

Induction of Salinity Tolerance through Seed Priming with *Chenopodium* Salt Bladders in Rice (*Oryza sativa* L.) and Black gram (*Vigna mungo* L.)

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ABSTRACT: *Chenopodium* is a halophytic plant that has the ability to absorb salt from soil and deposits it in aerial plant parts, more particularly in leaves. It helps the plant to survive in salinity affected soil conditions. The morpho-physiological characters of the plant act as protective mechanism against mechanical damages, pests, excessive light and drought. Also, salt bladders have macro and micro nutrients. Therefore, experiments were conducted to induce salt tolerance in rice and black gram through seed priming treatment with the salt glands of *Chenopodium*. The results showed that the seed quality parameters such as germination, speed of germination, seedling vigour etc. were reduced with the increase in the salinity concentrations irrespective of the treatments. In addition, both the seeds tolerated the salinity level up to 75 mM NaCl with good seed quality attributes. The rice seeds treated with *Chenopodium* salt bladder @ 0.6 per cent have recorded higher germination (72 %) than the control (36 %) under 100 mM salinity condition. Seedling survival was also higher (67 %) in this saline condition due to *Chenopodium* treatment. Similarly, the black gram seeds primed with *Chenopodium* salt bladder @ 0.2 per cent have recorded higher germination (71 %) at 75 mM NaCl concentration than the control (65 %) and the rate of survival is also better in case of *Chenopodium* salt bladder (52 %) than the control (48 %). Consequently, it is concluded that the *Chenopodium* salt bladder can be used to treat the rice and black gram seeds for the induction of salt tolerance up to 75 mM saline level.

Keywords: *Chenopodium*, halophytes, salinity tolerance, seed priming, rice, black gram, seed germination, seedling vigour

INTRODUCTION

Rice (*Oryza sativa* L.) and black gram (*Vigna mungo* L.) are important crops of our Indian food system. The seeds of both the crops are sensitive to saline or sodic conditions except some varietal seeds which can tolerate these conditions. Also, salinity affects the seed germination at earlier stage of seed germination process including imbibition, emergence of embryonic axis, activation of metabolism, destruction of cell covering structure, nutrient imbalance, reduction in enzyme activity as well as further establishment of seedlings [1]. Therefore, seedling establishment, growth and yield affected due to high salinity [2, 3].

Under this situation, Lambs quarters (*Chenopodium album* L.) is one of the halophytic edible plants that has potential to gather the salts from soil and encrust through the above ground plant parts known as 'salt bladders' which act as obstacle against mechanical damages, pests, excessive light and drought [4, 5, 6]. Also, the salt bladders have rich of macro and micronutrients [7].

Nevertheless, Na⁺ ions accretion in vacuole helps the plants for salinity tolerance [8]. Also, *Chenopodium* plant has antifungal [9], antibacterial and antioxidant properties [10]. It has more of total phenolic compounds and flavanoids, tannins, saponins, phytic acid and alkaloids [11]. Apart from stress tolerance, it has drought tolerance and defensive mechanism against pest herbivores [12]. Hence, the present study was conducted to evaluate the effect of *Chenopodium* salt bladders on the induction of saline tolerance in rice and black gram seeds through the way of seed priming treatment.

MATERIALS AND METHODS

Preparation of salt bladder

The present experiment was conducted in the Department of Seed Science and Technology, Directorate of Seed Centre, Tamil Nadu Agricultural University, Coimbatore during 2019 - 2021. In this experiment, the rice variety CO 51 and black gram variety VBN 8 seeds were soaked in *Chenopodium* leaf extract and salt

bladders. Thus, the *Chenopodium* crop was raised as bulk and used as treatment material. In which, the leaf extract of *Chenopodium* was prepared by macerating the leaves containing salt bladders in double distilled water. This leaf extract solution was used for seed treatment according to treatment concentration.

Similarly, the salt bladders were collected from the young leaves by using a camel brush (Figure 1). The granules were scrubbed off and dried under shade. The salt bladder solution was prepared by dissolving these granules in little quantity of ethanol and then, the volume was made up with distilled water.

Seed treatment with salt bladder in rice and black gram

The rice seeds were subjected for soaking treatment in these solutions viz., leaf extract @ 0.5 % and salt bladders extract @ 0.6% for 16 h with equal volume of seed to solution ratio. In case of black gram, the seeds were soaked in the solutions at 1.0% and 0.2% of leaf extract and salt bladders extract respectively for 3 h at 1:0.3 (w/v) seed to solution ratio. After that, the seeds were dried under shade to original moisture content. Then, the seeds were evaluated for germination and seedling vigour.

Seed germination and seedling vigour

Seed germination test for both rice and black gram was conducted with 400 seeds in four replications containing 100 seeds in each replication [13]. The rate or speed of germination was computed by the formula given by Maguire [14].

$$\text{Speed of germination} = \frac{X_1}{Y_1} + \frac{X_2 - X_1}{Y_2} + \dots + \frac{X_n - X_{n-1}}{Y_n}$$

Where, X_1 - number of seeds germinated at 1st count; X_2 - number of seeds germinated at 2nd count; X_n - 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; Y_n - number of days from sowing to nth count. The final count was made on 14th and 7th day after sowing for rice and black gram respectively and the shoot length and root length were measured in ten arbitrarily selected normal seedlings.

Vigour index was computed by following the formula, vigour index = germination percentage x seedling length (shoot length + root length) [15].

Salt bladder on stress tolerance

The treated seeds were sown in the pots as three

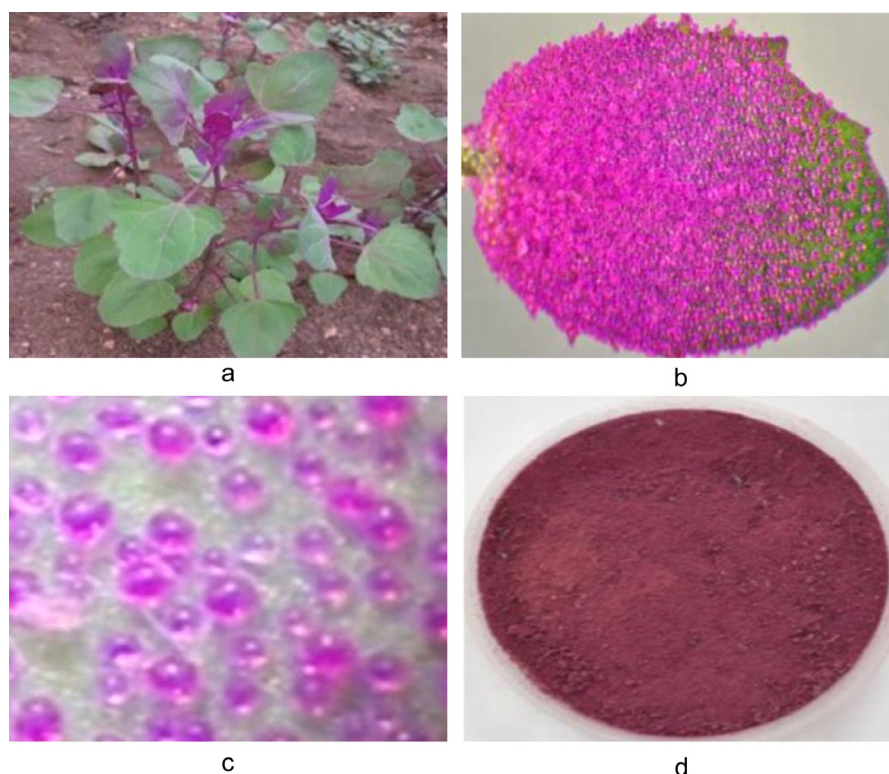


Figure 1. a) *Chenopodium* plant b) Leaf with salt bladders c) Microscopic view of salt bladders d) Scrubbed salt bladders

numbers per pot and watered regularly. During which, the water with different salinity levels such as 25, 50, 75, 100, 125, 150 and 175 mM were prepared by using sodium chloride and used for watering the plants. In which, the normal tap water was considered as control. In which, the seedling emergence percent was computed based on the number of seeds germinated under saline stress condition by following the formula,

$$\text{Emergence (\%)} = \frac{\text{Number of seeds germinated}}{\text{Number of seeds sown initially}} \times 100$$

In addition, seedlings that survived at 20 days after transplanting were counted and the survival percent was calculated based on the following formula.

$$\text{Survival (\%)} = \frac{\text{Seedlings survived at 20 days after transplanting}}{\text{Total number of seedlings transplanted at initial stage}} \times 100$$

Statistical analysis

The collected data were subjected for statistical analysis and the critical difference (CD) values were calculated at 5% probability level [16]. Further, statistical analysis of the data was performed with SPSS 16.0 software.

RESULTS AND DISCUSSION

Various priming treatments are mainly employed to increase the speed of germination, germination per cent, seedling vigour and uniform establishment for better crop performance under various stress conditions. In which, salinity condition is one of the major threat to the germinating seed. Salinity condition affects the seed germination by oxidative stress, osmotic stress and ion-

specific effects and reducing the hydrolysis of endosperm reserves [17]. Sometimes, the lower level of salt concentration induces the seed dormancy and higher concentration inhibits germination [18, 19] and this might be adaptive mechanism of the seed to prevent germination in stressful situations [20].

In such a case, seed priming repairs cell membranes, leaches inhibitors and hastens germination [21, 22]. In addition, seed priming enables the seed to tolerate both optimal and unfavorable conditions [23]. In the present study, both rice and black gram seeds were affected with increased salinity levels. In which, both the seeds tolerated upto 50 mM NaCl salinity level and recorded 85 and 77 per cent germination. Thereafter, severe reduction in germination was observed and recorded nil germination at 150 mM NaCl in rice and 100 mM NaCl in black gram seeds. It shows that the rice can able to tolerate certain level of salinity rather than black gram. In this regard, exposure of *Chenopodium* leaf extract and salt bladder treated rice seeds to various levels of salinity showed some level of tolerance to the salinity (Table 1). In which, *Chenopodium* salt bladder @ 0.6 per cent treatment recorded maximum of 36 per cent germination at 125 mM NaCl which is 33.3 per cent higher than the control. Even under stress conditions, halopriming increased germination due to enhanced oxygen uptake by the seed and facilitate the translocation of nutrients from cotyledon to embryonic axis. Presence of micronutrients in the *Chenopodium* salt bladders might be favoured to active absorption by plant and increased the enzyme and hormonal activity which promoted the growth and productive tillers.

Table 1. Effect of *Chenopodium* treatments on germination and speed of germination under different NaCl salt stress conditions in rice

Treatment	Germination (%)			Mean	Speed of germination			Mean
	Control	C.L.E. (0.5%)	C.S.B. (0.6%)		Control	C.L.E. (0.5%)	C.S.B. (0.6%)	
Control	91 (72.54)	94 (75.82)	98 (81.87)	94 (75.82)	8.42	8.71	9.00	8.71
25 mM	88 (69.73)	91 (72.54)	94 (75.82)	91 (72.54)	8.14	8.33	8.65	8.37
50 mM	85 (67.21)	88 (69.73)	91 (72.54)	88 (69.73)	7.62	7.83	8.25	7.90
75 mM	76 (60.67)	80 (63.43)	84 (66.42)	80 (63.43)	7.16	7.34	7.62	7.37
100 mM	60 (50.77)	64 (53.13)	72 (58.05)	65 (53.73)	6.55	6.94	7.28	6.92
125 mM	44 (41.55)	48 (43.85)	56 (48.45)	49 (44.43)	5.54	5.83	6.16	5.84
150 mM	16 (23.58)	22 (27.97)	28 (31.95)	22 (27.97)	4.33	4.70	5.07	4.70
Mean	66 (54.33)	70 (56.79)	75 (60.00)		6.82	7.10	7.43	
	T	S	TXS		T	S	TXS	
SE d	0.20	0.30	0.53		0.01	0.02	0.03	
CD (P=0.05)	0.40	0.61	1.05		0.03	0.04	0.07	

(Figures in parenthesis indicates arcsine transformed value) (C.L.E.- *Chenopodium* leaf extract; C.S.B.- *Chenopodium* salt bladder)

The vigour of the rice seedlings was reduced with the increased salinity levels. Therefore, at higher salinity levels like 100, 125 and 150 mM, the per cent abnormal seedlings were more irrespective of treatments (Table 2). Also, the seedlings transplanted in pot culture survived upto 100 mM NaCl and the *Chenopodium* salt bladder @ 0.6 per cent treatment recorded higher survival per cent (66 %) than control (33 %). Under high salinity (125-150 mM NaCl) range, the seedlings were not survived (Table 3).

In black gram, the seed treatment with *Chenopodium* salt bladder @ 0.2 per cent has recorded higher germination (71 %) at 75 mM NaCl concentration which is 8.5 per cent higher than control (Table 4). Also, the abnormal seedling percentage was lesser upto 75 mM NaCl concentrations and the seedling vigour was not affected much. Thereafter, the entire seedlings produced were in

abnormal condition (Table 5). Similarly, the seedling emergence was recorded upto 100 mM NaCl concentrations in pot culture, but it couldn't survive further. In which, the maximum survival (100 %) was recorded upto 50 mM NaCl concentration irrespective of the treatments and thereafter, it declined rapidly. Also, the seedling survival was noticed upto 75 mM NaCl concentration only. Where, *Chenopodium* salt bladder @ 0.2 per cent treated seeds recorded maximum survival (66 %) which is 50 per cent higher than untreated seed (Table 6).

Therefore, the results clearly indicates that the *Chenopodium* salt bladder (0.6 % in rice, 0.2 % in black gram) treated seeds tolerated the salinity conditions due to presence of sodium and chloride ions. Absorption of these ions might be responsible for the improvement in seed germination, seedling vigour and salinity tolerance

Table 2. Effect of *Chenopodium* treatments on seedling vigour and field emergence under different NaCl salt stress conditions in rice

Treatment	Vigour index			Mean	Field emergence (%)			Mean
	Control	C.L.E. (0.5%)	C.S.B. (0.6%)		Control	C.L.E. (0.5%)	C.S.B. (0.6%)	
Control	2120	2256	2430	2269	92 (73.57)	96 (78.46)	96 (78.46)	95 (76.65)
25 mM	1936	2057	2190	2061	80 (63.43)	84 (66.42)	90 (71.57)	85 (66.95)
50 mM	1717	1848	1984	1850	72 (58.05)	75 (60.00)	80 (63.43)	76 (60.44)
75 mM	1277	1392	1520	1396	68 (55.55)	71 (57.42)	74 (59.34)	71 (57.42)
100 mM	822	928	1102	951	56 (48.45)	61 (51.35)	65 (53.73)	61 (51.16)
125 mM	493	576	706	591	40 (39.23)	43 (40.98)	46 (42.71)	43 (40.98)
150 mM	154	227	308	229	12 (20.27)	12 (20.27)	15 (22.79)	13 (21.13)
Mean	1217	1326	1463		60 (50.77)	63 (52.62)	67 (54.68)	
	T	S	TXS		T	S	TXS	
SE d	5.64	8.61	14.91		0.28	0.42	0.73	
CD (P=0.05)	11.26	17.20	29.80		0.56	0.85	1.47	

(Figures in parenthesis indicates arcsine transformed value) (C.L.E.- *Chenopodium* leaf extract; C.S.B.- *Chenopodium* salt bladder)

Table 3. Effect of *Chenopodium* treatments on seedling survival and plant height under different NaCl salt stress conditions in rice

Treatment	Survival (%)			Mean	Plant height (cm)			Mean
	Control	C.L.E. (0.5%)	C.S.B. (0.6%)		Control	C.L.E. (0.5%)	C.S.B. (0.6%)	
Control	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)	72.0	74.0	75.5	73.8
25 mM	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)	71.5	73.0	74.8	73.1
50 mM	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)	70.0	71.5	73.0	71.5
75 mM	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)	70.0	71.0	72.0	71.0
100 mM	66 (54.33)	100 (90.00)	100 (90.00)	89 (70.63)	68.0	68.0	69.0	68.3
125 mM	33 (35.06)	66 (54.33)	100 (90.00)	66 (54.33)	65.0	66.7	68.5	66.7
150 mM	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0.0	0.0	0.0	0.0
Mean	71 (57.42)	81 (64.16)	86 (68.03)		59.5	60.6	61.8	
	T	S	TXS		T	S	TXS	
SE d	0.05	0.077	0.13		0.30	0.46	0.80	
CD (P=0.05)	0.10	0.15	0.27		0.61	0.93	NS	

(Figures in parenthesis indicates arcsine transformed value) (C.L.E.- *Chenopodium* leaf extract; C.S.B.- *Chenopodium* salt bladder)

Table 4. Effect of *Chenopodium* treatments on germination and speed of germination under different NaCl salt stress conditions in black gram

Treatment	Germination (%)			Mean	Speed of germination			Mean
	Control	C.L.E. (1%)	C.S.B. (0.2%)		Control	C.L.E. (1%)	C.S.B. (0.2%)	
Control	88 (69.73)	93 (74.66)	97 (80.03)	93 (74.29)	12.08	12.4	12.74	12.41
25 mM	85 (67.21)	90 (71.57)	93 (74.66)	89 (70.94)	10.33	11.82	12.16	11.44
50 mM	77 (61.34)	81 (64.16)	86 (68.03)	81 (64.40)	9.24	9.43	9.85	9.51
75 mM	65 (53.73)	68 (55.55)	71 (57.42)	68 (55.55)	8.46	8.83	9.18	8.82
100 mM	42 (40.40)	44 (41.55)	49 (44.43)	45 (42.13)	7.28	7.81	8.28	7.79
125 mM	14 (21.97)	17 (24.35)	20 (26.57)	17 (24.35)	5.66	6.15	6.85	6.22
150 mM	4 (11.54)	6 (14.18)	8 (16.43)	6 (14.18)	4.30	4.90	5.60	4.93
Mean	54 (47.05)	57 (49.02)	61 (51.10)		8.19	8.76	9.24	
	T	S	TXS		T	S	TXS	
SE d	0.28	0.43	0.74		0.02	0.03	0.056	
CD (P=0.05)	0.56	0.85	1.48		0.04	0.06	0.11	

(Figures in parenthesis indicates arcsine transformed value) (C.L.E.- *Chenopodium* leaf extract; C.S.B.- *Chenopodium* salt bladder)**Table 5.** Effect of *Chenopodium* treatments on seedling vigour and field emergence under different NaCl salt stress conditions in black gram

Treatment	Vigour index			Mean	Field emergence (%)			Mean
	Control	C.L.E. (1%)	C.S.B. (0.2%)		Control	C.L.E. (1%)	C.S.B. (0.2%)	
Control	1874	2055	2241	2057	92 (73.57)	100 (90.00)	100 (90.00)	97 (80.60)
25 mM	1734	1908	2046	1896	84 (66.42)	86 (68.03)	92 (73.57)	87 (69.15)
50 mM	1348	1499	1677	1508	70 (56.79)	76 (60.67)	84 (66.42)	77 (61.12)
75 mM	956	1074	1193	1074	60 (50.77)	65 (53.73)	70 (56.79)	65 (53.73)
100 mM	538	598	711	616	42 (40.40)	44 (41.55)	48 (43.85)	45 (41.94)
125 mM	143	187	240	190	20 (26.57)	25 (30.00)	28 (31.95)	24 (29.56)
150 mM	36	57	82	58	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
Mean	947	1054	1170		46 (42.71)	57 (48.78)	60 (50.94)	
	T	S	TXS		T	S	TXS	
SE d	2.61	3.98	6.90		0.17	0.25	0.44	
CD (P=0.05)	5.22	7.98	13.82		0.33	0.51	0.88	

(Figures in parenthesis indicates arcsine transformed value) (C.L.E.- *Chenopodium* leaf extract; C.S.B.- *Chenopodium* salt bladder)**Table 6.** Effect of *Chenopodium* treatments on seedling survival and plant height under different NaCl salt stress conditions in black gram

Treatment	Survival (%)			Mean	Plant height (cm)			Mean
	Control	C.L.E. (1%)	C.S.B. (0.2%)		Control	C.L.E. (1%)	C.S.B. (0.2%)	
Control	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)	24.0	27.6	29.5	27.0
25 mM	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)	19.5	21.0	24.7	21.7
50 mM	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)	16.3	17.5	21.8	18.5
75 mM	66 (54.33)	100 (90.00)	100 (90.00)	89 (70.63)	15.5	16.9	18.0	16.8
100 mM	33 (35.06)	66 (54.33)	100 (90.00)	66 (54.33)	10.7	11.4	12.7	11.6
125 mM	33 (35.06)	33 (35.06)	66 (54.33)	44 (41.55)	8.0	8.4	8.8	8.4
150 mM	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0.0	0.0	0.0	0.0
Mean	62 (51.77)	71 (57.60)	81 (64.05)		13.4	14.7	16.5	
	T	S	TXS		T	S	TXS	
SE d	0.11	0.17	0.30		0.17	0.26	0.45	
CD (P=0.05)	0.22	0.34	0.58		0.34	0.52	0.90	

(Figures in parenthesis indicates arcsine transformed value) (C.L.E.- *Chenopodium* leaf extract; C.S.B.- *Chenopodium* salt bladder)

[24, 25]. Similar results of seed treatment with NaCl were reported earlier in wheat [26] and sunflower [27]. In the present study, *Chenopodium* salt bladder treatment acted like halopriming and improved the seed and seedling vigour. However, at higher concentrations of NaCl, tend to produce abnormal seedlings both in rice and black gram. In evidence to this, many works were recorded that the higher salinity affected the seed germination [18, 20, 28] and produced the non-uniform seedlings [29]. However, seed germination and seedling development can be managed by primed seeds [30]. Rafiq *et al.* [31] supported the present study that the impacts of salinity can be reduced by seed priming. However, the response of crop under salinity varied according to the genetic makeup of the crop as witnessed in the present study.

The further plant growth and yield parameters were also affected due to higher saline conditions both in rice and black gram. However, better performance in *Chenopodium* leaf extract and salt bladder treated seeds were recorded than control. Khan *et al.* [32] reported the similar results that the NaCl halopriming in pepper seeds alleviated the negative effect of salinity. Also, unavailability of phosphorous under salinity causes reduction in the vegetative growth and seedling height [33]. Salinity also affects the vegetative growth by chlorophyll and photosynthesis reduction at higher levels [34]. Rehman *et al.* [35] found that the maize crop growth and yield were improved by osmopriming with moringa leaf extract at 5 per cent. Under salinity stress, antioxidant enzymes activity (catalase and peroxidase) could be reduced in rice [36] except of very sensitive genotypes. Seed priming also increased the activities of amylase and dehydrogenase enzymes in wheat [37].

CONCLUSION

The study clearly indicates that the *Chenopodium* salt bladder can be used for the induction of salinity tolerance in rice and black gram seeds. In which, seed priming with *Chenopodium* salt bladder @ 0.6 per cent induces salinity tolerance upto 100 mM in rice and 0.2 per cent in black gram with tolerance upto 75 mM.

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