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Seed Germination and Seedling Vigour Improvement by Halophytic Seed Treatment in Black gram (*Vigna mungo* L.)

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ABSTRACT

Background: *Chenopodium* plant is halophytic in nature in which the plant absorbs salt from soil and secrets the salts in aerial parts particularly in leaves and also has lot of macro and micro nutrients. This salt secretion by salt glands helps to survive the plants in saline conditions. The morpho-physiological characters act as barrier against mechanical damages, insects, excessive light and loss of water. Therefore, an experiment was conducted to enhance the seed quality traits *viz*., germination, speed of germination and seedling vigour in black gram by treating with the salt glands of *Chenopodium*.

Methods: The experiment was conducted in the Department of Seed Science and Technology, Tamil Nadu Agricultural University, Coimbatore during 2019 - 2020. The black gram variety VBN 8 seeds were treated with different concentrations of *Chenopodium* leaf extract and salt bladders. Then, the seeds were assessed for its quality traits.

Result: The experimental results showed that seeds soaked in *Chenopodium* leaf extract along with salt bladders @ 1.0% or salt bladders alone @ 0.2% for 3 h at 1:0.3 (w/v) ratio have recorded highest germination (97% and 96%) and seedling vigour (2280 and 2102). Nevertheless, analytical results indicated that the *Chenopodium* leaf extract and its salt bladders contain more amount of minerals particularly phosphorous (0.50%, 0.15%), potassium (0.83%, 1.11%), nitrogen (2.52%, 2.21%), calcium (16.00 ppm, 22.40 ppm), magnesium (190.56 ppm, 193.40 ppm), sodium (4.14 mg 100 g^{-1}, 6.57 mg 100 g^{-1}), chloride (0.14 mol. L^{-1}, 0.17 mol. L^{-1}), respectively, which favored the enhancement of seed qualities in black gram.

Key words: *Chenopodium,* Halophytes, Seed germination, Seedling vigour.

INTRODUCTION

Black gram (*Vigna mungo* L.) is an important legume crop of the Asian countries. The black gram seeds are sensitive to saline or sodic conditions except some varietal seeds which can tolerate these conditions. In this regard, Lamb's quarters (*Chenopodium album* L.), a halophytic plant which absorbs the salts from the soil and accumulates on its parts known as 'salt bladders' which act as barrier against mechanical damages, insects, excessive light and loss of water (Flowers and Yeo, 1986). These salt bladders have one bladder cell, with or without one or more stalk cells. But, salt glands consist of either two or multi cellular structures called 'recretohalophytes' (Reimann, 1988). In this plants, the combination of apoplastic and symplastic pathway helps to transport the salt (Na^+) from roots to salt gland by transpiration stream (Reimann and Breckle, 1988; Reimann, 1992; Flowers, 2015). This salt secretion by salt gland helps to survive the plants in saline lands (Yuan and Wang 2015). In which, the Na^+ ions accumulation promotes the salinity tolerance in the *Chenopodium* plants (Pan *et al*., 2016).

It was evidenced that the chenopod salts have rich of total phenolics and flavanoids (Kumar and Kumar, 2009), tannins, saponins, phytic acid and alkaloids (Al-Snafi, 2015). Therefore, it has antifungal, antibacterial and antioxidant properties (Singh *et al.,* 2011). In addition, it has drought tolerance and defensive mechanism against insect herbivores (LoPresti, 2014). Hence, a study was conducted to assess the effect of these *Chenopodium* salt bladders on seed germination and seedling vigour in black gram by seed treatment.

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MATERIALS AND METHODS

The experiment was conducted in the Department of Seed Science and Technology, Tamil Nadu Agricultural University, Coimbatore during 2019 - 2020. In this experiment, the black gram variety VBN 8 seeds were treated with *Chenopodium* leaf extract and salt bladders. Hence, *Chenopodium* crop was raised as bulk and used as treatment material. In which, the *Chenopodium* leaf extract was prepared by grinding the

leaves containing salt bladders in distilled water at different concentrations *viz*., 0.5 (T_2), 1 (T_3), 2 (T_4), 4 (T_5), 6 (T_6), 8 (T_7) and 10% (T_8). Then, the black gram seeds were soaked in those solutions and water (T_1) for 3 h at 1:0.3 (w/v) seed to solution ratio. After that, the seeds were dried back to original moisture content and evaluated for germination and seedling vigour. In which, the unsoaked seeds were considered as control (T_0).

Salt bladders are pink pigmented salts present on leaves, inflorescence and partially on stems of the *Chenopodium* plant (Fig 1). In the present experiment, the salt bladders present in the leaves were scrubbed off by using camel brush (Fig 2). Then, scrubbed salt bladders were dissolved in few drops of ethanol and then with distilled water. The bladder extract was prepared at different concentrations *viz*., 0.1 (T_2), 0.2 (T_3), 0.3 (T_4), 0.4 (T_5), 0.5 (T_6), 0.6 (T_7), 0.7 (T_8), 0.8 (T_9), 0.9 (T_{10}) and 1.0% (T_{11}). Afterwards, the black gram seeds were soaked in those solutions as described earlier along with water (T_1) and control (T_0). Then, the seeds were dried back to the original moisture content and tested for their germination and seedling vigour.

Germination test was conducted with 400 seeds in four replications comprising of 100 seeds in each replication

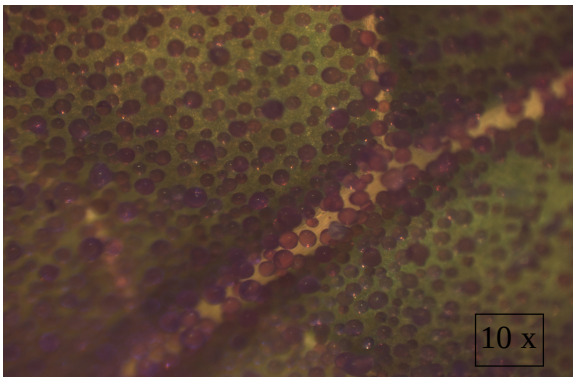

Fig 1: Salt bladders in *Chenopodium album* leaves.

Fig 2: Salt bladders collected from the *Chenopodium album* leaves.

(ISTA, 2013). The speed of germination was calculated using the formula given by Maguire (1962).

$$\text{Speed of germination} = \frac{X_1}{Y_1} + \frac{X_2 - X_1}{Y_2} + \ldots\ldots + \frac{Xn - Xn\text{-}1}{Yn}$$

Where

X_1 - number of seeds germinated at 1st count; X_2 - number of seeds germinated at 2nd count; Xn - number of seeds germinated on nth count; Y_1 - number of days from sowing to 1st count; Y_2 - number of days from sowing to 2nd count; Yn - number of days from sowing to nth count. The final count was made on seventh day after sowing and the shoot and root length were measured from ten randomly selected normal seedlings. Then, the seedlings dry matter was recorded by drying the seedlings in shade followed by hot air oven at 80^0C for 36 h. Vigour index (I) and (II) were computed by following the formula, vigour index (I) = germination percentage x seedling length (shoot length + root length); vigour index (II) = germination percentage x dry matter production (g 10 seedlings^{-1}) (Abdul Baki and Anderson, 1973).

In addition, the mineral composition of the *Chenopodium* leaves, stems and its salt bladders were analyzed at 30, 60 and 90 days after sowing . In which, the minerals such as total nitrogen by Micro Kjel dahl method using diacid extract (Humphries, 1956), total phosphorous by Vanodomolybdate yellow colour method using triple acid extract (Jackson, 1973a), total potassium by flame photometry using triple acid extract (Jackson, 1973a), total calcium and magnesium by Versanate method (Jackson, 1973b) were estimated and tabulated (Table 1).

The data collected were subjected to statistical analysis (Panse and Sukhatme, 1967) and the critical difference values were calculated at 5% probability level.

RESULTS AND DISCUSSION

Seed treatment with leaf extract is generally followed as one of the pre-sowing treatment for improvement of germination, seedling vigour, stress-tolerance etc. In the present study, the results showed that the black gram seeds soaked in *Chenopodium* leaf extract @ 1.0% have recorded the highest germination (97%) when compared with control (88%) (Table 2). However, the germination per cent got reduced and recorded the lowest (84%) at 10 per cent concentration. The improvement in germination might be due to presence of growth promoting substances such as flavanoids, tannins, coumarins, lignans, quinones, stilbens, curcuminoids *etc*. and mineral salts in the *Chenopodium* leaf extract. This was evidenced by many scientists who confirmed the presence of growth promoting and bioactive substances *viz*., α- amylase, biosynthesis of gibberellins (Lee *et al*., 1998; Lee and Kim, 2000; Basra *et al*., 2005) and synthesis of hydrolytic enzymes during the II phase of the germination. This resulted with early DNA replication (Bray *et al*., 1989), increased RNA and protein synthesis (Fu *et al*., 1988), enzyme activation for radical protrution,

antioxidant mechanism for repairing of DNA damage (Fu *et al*., 1988; Saha *et al*., 1990; Macovei *et al*., 2010).

Also, the analytical results showed that the *Chenopodium* leaf contains the minerals such as nitrogen (2.52%), phosphorous (0.50%), potassium (0.83%), calcium (16 ppm) and magnesium (190.56 ppm) (Table 1) which was higher at early stages of plant growth *i.e.* at 30 days after sowing when compared with 60 and 90 days old plants. Similar findings were reported earlier in which the nutritive values were declined due to advancement of plant age (Shahi, 1977). In addition, it contains more of sodium (4.14 mg 100 g^{-1}) and zinc (0.75 mg 100 g^{-1}) (Yildirim *et al*., 2001). In which, calcium may act as cofactor for enzymes for improvement in germination (Christansen and Foy, 1979). However, presence of higher amount of these minerals particularly the sodium salts has resulted with the deleterious effect on seed germination when the seeds soaked in higher concentrations. Similarly, the speed of germination was maximum (4.02) when the seeds soaked in *Chenopodium* leaf extract at 1.0 per cent concentration (Table 2). Root length (13.80 cm), shoot length (9.70 cm) and seedling dry matter (235.8 mg / 10 seedlings) were also highest at this concentration (Table 2). Computed vigour index I (2280) and II (22.87) were higher when the black gram seeds were soaked in *Chenopodium* leaf extract at 1.0 % concentration (Fig 3 and Table 2). The vigour improvement was mainly due to the greater synthesis of growth hormones, ATP availability and faster embryo growth (Dahal *et al*., 1990). Similar findings of germination and vigour improvement by soaking the seeds in leaf extracts were studied earlier in many crops (Shakuntala *et al*., 2012; Mansur Ahmed *et al*., 2013; Gunasekar *et al.,* 2017; Kamaraj *et al.,* 2019). Similarly, the black gram seeds soaked in *Chenopodium* salt bladders have showed the highest germination (96 %)

Table 1: Mineral composition of *Chenopodium* leaves, stems and its salt bladders.

Minerals	Plant parts	30 DAS	60 DAS	90 DAS
Nitrogen (N) (%)	Leaves	2.52	2.49	1.96
	Stems	2.30	1.34	1.06
	Salt bladders	2.21	1.34	0.81
Phosphorous (P) (%)	Leaves	0.50	0.46	0.42
	Stems	0.37	0.43	0.20
	Salt bladders	0.15	0.10	0.09
Potassium (K) (%)	Leaves	8.33	7.87	7.50
	Stems	11.57	8.92	5.38
	Salt bladders	11.14	10.27	6.99
Calcium (Ca) (ppm)	Leaves	16.00	15.20	12.00
	Stems	13.60	10.40	10.40
	Salt bladders	22.40	15.20	11.20
Magnesium (Mg) (ppm)	Leaves	190.56	231.84	229.44
	Stems	252.00	204.00	181.44
	Salt bladders	193.40	217.44	204.96

(Values are in dry weight basis) (*DAS - Days after sowing).

Table 2: Effect of *Chenopodium* leaf extract on seed germination and seedling vigour in black gram.

Treatments	Germination (%)	Root length (cm)	Shoot length (cm)	Dry matter production (mg/10 seedlings)	Vigour Index (II)
T_0 - Control	88 (69.7)	12.2	9.1	209.2	18.40
T_1 - Soaking in water for water for 3 h	92 (73.6)	13.7	9.3	219.5	20.19
T_2 - Soaking in *Chenopodium* leaf extract @ 0.5% for 3 h	92 (73.6)	12.7	9.2	227.3	21.91
T_3 - Soaking in *Chenopodium* leaf extract @ 1.0% for 3 h	97 (80.0)	13.8	9.7	235.8	22.87
T_4 - Soaking in *Chenopodium* leaf extract @ 2.0% for 3 h	93 (74.7)	12.8	9.4	224.4	20.86
T_5 - Soaking in *Chenopodium* leaf extract @ 4.0% for 3 h	90 (71.6)	12.5	9.2	215.7	19.41
T_6 - Soaking in *Chenopodium* leaf extract @ 6.0% for 3 h	93 (74.7)	12.7	9.1	212.5	19.76
T_7 - Soaking in *Chenopodium* leaf extract @ 8.0% for 3 h	86 (68.0)	12.4	9.0	209.6	18.02
T_8 - Soaking in *Chenopodium* leaf extract @ 10.0% for 3 h	84 (66.4)	12.4	8.8	201.3	16.90
Mean	72.0 (58.0)	12.8	9.2	217.3	16.00
SEd	1.44	0.23	0.14	2.60	0.20
CD (P=0.05)	2.96	0.47	0.28	5.34	0.41

(Figures in parenthesis indicate arcsine transformed value).

Table 3: Effect of *Chenopodium* salt bladders on seed germination and seedling vigour in black gram.

Treatments	Germination (%)	Root length (cm)	Shoot length (cm)	Dry matter production (mg/10 seedlings)	Vigour Index (II)
T_0 - Control	88 (69.7)	12.3	8.4	190.7	16.78
T_1 - Soaking in water for water for 3 h	90 (71.5)	12.7	8.8	195.9	17.63
T_2 - Soaking in *Chenopodium* salt bladders @ 0.1% for 3 h	93 (74.6)	12.1	8.6	201.7	18.76
T_3 - Soaking in *Chenopodium* salt bladders @ 0.2% for 3 h	96 (78.4)	12.8	9.1	207.5	19.92
T_4 - Soaking in *Chenopodium* salt bladders @ 0.3% for 3 h	90 (71.5)	12.1	8.7	200.2	18.02
T_5 - Soaking in *Chenopodium* salt bladders @ 0.4% for 3 h	87 (68.8)	11.6	8.3	194.8	16.95
T_6 - Soaking in *Chenopodium* salt bladders @ 0.5% for 3 h	84 (66.4)	11.4	8.2	190.6	16.01
T_7 - Soaking in *Chenopodium* salt bladders @ 0.6% for 3 h	81 (64.1)	10.8	7.9	185.4	15.02
T_8 - Soaking in *Chenopodium* salt bladders @ 0.7% for 3 h	80 (63.4)	10.7	7.6	180.4	14.43
T_9 - Soaking in *Chenopodium* salt bladders @ 0.8% for 3 h	78 (62.0)	10.3	7.5	175.2	13.67
T_{10} - Soaking in *Chenopodium* salt bladders @ 0.9% for 3h	75 (60.0)	9.8	7.2	174.2	13.07
T_{11} - Soaking in *Chenopodium* salt bladders @ 1% for 3 h	72 (58.0)	9.4	6.8	169.4	12.20
Mean	67.4 (55.2)	11.3	8.0	188.8	16.04
SEd	1.17	0.15	0.08	2.75	0.23
CD (P=0.05)	2.38	0.31	0.17	5.57	0.47

(Figures in parenthesis indicate arcsine transformed value).

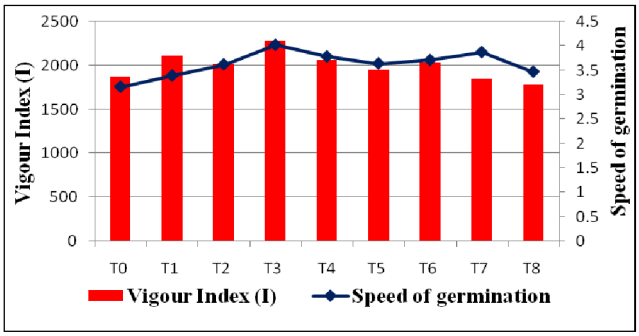

Fig 3: Effect of *Chenopodium* leaf extract on speed of germination and vigour index (I) in black gram.

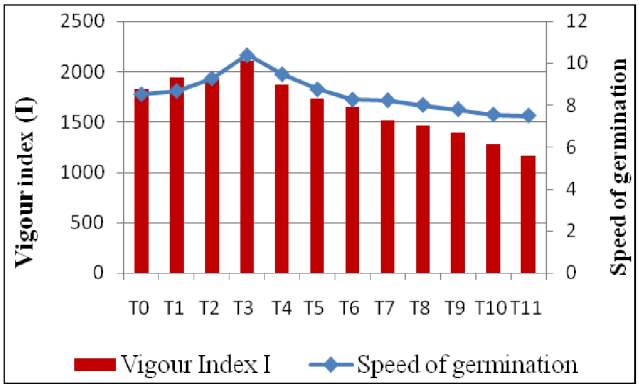

Fig 4: Effect of *Chenopodium* salt bladders on speed germination and vigour index (I) in black gram.

at 0.2 per cent when compared with control (88%) (Table 3). The presence of flavonoids, tannins, saponins, phenolic compounds and glycosides in *prosopis* and *pungam* leaf extract would have triggers the germination (Rathinavel *et al.,* 2000; Behera *et al.,* 2012).

The improvement in germination might be due to the presence of minerals such as nitrogen (2.21%), phosphorous (0.15%), potassium (1.11%), calcium (22.40 ppm), magnesium (193.40 ppm), sodium (6.57 mg 100 g^{-1}) and chloride (0.17 mol $.L^{-1}$) (Table 1). However, the reduction in germination was noticed at higher concentrations and it might be due to the higher concentrations of Na^+ and Cl^- ions in salt bladders. Similarly, speed of germination (10.39), root length (12.8 cm), shoot length (9.1 cm), dry matter (207.5 mg / 10 seedlings) and vigour index I (2102) and vigour index II (19.92) were the highest in the seeds soaked in *Chenopodium* salt bladders @ 0.2 per cent (Fig 4 and Table 3). Similar results were recorded by the effect of NaCl on seed germination and seedling vigour (Jeannette *et al.,* 2002; Mavi *et al.,* 2006).

CONCLUSION

It is concluded that the *Chenopodium* leaf extract @ 1% or salt bladders @ 0.2% have showed significant effect on improvement in seed germination (97% and 96%) and seedling vigour (2280 and 2102) in black gram. The cost involved for the seed treatment was also cheaper and it was about 25 paise for *Chenopodium* leaf extract and 50 paise for salt bladder treatments. Also, future studies are needed on the effect of salt bladders for the induction of salt tolerance in other crop seeds with different salinity levels under field condition.

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