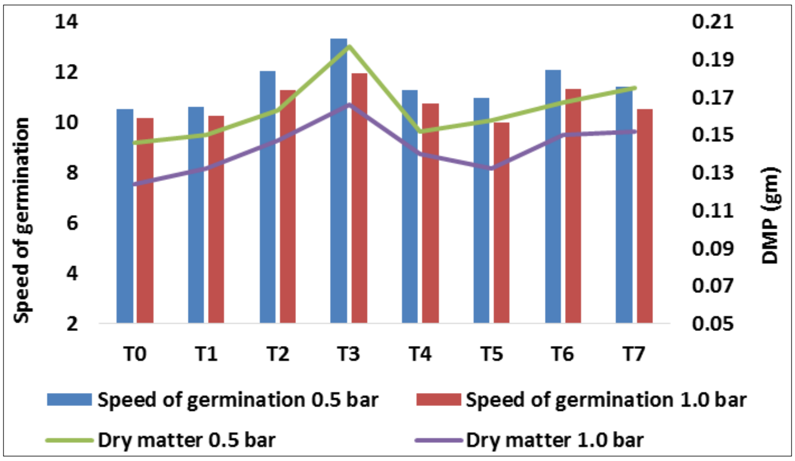

Fig 2: Effect of seed priming with osmoprotectants on speed of germination and dry matter production (gm) of green gram var.VBN 4

Significantly higher seed water uptake percentage was observed in seeds primed with proline @ 350 ppm (14.97%) followed by glycine betaine @ 150 ppm (12.42%) at 3 hrs duration. At 6 hours, there was a increment in the seed water uptake of seeds primed with proline @ 350 ppm (32.66%) and glycine betaine @ 150 ppm (26.27%) 150 ppm respectively over control (Table 1). Primed seeds initiate germination, emergence, and establish themselves earlier than unprimed seeds due to its rapid uptake of water (Lutts *et al*., 2016) [10].

Table 1: Effect of seed priming with osmoprotectants on seed water uptake of green gram var. VBN 4.

Treatments	Seed water uptake%	
	3h	6h
T_0	7.11 (15.40)	15.28 (22.80)
T_1	9.06 (17.48)	20.54 (26.59)
T_2	11.35 (19.43)	24.76 (29.45)
T_3	14.67 (21.97)	27.43 (31.32)
T_4	12.02 (20.33)	25.51 (30.28)
T_5	11.18 (19.42)	24.23 (29.39)
T_6	12.92 (20.56)	26.31 (30.76)
T_7	11.96 (19.40)	24.88 (29.38)
Mean	11.29 (19.53)	23.62 (28.99)
S.Ed	0.217	0.286
CD (0.05)	0.459	0.606

The highest stress tolerance index 87.14% was noticed in proline @ 350 ppm followed by glycine betaine @ 150 ppm 77.65% and control seeds registered the lowest value of 51.18% (Table 5), similar results were obtained for Sindhu *et al*., 2021 [14]. Due to the highest stress tolerance index, the root (16.03) and shoot growth (11.89) of green gram seeds priming with proline @ 350ppm had increased (Table 2 & 3). According to Vendruscolo *et al*. (2007) [15], the improvement in proline stress tolerance against the negative effects of water is primarily due to its oxidative stress protection mechanisms. Moisture stress tolerance is highly related with tissue water potential and the antioxidant properties, exogenous application of glycine betaine significantly reduced water-stress adversities in fine grain aromatic rice seedlings and improved leaf water potential and photosynthetic ability under water-deficit conditions (Farooq *et al*., 2008) [7].

Table 2: Effect of seed priming with osmoprotectants on root length (cm) of green gram var. VBN 4.

Treatments	PEG concentration		Mean
	-0.5 bar	-1.0 bar	
T_0	13.35	11.70	12.53
T_1	14.50	12.35	13.43
T_2	15.75	14.49	15.12
T_3	16.54	15.52	16.03
T_4	15.27	14.08	14.68
T_5	14.86	13.18	14.02
T_6	15.97	14.92	15.45
T_7	14.65	13.62	14.12
Mean	15.11	13.73	14.42
S.Ed	0.218	0.109	0.308
CD (0.05)	0.444	0.222	0.628

Table 3: Effect of seed priming with osmoprotectants on shoot length (cm) of green gram var. VBN 4

Treatments	PEG concentration		Mean
	-0.5 bar	-1.0 bar	
T_0	10.16	9.51	9.84
T_1	10.71	10.03	10.37
T_2	11.26	10.87	11.07
T_3	12.57	11.21	11.89
T_4	11.68	10.80	11.24
T_5	11.00	10.02	10.51
T_6	12.01	11.00	11.51
T_7	10.86	10.63	10.75
Mean	11.28	10.51	10.90
S.Ed	0.147	0.074	0.209
CD (0.05)	0.301	0.150	0.426

Table 4: Effect of seed priming with osmoprotectants on vigour index of green gram var. VBN 4

Treatments	PEG concentration		Mean
	-0.5 bar	-1.0 bar	
T_0	8.76	5.95	7.35
T_1	10.20	6.86	8.53
T_2	14.36	10.20	12.28
T_3	17.73	12.16	14.94
T_4	13.04	9.40	11.22
T_5	11.55	7.92	9.74
T_6	14.70	10.33	12.52
T_7	12.64	8.68	10.66
Mean	12.87	8.94	10.91
S.Ed	0.231	0.115	0.327
CD (0.05)	0.471	0.235	0.666

Table 5: Effect of seed priming with osmoprotectants on stress tolerance index of green gram var. VBN 4

Treatments	PEG concentration		Mean
	-0.5 bar	-1.0 bar	
T_0	52.27 (46.24)	50.09 (45.24)	51.18 (45.61)
T_1	56.78 (48.44)	53.17 (46.82)	54.98 (47.57)
T_2	74.89 (59.34)	71.00 (57.41)	72.95 (58.05)
T_3	88.36 (69.78)	85.92 (67.21)	87.14 (68.89)
T_4	74.53 (59.10)	69.78 (56.16)	72.16 (58.07)
T_5	71.04 (57.41)	67.42 (54.43)	69.23 (56.18)
T_6	80.02 (63.43)	78.23 (62.06)	79.13 (62.73)
T_7	74.26 (59.37)	68.00 (55.55)	71.13 (57.42)
Mean	71.52 (57.41)	67.95 (54.96)	69.74 (56.18)
S.Ed	1.052	0.526	1.488
CD (0.05)	2.144	1.072	3.032

Conclusion
From the present study, it is proved that seed priming with osmoprotectants improved the seed quality parameters than the unprimed seeds. The results revealed that increasing concentration of polyethylene glycol (6000) decreases the germination percentage of both primed and unprimed seeds. Proline @ 350 ppm registered the highest germination percentage, seedling length and vigour index and glycine betaine @ 150 ppm is the next best treatment among various priming treatments. Hence it is concluded that proline @ 350 ppm as the suitable concentration for priming the green gram seeds before sowing of green gram seeds under drought stress conditions.

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