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RESEARCH ARTICLE

Seed Germination and Seedling Vigor Improvement by *Chenopodium* (common lambs quarters) leaf extract and *Chenopodium* Salt Bladders in Rice (*Oryza sativa* L.)

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ABSTRACT

Chenopodium is a halophyte which absorbs salt from the soil and encrusts in aerial parts particularly in leaves which is known as chenopod salt bladders. Therefore, an experiment was conducted to enhance the seed quality in rice by treating with these bladders. Results showed that significant improvement in seed germination and seedling vigor was recorded in rice. The seeds soaked in *Chenopodium* leaf extract along with salt bladders @ 0.5 % or salt bladders alone @ 0.6 % for 16 h at 1:1 (w/v) ratio recorded the highest germination and seedling vigor. It is analyzed that the *Chenopodium* leaf extract and its salt bladders contains 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}), total phenol (5.00 mg g^{-1}, 16.12 mg g^{-1}) and poly phenol oxidase (0.20 ΔA $g^{-1}min^{-1}$, 0.10 ΔA $g^{-1}min^{-1}$), respectively which favoured the enhancement of seed quality in rice.

Keywords: *Rice; Chenopodium leaf extract; chenopod salt bladders; seed germination; seedling vigor.*

INTRODUCTION

Rice (*Oryza sativa* L.) is an important food crop of the world, grown in most of the countries. The rice seeds tend to germinate even under anaerobic conditions. Salinity hinders the rice seed germination at early stages such as imbibition, emergence of embryonic tissues, metabolism activation, cell membrane destruction, nutrient imbalance, reduction of enzyme activity and seedling establishment (Rahman *et al.,* 2017; Xiong and Zhu, 2002). On the basis of tolerance ability towards salinity, rice is salt-sensitive crop and hence, growth and yield are affected due to excess salinity (Aslam *et al.,*1989; Maas and Hoffman, 1977). Even though, some varietal seeds could tolerate these adverse conditions, including treated seeds. In this regard, lambs quarters (*Chenopodium album* L.) is a halophytic edible weed has the potential to accumulate the salts from the soil and these salts are encrusted through the aerial parts (Flowers, 2015; Reimann and Breckle, 1988; Reimann, 1992). Nevertheless, Na^+ ions accumulation in vacuole promotes salinity tolerance in plants (Pan *et al*., 2016). *Chenopodium* has antifungal (Singh *et al.,* 2011), antibacterial, antioxidant properties and rich of total phenolic contents (Kumar and Kumar, 2009), tannins, saponins, phytic acid, alkaloids and flavanoids (Al-Snafi, 2015). Not only stress tolerance, additionally, it has drought tolerance and defensive mechanism against insect herbivores (LoPresti, 2013). Hence, a study was conducted to assess the effect of these *Chenopodium* salt bladders on seed germination and seedling vigor in rice by way of seed treatment.

MATERIAL AND METHODS

The experiment was conducted at the Department of Seed Science and Technology, Tamil Nadu Agricultural University, Coimbatore during 2019 - 2020. The *Chenopodium* leaf extract, *Chenopodium* salt bladders and rice seeds of variety CO 51 constituted as study materials.

Culturing, mineral composition and enzyme activity analysis of Chenopodium

The mineral composition of the *Chenopodium* leaves, stems and its salt bladders were analyzed at 30, 60 and 90 days after sowing (DAS), in which, the minerals such as total nitrogen by micro Kjeldahl 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

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and magnesium by versanate method (Jackson, 1973b) and total phenol (Bray and Thorpe, 1954) and poly phenol oxidase activity (Mayer and Harel, 1979; Mishra, 2012) were estimated and tabulated (Table 1).

Preparation of Chenopodium Leaf extract

The physiologically active *Chenopodium* leaf samples were collected at 30 days after sowing (Figure 1). The leaf extract was prepared by grinding the leaves along with the salt bladders in distilled water at different concentrations *viz*., 0.5, 1, 2, 4, 6, 8 and 10% by (w/v).

In case of salt bladder extract preparation, the salt bladders on the leaves were scrubbed off and dissolved in few drops of ethanol followed by distilled water. The solutions were prepared at different concentration *viz*., 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1.0% by (w/v).

Chenopodium leaf extract and Chenopodium salt bladders seed invigoration on seed quality

The experiment was attempted using one-month-old rice seeds with 90 per cent germination. The seeds were soaked in leaf extract and salt bladder extract for 16 h at equal seed to solution ratio (w/v) as per the treatments schedule given below and dried back to original moisture content.

Chenopodium leaf extract seed invigoration

T_0 - Control

T_1 - Soaking in water for 16 h

T_2 - Soaking in *Chenopodium* leaf extract @ 0.5% for 16 h

T_3 - Soaking in *Chenopodium* leaf extract @ 1.0% for 16 h

T_4 - Soaking in *Chenopodium* leaf extract @ 2.0% for 16 h

T_5 - Soaking in *Chenopodium* leaf extract @ 4.0% for 16 h

T_6 - Soaking in *Chenopodium* leaf extract @ 6.0% for 16 h

T_7 - Soaking in *Chenopodium* leaf extract @ 8.0% for 16 h

T_8 - Soaking in *Chenopodium* leaf extract @ 10.0% for 16 h

Chenopodium salt bladder seed invigoration

T_0 - Control

T_1 - Soaking in water for 16 h

T_2 - Soaking in *Chenopodium* salt bladders @ 0.1% for 16 h

T_3 - Soaking in *Chenopodium* salt bladders @ 0.2% for 16 h

T_4 - Soaking in *Chenopodium* salt bladders @ 0.3% for 16 h

T_5 - Soaking in *Chenopodium* salt bladders @ 0.4% for 16 h

T_6 - Soaking in *Chenopodium* salt bladders @ 0.5% for 16 h

T_7 - Soaking in *Chenopodium* salt bladders @ 0.6% for 16 h

T_8 - Soaking in *Chenopodium* salt bladders @ 0.7% for 16 h

T_9 - Soaking in *Chenopodium* salt bladders @ 0.8% for 16 h

T_{10} - Soaking in *Chenopodium* salt bladders @ 0.9% for 16 h

T_{11} - Soaking in *Chenopodium* salt bladders @ 1.0% for 16 h

The germination test was conducted using 400 seeds in four replications comprising of 100 seeds each (ISTA, 2013). The speed of germination was calculated using the formula, $X_1/Y_1 + X_2-X_1/Y_2 +.....+ X_n (X_{n-1})/Y_n$, where, X_n- number of seeds germinated at n^{th} count, Y_n- number of days from sowing on n^{th} count (Maguire, 1962). Shoot and root length were measured from ten randomly selected normal seedlings from the germination test. Seedling dry matter was determined by drying the seedlings in a hot air oven at 85±2^{0}C for 24 h. Vigor index I and II were calculated by multiplying the germination percentage with seedling length and germination percentage with dry matter production, respectively (Abdul-Baki and Anderson, 1973; Reddy and Khan, 2001). In the treated seeds, α-amylase (Paul *et al.*, 1970) and poly phenol oxidase activities were also estimated. The experiment was conducted by following completely randomized design (CRD) with four replications.

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 botanical extract is generally followed as one of the pre-sowing treatment to improve the germination, seedling vigor and stress-tolerance.

Figure 1. *Chenopodium* plants with salt bladders and its microscopic view

In the present study, the results showed that the rice seeds soaked in *Chenopodium* leaf extract @ 0.5 % have recorded the highest germination (97%) when compared with control (90%) (Table 2). However, the germination got declined as the concentration increased and recorded the lowest (81%) at 10 per cent concentration. The improvement in germination might be due to the presence of growth-promoting substances 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 protrusion, antioxidant mechanism for repairing of DNA damage (Fu *et al*., 1988; Saha *et al*., 1990; Macovei *et al*., 2010).

Table 1. Mineral composition and enzyme activity 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	0.83	0.79	0.75
	Stems	1.16	0.89	0.54
	Salt bladders	1.11	1.00	0.70
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
Total phenol (mg/g)	Leaves	5.00	9.50	4.55
	Stems	1.80	4.50	2.85
	Salt bladders	16.12	9.00	8.50
Polyphenol oxidase (ΔA/g/min)	Leaves	0.20	0.10	0.05
	Stems	0.10	0.10	0.05
	Salt bladders	0.10	0.10	0.05

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

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) magnesium (190.56 ppm), total phenol (5.00 mg g^{-1}) and poly phenol oxidase (0.20 ΔA $g^{-1}min^{-1}$) which were higher at early stages of plant growth *i.e*. at 30 days after sowing when compared with 60 and 90 days old plants (Table 1).

Table 2. Effect of *Chenopodium* leaf extract on seed germination and seedling vigor in rice

Treatments	Germination (%)	Root length (cm)	Shoot length (cm)	Dry matter production (mg/10 seedlings)	Vigor Index (II)
T_0- Control	90 (71.6)	16.20	6.14	64.25	5.78
T_1- Soaking in water for 16 h	92 (73.6)	17.30	6.50	77.73	7.15
T_2- Soaking in *Chenopodium* leaf extract @ 0.5% for 16 h	97 (80.0)	18.50	7.60	84.55	8.20
T_3- Soaking in *Chenopodium* leaf extract @ 1.0% for 16 h	94 (75.8)	17.80	7.26	76.13	7.16
T_4- Soaking in *Chenopodium* leaf extract @ 2.0% for 16 h	90 (71.6)	17.65	7.05	69.88	6.29
T_5- Soaking in *Chenopodium* leaf extract @ 4.0% for 16 h	88 (69.7)	17.41	6.64	64.05	5.64
T_6- Soaking in *Chenopodium* leaf extract @ 6.0% for 16 h	85 (67.2)	17.20	6.32	62.70	5.33
T_7- Soaking in *Chenopodium* leaf extract @ 8.0% for 16 h	83 (65.6)	16.91	6.20	61.75	5.13
T_8- Soaking in *Chenopodium* leaf extract @ 10.0% for 16 h	81 (64.2)	16.10	6.03	60.83	4.93
Mean	71 (57.4)	17.23	6.64	69.10	6.17
SEd	1.55	0.20	0.09	0.81	0.07
CD (P = 0.05)	3.18	0.41	0.18	1.66	0.15

Calcium may act as co-factor for enzymes for improvement in germination and vigor (Christansen and Foy, 1979). However, presence of higher amount of minerals particularly the sodium salts have resulted with the deleterious effect on seed germination when the seeds were soaked in higher concentrations.

Table 3. Effect of *Chenopodium* salt bladder on seed germination and seedling vigor in rice

Treatments	Germination (%)	Root length (cm)	Shoot length (cm)	Dry matter production (mg/10 seedlings)	Vigor Index II
T_0. Control	90 (71.57)	16.4	6.0	67.55	6.08
T_1. Soaking in water for 16 h	92 (73.57)	16.9	6.2	65.85	6.06
T_2. Soaking in *Chenopodium* salt bladders @ 0.1% for 16 h	86 (68.03)	16.6	5.9	66.90	5.75
T_3. Soaking in *Chenopodium* salt bladders @ 0.2% for 16 h	89 (70.63)	17.2	6.0	71.65	6.38
T_4. Soaking in *Chenopodium* salt bladders @ 0.3% for 16 h	87 (68.87)	17.4	6.4	67.51	5.87
T_5. Soaking in *Chenopodium* salt bladders @ 0.4% for 16 h	90 (71.57)	17.0	6.3	62.80	5.65
T_6. Soaking in *Chenopodium* salt bladders @ 0.5% for 16 h	92 (73.57)	17.4	6.7	62.63	5.76
T_7. Soaking in *Chenopodium* salt bladders @ 0.6% for 16 h	98 (81.87)	18.7	7.2	74.13	7.26
T_8. Soaking in *Chenopodium* salt bladders @ 0.7% for 16 h	92 (73.57)	16.5	6.7	67.40	6.20
T_9. Soaking in *Chenopodium* salt bladders @ 0.8% for 16 h	90 (71.57)	17.1	6.3	66.85	6.02
T_{10}. Soaking in *Chenopodium* salt bladders @ 0.9% for 16 h	87 (68.87)	17.4	6.0	67.00	5.83
T_{11}. Soaking in *Chenopodium* salt bladders @ 1.0% for 16 h	85 (67.21)	17.0	5.9	66.93	5.69
Mean	72 (58.05)	17.1	6.30	67.27	6.05
SEd	1.76	0.23	0.09	0.94	0.08
CD (P = 0.05)	3.58	0.46	0.18	1.91	0.17

Similarly, the speed of germination was maximum (9.0) when the seeds were soaked in *Chenopodium* leaf extract @ 0.5% (Figure 2).

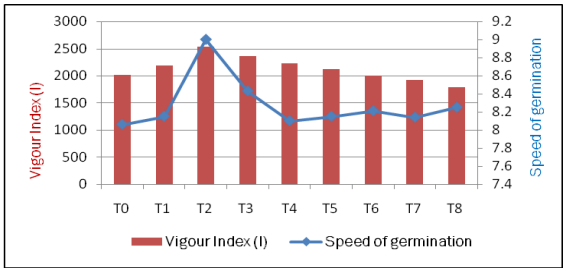

Figure 2. Effect of *Chenopodium* leaf extract on speed of germination and seedling vigor index (I) in rice

Root length (18.50 cm), shoot length (7.60 cm) and seedling dry matter (84.55 mg / 10 seedlings) were also higher at 0.5% concentration (Table 2).

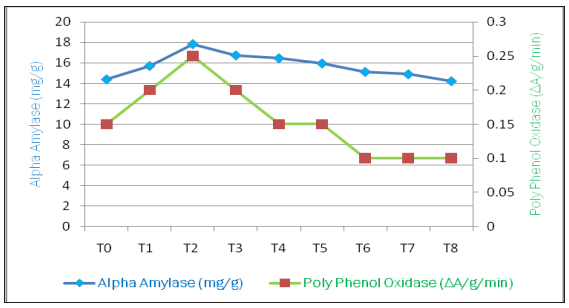

Figure 3. Effect of *Chenopodium* leaf extract on α-amylase and poly phenol oxidase in rice

Computed vigor index I (2531) and II (8.20) were higher when the rice seeds soaked in *Chenopodium* leaf extract at 0.5% concentration. This vigor improvement is mainly due to the greater synthesis of growth hormones, ATP availability and faster embryo growth (Dahal *et al*., 1990) due to leaf extract soaking. Similar findings of germination and vigor improvement by soaking the seeds in leaf extracts *viz*., *Prosopis*, neem, moringa and *Pongamia* were studied earlier in paddy (Shakuntala *et al*., 2012; Ahmed *et al*., 2013; Gunasekar *et al*., 2017; Kamaraj *et al*., 2019). The presence of flavonoids, tannins, saponins, phenolic compounds and glycosides in *Prosopis* and *Pongamia* leaf extracts would have triggered the germination (Rathinavel *et al*., 2000; Behera *et al*., 2012). In *Chenopodium* leaf extract @ 0.5% treated seeds, α-amylase (17.84 mg g^{-1}) and poly phenol oxidase (0.25 ΔA.g $^{-1}$$min^{-1}$) activities recorded were also maximum (Figure 3).

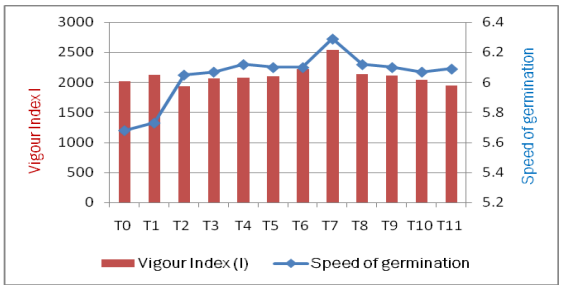

Figure 4. Effect of *Chenopodium* salt bladders on speed germination and vigor index (I) in rice

Regarding *Chenopodium* salt bladders, the highest germination (98%) was observed at 0.6 per cent as compared to control (90%) (Table 3). This might be due to the presence of minerals in salt

bladders 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}), chloride (0.17 mol. L^{-1}), total phenol (16.12 mg g^{-1}) and polyphenol oxidase (0.10 ΔA g^{-1} min^{-1}) at early stage of plants (30 DAS). However, the reduction in germination was noticed at higher concentrations. This might be due to the higher concentrations of Na^+ and Cl^- ions in salt bladders. Similarly, speed of germination (6.29), root length (18.7 cm), shoot length (7.2 cm) and dry matter (74.13 mg / 10 seedlings) were maximum in the seeds soaked in *Chenopodium* salt bladders @ 0.6% concentration (Table 3). Vigor index I (2538) and II (7.26) were also higher at this concentration (Figure 4).

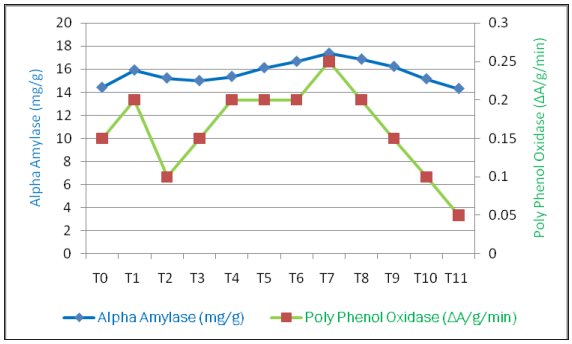

Figure 5. Effect of *Chenopodium* salt bladders on α-amylase and poly phenol oxidase in rice

Alpha amylase (17.38 $mg.g^{-1}$) and poly phenol oxidase (0.25 $\Delta A.g^{-1}.min^{-1}$) activities were also higher in the seeds soaked in *Chenopodium* salt bladders @ 0.6% (Figure 5). Similar studies were also carried out earlier by the effect of NaCl on seed germination and seedling vigor (Jeannette *et al.,* 2002; Mavi *et al.,* 2006).

CONCLUSION

It is concluded that the *Chenopodium* leaf extract at 0.5 per cent or *Chenopodium* salt bladders at 0.6 per cent concentration have beneficial effect on improvement of seed germination and seedling vigor in rice. Further study is needed on the effect of these salt bladders for the induction of salt tolerance in other crop seeds.

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