SALINITY TOLERANCE OF RICE (*ORYZA SATIVA* L.) SEEDLINGS IN PRESENCE OF AQUEOUS EXTRACT OF TESTA OF CASHEW NUT (*ANACARDIUM OCCIDENTALE* L.)

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Abstract— Salinity tolerance of cv. Kasturi (basmati) and cv. Pant Dhan 11 (non-basmati) of rice (*Oryza sativa* L.) in presence of aqueous extracts of testa of *Anacardium occidentale* L. during seedling stage was studied in laboratory. Basmati and non-basmari seeds were sown at 0, 0.5, 1.0 and 2% (w/v) NaCl concentrations along with 5% of testa extract (TE=1.5gm/100mL). The performance of seed germination improved at 1.0 and 2.0% NaCl concentrations in both the cultivers. Presence of testa extract decreased the effect of salinity on shoot and root length, fresh and dry weight in both the cultivers at 10 and 15 days.Relative improvements in germination, shoot and root growth was better in cv. Pant Dhan 11 in presence of testa extract under salinity.

Keywords— aqueous extract, cashew testa, tolerance, salinity

I. INTRODUCTION

Testa of cashew nut kernal is rich in hydrolysable tannins and proanthrocyanins as major polyphenols [1]. Studies on flavan-3-ols which is widely distributed in fruits of plants and it nutritional value of well established [2]. Diverse biological roles of polyphenols include antioxidant properties [3] in defense mechanism attracted many reserchers. Chemical nature of polyphenols of cashew nut testa was identified [1].Aqueous extracts of cashew nut testa consists of 19.9-22% tannins [4]. Antioxidant defence mechanism is an option to reduce the oxidative damage [5]. Antiradical scavenging properties of cashew nut skin extract *in vitro* antioxidant assay system [6] and protective role against organophosphorous insecticide toxicity in rats were reported [7]. Stress often results in production of reactive oxygen species results in oxidative stress [8]. Salt stress response may be immediate, slow or continuous and differ in dose periodically or continuous during any stage of life cycle of a plant [9]. Abiotic stresses are responsible for poor seeding growth and reduction in crop yield [10-12]. Germination of seeds affected by salinity [9, 13] and reduction in growth parameters were reported [14]. Salt stress inhibits shoot growth reported in rice [15]. Rice is an important cereal crop and is moderately salt tolerant [16]. Salt effects dry matter allocation results in reduction of growth [17]. Salinity usually affects shoot growth results in increase in root: shoot ratio [18]. The first line of defense of plant is root [19]. Reports show in some plants low salinity level stimulated root growth [20]. Aqueoous extracts of sunflower improved salinity tolerance in rice [21]. Basmati is a GI (Geographical Indication) crop of Uttarakhand [22]. Cv. Kasturi (aromatic Basmati) and cv. Pantdhan 11(non-basmati) are one among the suitable popular varieites of Uttarakhand state [23, 24]. Present study undertaken to know whether aqueous extracts of testa has supportive role on seed germination and seedling growth under NaCl salt salinity taking rice as testing plant species.

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II. MATERIALS AND METHODS

Testa of cashew nut seed was procured from cashew processing unit located around Chirala-Andhra Pradesh, India. Freshly harvested seeds of cv. Kasturi (Pedigree: Basmati370/CR 88-17-1-5, IET No.: 8580 [22] and cv. Pant Dhan 11(hence onwards cv. Pant 11) collected from agriculture field located near Selaqui region, Dehradun, Uttarakhand, India. Testa was powdered and passed through 150 µm sieve. 1.5 Gm of 150 µm size powder soaked in 100 ml water for 24 hours at 29±1^{o}C [25] and the aqueous extracts collected by filtering through Whatman filter paper 1 and used for preparing 5% dilution and passed through 0.2 µm membrane filter before applying on rice seeds. Rice seeds surface sterilized with 0.1% (w/v) $HgCl_2$ for 10 minutes and washed with sterile distilled water for 5 times. Petri dish (14 cm diameter in size) lined with a thin layer of cotton and Whatman filter paper 1 moistened with 20 mL salinity treatments of 0.5%, 1.0% and 2% (w/v) NaCl with or without aqueous testa extract, 30 rice seeds were disposed on it [26, 27] and distilled sterilized water used as control treatments. There were duplicates for each treatment in completely randomized block design. Petri dishes were incubated at room temperature (29±1^{o}C). Germination test was performed as per ISTA rules, 1999. Germination of seeds was recorded for seven days after every 24 hours. Seedling growth was measured on 7th, 10th and 15th day. Germination percentage [28], shoot, root and seedling height (mm), fresh and dry weight of shoot and root were measured. Speed of germination, response index [29], seedling vigour index [30] and seedling growth parameters were used for evaluation of effect of testa aqueous extract on salinity tolerance. Data was subjected to ANOVA [31] and excel 2007 was used for data analysis and preparing graphs.

III. RESULTS

Table 1 shows the seed germination percent (%) of cv. Kasturi (basmati) and cv. Pant 11 (non-basmati) at 7 days. In both the cultivers, seed germination was totally retarded in NaCl treatments more than 1.0% (w/v). In presence of testa

extract (+TE), there was significant improvement in germination and seedling growth under 0-2% (w/v) salinity in both the cultivers. However little decrease in germination % in cv. Kasturi.

Response index of cv. Kasturi and cv. Pant 11shows, in presence of testa extract reduced the effect of inhibition above 0.5% (w/v) salinity (figure 1). In cv. Kasturi, presence of testa extract, effect of inhibition reduced in 1.0% and in cv. Pant 11 in 2.0% (w/v) NaCl.

With increase in salinity, decrease in germination %, shoot and root growth at 7 days in both the cultivers (table 1). However, root length increased in 0.5% (w/v) salinity in cv. Kasturi. In +TE, the effect salinity reduced on shoot and root length in both the cultivers. However, in presence of testa extract, in 0.5% (w/v) salinity, shoot length increased over control and root length decreased in non-basmati and both shoot and root length increased in basmati at 7 days.

In +TE, cv. Kasturi showed 46% decrease in root: shoot length ratio at 1.0% (w/v) salinity and 54% decrease in 0.5% (w/v) salinity treatment in cv. Pant 11 (Table 1). Growth in TE+2.0% (w/v) NaCl treatment was negligible measure limit in both the cultivers.

NaCl (%)	cv. Kasturi Germination (%)		Shoot length (mm)		Root length (mm)	
	- TE	+ TE	- TE	+ TE	- TE	+ TE
0	96	93	20	20	30	29
0.5	93	86	16	22	32	39.9
1	63	90	3.6	16	13	27
2	0	70	0	!	0	!
Lsd (P=0.001)	1.84		0.55		0.782	
NaCl (%)	cv. Pant Dhan 11 Germination (%)		Shoot length (mm)		Root length (mm)	
	- TE	+ TE	- TE	+ TE	- TE	+ TE
0	100	100^{ns}	20	24	34	37
0.5	96	96	12	21	31	29.5
1	73	90	3	18.5	7	26.5
2	0	83	0	!	0	!
Lsd (P=0.001)	0.8316		0.47		1.15	

Table 1: Seed germintion and seedling growth of cv. Kasturi and cv.Pant 11 at 7 days in absence (-TE) & presence (+TE) of testa extract under salinity treatments. Lsd= least significant difference; NS= not significant; !=negligible growth

Table 2 shows seedling growth of cv. Kasturi, shoot length decreased with increase in salinity and effect was more at 10 days compared to 15 days. In cv. Pant 11, shoot and root length decreased with salinity and varied with time (table 3).

In cv. Pant 11, in 0.5% (w/v) NaCl, shoot length decreased with time and no further decrease in root length at 15 days. Both basmati and non-basmati showed decrease in shoot length under salinity both at 10 and 15 days. Salinity affected more at 10 days and affect was more with increase in salt concentration. However percentage decrease was more in cv. Pant 11 compared to cv. Kasturi.

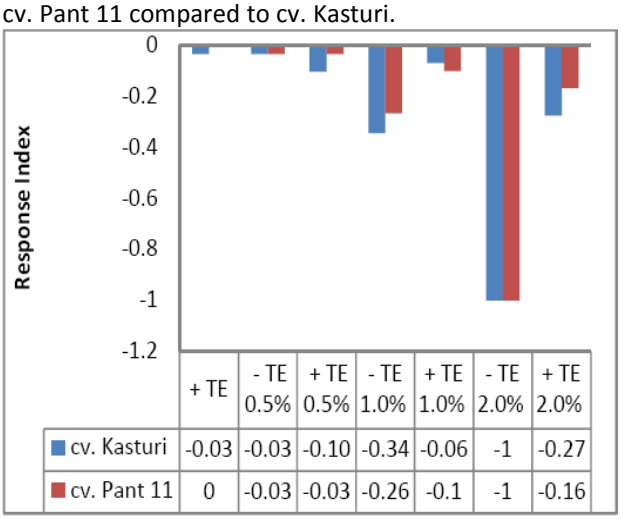

Figure 1: Response index of cv. Kasturi and cv.Pant 11 at 7 days in absence (-TE) & presence (+TE) of testa extract under (0-2% w/v NaCl) salinity treatments.

In presence of testa extract, effect of salinity on shoot and root

length reduced both at 10 and 15 days in both the cultivers. In cv. Kasturi, improvement in shoot and root length was more at 10 days in 0.5 and 1.0% (w/v) +TE treatments. In cv. Kasturi, +TE 1.0% (w/v) NaCl treatment showed 65% and 52% increase in shoot length at 10 and 15 days respectively compared to salinity in absence of TE. The performance of reducing the effect of salinity on shoot length in cv. Pant 11 was better over cv. Kasturi.

In +TE 1.0% (w/v) NaCl treatment, in cv. Pant 11, 56% and 37% increase in root growth at 10 and 15 days respectively. No germination in 2% (w/v) NaCl in both the cultivers. However in presence of testa extract, 2% (w/v) NaCl treatment in basmati showed 33% shoot and 39% root growth and non-basmati showed 30% shoot and 26% root growth at 15 days. In cv. Pant 11, presence of testa extract increased root length over control at 10 days and increase was not significant in basmati. Testa extract stimulated root length at 15 days in basmati and decreased in non-basmati.

In cv. Pant 11, presence of testa extract showed no effect on salinity in root fresh weight in 0.5% (w/v) salinity at 10 days and effect of salinity decreased at 15 days. Root fresh weight increased over control in +TE 1.0% (w/v) NaCl treatment in non-basmati at 10 days.

In cv. Pant 11, testa extract decreased the shoot fresh weight and decrease was more with time. Basmati response to testa extract was reverse, it increased shoot fresh weight and increase was more with time.

In cv. Kasturi, root dry weight and in cv. Pant 11, shoot and root dry weight decreased under salinity with time. In cv. Kasturi, in 0.5% (w/v) salinity shoot dry weight increased and in 1.0% (w/v) salinity shoot dry weight decreased at 10 days. However, in 0.5% and 1.0% (w/v) salinity, shoot dry weight decreased in basmati at 15 days.

The effects of salinity in presence of testa extract reduced on shoot dry weight in both the cultivers at 10 and 15 days. In cv. Kasturi, increase in shoot dry weight in 0.5% (w/v) NaCl +TE treatment over control at 10 and 15 days. However, two fold sharp increase in shoot dry weight at 10 days.

Growth parameter cv. Kasturi	Days	0 % NaCl - TE	0 % NaCl + TE	0.5% NaCl - TE	0.5% NaCl + TE	1.0 % NaCl - TE	1.0 % NaCl + TE	2.0% NaCl - TE	2.0% NaCl + TE	Lsd (P=.001)
Germination (%)	10	96.6	93.33***	93.33***	86.67***	63.33***	90***	0***	70***	1.84
Shoot length (mm)	10	29	31.5*	23***	32.5**	5.15***	24***	0***	0	4.37
	15	33	39.25***	25***	33.25^{ns}	12.75***	30.5^{ns}	0***	11***	6.27
Root length (mm)	10	42.25	43^{ns}	47.75*	53**	19.75***	39.75*	0***	0	9.36
	15	56.25	63.5**	60.75^{ns}	57.5^{ns}	24.25***	40.25***	0***	22***	9.7
Shoot fresh weight (mg)	10	12.2	12.83***	9.63***	9.63***	4.86***	9.56***	!	!	0.33
	15	14	16***	10.5***	13.36***	6.8***	12.98***	!	6.66***	0.45
Root fresh weight (mg)	10	9.6	6.9***	8.33***	9.06***	3.5***	9.1***	!	!	0.048
	15	17	19.56***	19.72***	25.56***	8.54***	20.92***	!	6.37***	0.29
Shoot dry weight (mg)	10	1.53	1.83***	1.7***	4.2***	0.36***	1.1***	!	!	0.13
	15	1.32	1.94***	0.92***	1.58***	0.48***	1.14***	!	0.58***	0.014
Root dry weight (mg)	10	1.9	1.16***	1.06***	0.9***	0.2***	0.8***	!	!	0.04
	15	2.74	2.98***	2.04***	2.52***	0.77***	2***	!	0.925***	0.07

Table 2: Seed germintion and seedling growth of cv. Kasturi at 10 & 15 days in absence (-TE) & presence (+TE) of testa extract under salinity treatments. Lsd= least significant difference; NS= not significant; *, ** & *** are P= 0.05, 0.01 & 0.001 respectively; ! = no germination; !!= negligible growth

Growth parameter cv. Pant Dhan 11	Days	0 % NaCl - TE	0 % NaCl + TE	0.5% NaCl - TE	0.5% NaCl + TE	1.0 % NaCl - TE	1.0 % NaCl + TE	2.0% NaCl - TE	2.0% NaCl + TE	Lsd (P=.001)
Germination (%)	10	100	100^{ns}	96***	96***	73***	90***	0	83***	0.8316
Shoot length (mm)	10	30	35.3^{ns}	18.25	31.25^{ns}	5***	27.75^{ns}	0	6.75***	8.4
	15	39	46.8***	21***	36.75*	11***	36.25*	0	11.75**	6.86
Root length (mm)	10	51	55.5^{ns}	46.5^{ns}	44.25*	10.5***	39.75***	0	11.75**	10.43
	15	66	62.25^{ns}	60.25^{ns}	51.25***	26.5***	51.25***	0	17.5***	14.06
Shoot fresh weight (mg)	10	13.23	12.53**	7.8***	12.5***	3.66***	11.66***	0	0	0.09
	15	17.06	15.26**	9.3***	16.34***	10.28***	14.76***	0	7.77***	0.15
Root fresh weight (mg)	10	10.86	10.03**	9.6***	9.2***	3***	11.2**	0	0	0.052
	15	23.7	17.82**	15.9***	19.98***	11.44***	16.96***	0	6.11***	0.094
Shoot dry weight (mg)	10	1.46	1.7***	0.85***	1.4***	0.36***	1.43***	0	0	0.04
	15	1.88	1.9*	0.8***	1.86^{ns}	1.5***	1.36***	0	0.77***	0.12
Root dry weight (mg)	10	1.93	2***	1.56***	0.96***	0.23***	1.06***	0	0	0.02
	15	3.72	3.26***	1.9***	2.2***	0.61***	2.04***	0	0.72***	0.06

Table 3: Seed germintion and seedling growth cv.Pant 11 at 10 & 15 days in absence (-TE) & presence (+TE) of testa extract under salinity treatments. Lsd= least significant difference; NS= not significant; *, ** & *** are P= 0.05, 0.01 & 0.001 respectively; != no germination; !!= negligible growth

In cv. Kasturi, effect of salinity reduced in decrease in root dry weight in presence of testa extract at 10 and 15 days. However, performance of reduction in presence of testa extract was better with increase in time. In cv. Pant 11, presence of testa extract in 0.5% (w/v) salinity, further decrease in root dry weight at 10 days, however, in the presence of testa extract, effect of salinity reduced at 15 days. In cv. Pant 11, 1.0% (w/v) NaCl in presence of testa extract decreased the effect of salinity on root dry weight at 10 and 15 days.

In presence of testa extract, increase in shoot dry weight both at 10 and 15 days, however cv. Pant 11 showed more increase at 15 days and cv. Kasturi at 10 days.

The analysis of shoot: root growth under salinity in presence of testa extract in both the cultivers shown in figures 2&3.

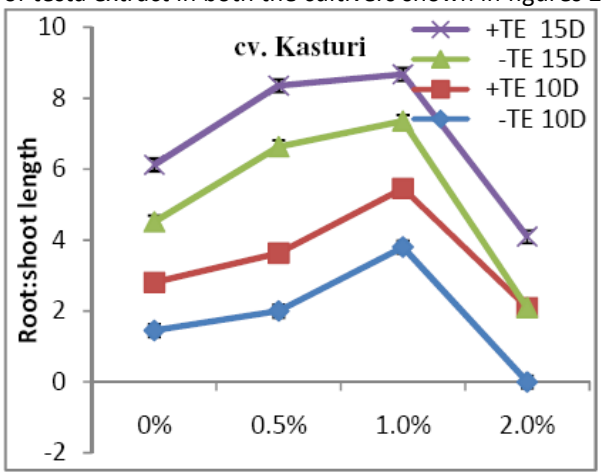

Figure 2: Root: shoot length ratio of cv. Kasturi at 10 & 15 days in absence (-TE) & presence (+TE) of testa extract under salinity treatments (P=0.01).

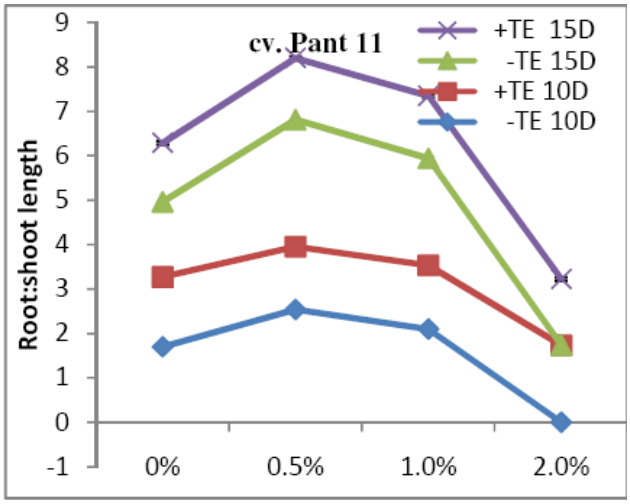

Figure 3: Root: shoot length ratio cv. Pant 11 at 10 & 15 days in absence (-TE) & presence (+TE) of testa extract under salinity treatments (P=0.01).

Increase in salinity increased root: shoot length ratio in both the cultivers. In cv. Kasturi, 0.5% (w/v) salinity, presence of testa extract further increased the root: shoot length at 10 and 15 days. In presence of testa extract, in basmati, the root: shoot length decreased over 1.0% (w/v) NaCl compared to absence of testa extract.

In cv. Pant 11, presence of testa extract decreased the root: shoot length ratio over salinity at 10 and 15 days (figure 3).

Figure 4 shows in cv. Kasturi root: shoot fresh weight increased under salinity, however root: shoot fresh weight decreased in 1.0% (w/v) salinity at 10 days. In cv. Kasturi,

root: shoot fresh weight further increased in presence of testa extract at 10 and 15 days.

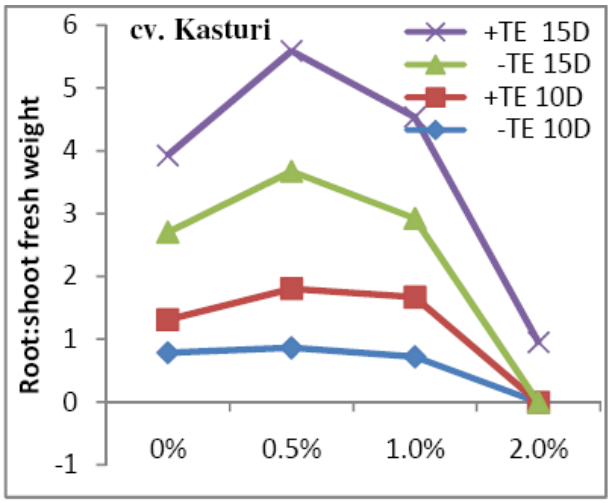

Figure 4: Root: shoot freshweight ratio of cv. Kasturi at 10 & 15 days in absence (-TE) & presence (+TE) of testa extract under salinity treatments (P=0.01).

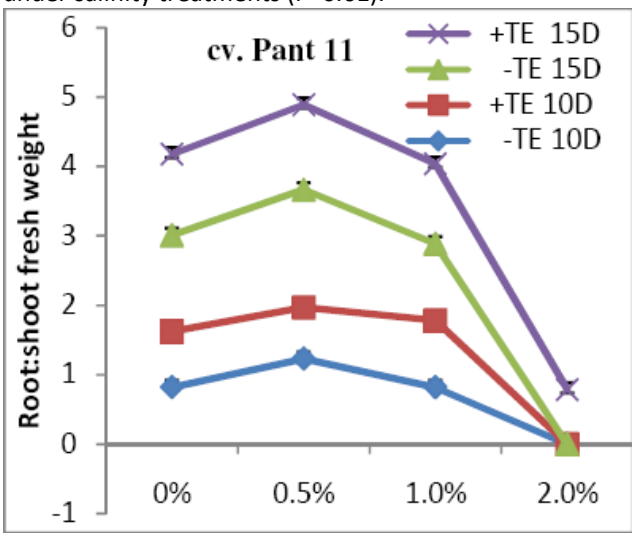

Figure 5: Root: shoot freshweight ratio of cv. Pant 11 at 10 & 15 days in absence (-TE) & presence (+TE) of testa extract under salinity treatments (P=0.01).

Figure 5 show in cv. Pant 11, root: shoot fresh weight increased in 0.5% (w/v) and decreased in 1.0% (w/v) salinity at 10 and 15 days. In presence of testa extract, root: shoot fresh weight decreased in 0.5% (w/v) at 10 and 15 days and decrease in 1.0% (w/v) at 15 days. However, presence of testa extract increased root: shoot fresh weight ratio in 1.0% (w/v) NaCl at 10 days.

Figure 6 shows, in basmati cv. Kasturi, presence of testa extract, further decrease in root: shoot dry weight ratio in 0.5% (w/v) salinity treatment at 10 days and 15 days. However, in presence of testa extract, no further decrease in root: shoot dry weight in 1.0% (w/v) salinity at 10 and 15 days.

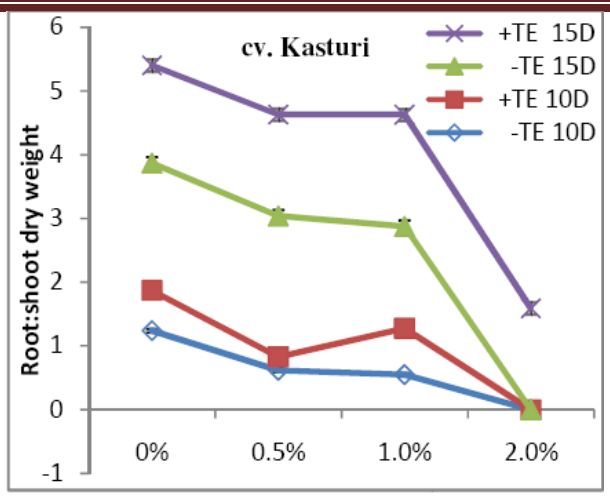

Figure 6: Root: shoot dry weight ratio of cv. Kasturi at 10 & 15 days in absence (-TE) & presence (+TE) of testa extract under salinity treatments (P=0.01).

In non-basmati cv. Pant 11, 0.5% (w/v) salinity increased the root: shoot dry weight, however, presence of testa extract decreased the root: shoot dry weight at 10 and 15 days. In cv. Pant 11, in presence of testa extract further decrease in root: shoot dry weight in 1.0% (w/v) salinity at 10 and 15 days (figure 7).

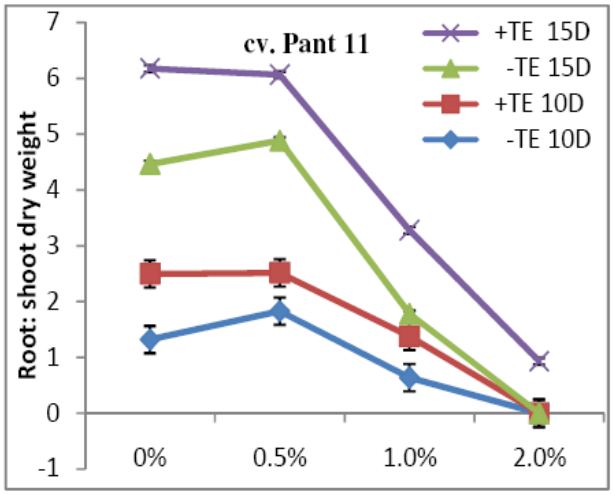

Figure 7: Root: shoot dry weight ratio of cv. Pant 11 at 10 & 15 days in absence (-TE) & presence (+TE) of testa extract under salinity treatments (P=0.01).

In cv. Kasturi, same pattern of performance was showed at 7 and 10 days (figure 8). In presence of testa extract, SVI increased over control in 0.5% (w/v) NaCl and reduced the effect of salinity in 1.0% (w/v) salinity at 7 and 10 days. However, SVI decreased in presence of testa extract at 0.5% (w/v) salinity and increase in 1.0% (w/v) salinity on 15th day.

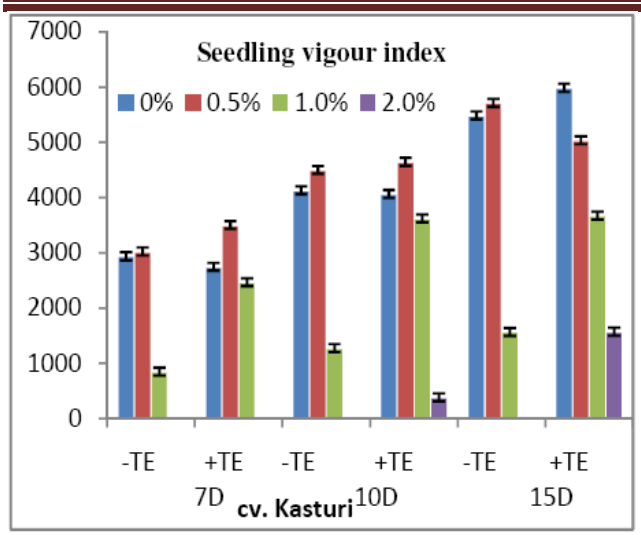

Figure 8: SVI of cv. Kasturi at 7, 10 & 15 days in absence (-TE) & presence (+TE) of testa extract under salinity treatments (P=0.001).

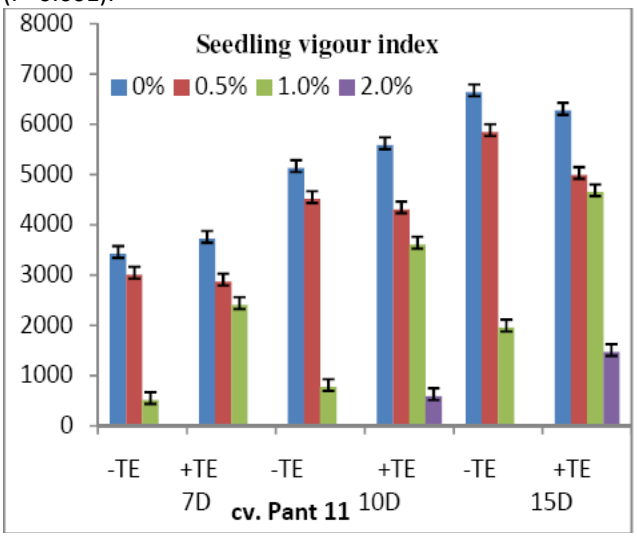

Figure 9: SVI of cv. Pant 11 at 7, 10 & 15 days in absence (-TE) & presence (+TE) of testa extract under salinity treatments (P=0.001).

SVI of cv. Pant 11 decreased over control values at 0.5-2.0% NaCl level with or without testa extract at 7, 10 and 15 days. In presence of testa extract, percentage improvement in SVI at 1.0% (w/v) was better. In presence of testa extract, SVI was decreased in 0.5% (w/v) salinity at 7, 10 and 15 days.

IV. DISCUSSION

Sensitivity of crops to salinity varies from different growth stages from seed germination to yeild of the crop [32]. Seedling stage is most sensitive stage to many stresses and allelochemicals [33]. Salinity affects growth of seedlings and salinity at mature stage of plant accelerates senescence [34]. Present study suggests treatment of rice seeds with testa of cashew nut may perform better under salinity stress.

Allelochemicals are usually secreted as secondary metabolities [35] and give protection against stress [36-38]. Basmai and non-basmati showed distinct pattern of germination and growth to salinity in presence of testa extract.

Germination performance and SVI of both the cultivers under salinity shows, basmati was relatively more susceptible to salinity at 7 days (table 1). Performance of both the cultivers in presence of testa extract under salinity suggests cv. Pant 11 responded better at 7 days. Both the cultivers improved germination under salinity in presence of testa extract. Present observation agrees with previous report of improvement in germination performance in presence of aqueous extracts of sunflower on rice [21].

Presence of testa extract decreased the effect of salinity in both the cultivers. Secondary metabolities have significant role in plant metabolism. [39]. Relative susceptibility for shoot and root length under salinity was more in non-basmati at 7, 10 and 15 days. Shoot inhibition was observed in rice in presence of salt more than 200 mM [15].

Significant decrease in shoot length under salinity reported [40-42]. In presence of testa extract, performance improved on shoot length under salinity relatively more in non-basmati at 7 days, in 1.0% (w/v) NaCl at 10 and 15 days. Increase in shoot length in presence of testa extract was more in non-basmati at 7, 10 and 15 days. Performance on root length was relatively improved more in cv. Kasturi at 7, 10 days and in 0.5% (w/v) NaCl at 15 days. Allelochemcials increase both root and shoot length [43] and the exact mechanism how secondary metabolities help in cop up the stress [44] is not known. These chemicals may induce detoxification mechanism or inactivation or sometimes elimination of toxins, oxidative modification of functional groups [44].

Root length increased more in presence of husk extract in cv. Pant 11 at 7 and 10 days and at 15 days root length increase more in cv. Kasturi. Increase in fresh weight in basmati in presence of aqueous extracts of sunflower was reported by Farooq *et al*., 2011 [21] under non-saline conditions. Growth of plant may be affected due to inhibitory or phytotoxic nature of allelochemicals [45].

Shoot fresh and dry weight decreased relatively more in non-basmati under stress at 10 days and 0.5% (w/v) at 15 days. Decrease in fresh weight observed under salt stress in maize [40]. Inhibition of shoot growth under stress may contribute to solute accumation to maintain osmotic balance [46-49] decrease in ROS results in reduction in weight.

In presence of testa extract, relatively better improvement on shoot fresh weight at 10 and 15 days and shoot dry weight at 10 days shown by cv. Pant 11. Root fresh weight decreased under salinity at 10 and 15 days and root dry weight at 15 days relatively more in cv. Pant 11. Salinity

restricts the availability of water to plant [50] and water availability is the one of the limiting factor in reduction of growth [51].

In presence of testa extract, basmati showed better improvement in root fresh weight at 15 days and in 0.5% (w/v) NaCl at 10 days. In contrast to shoots, ionic balance in roots is relatively good with lower Na+ and Cl- concentration to external environment [50, 52].

Relatively better improvement in basmati in presence of testa extract on shoot and root dry weight at 15 days. Root dry weight relatively affected in basmati under salinity at 10 days. Roots are directly exposed to salinity and they are usually robust. Reduction in shoot biomass may be due to reduced photosynthic active area [53]. Presence of testa extract, relatively better improvement on root dry weight in non-basmati at 10 days.

Dry weight increase in presence of allelochemicals under salinity reported in rice [21], similar observations reported [54, 55]. In basmati, presence of testa extract, relative increase in shoot fresh and dry weights at 10 and 15 days. Root dry relatively increased in non-basmati in presence of testa extract at 10 days and in basmati at 15 days. Decrease in root and shoot dry weight under salinity was observed in grasses [56].

Plant under optimal level of salt increase root and shoot growth and decrease in growth at low or high salinity conditions [57]. Increase in fresh weight may be due to high water content per unit area [9]. High antioxidant capacity of rice under salinity exhibits high antioxidant activity to cope up the stress [58].

Shoot vulnerbility to salt is more due to the accumulation Na+ and Cl- is more in shoots [59]. Accumulation capability may reflect the tolerance capability of a plant [60, 61]. Time of exposure and severity determine the physiological and molecular changes. High conc of salinity associated with morphological and low conc atleast induce physiological changes. Gradual increase in root to shoot ratio observed with increase in NaCl salinity [62, 63].

Both the varieties responded differently adjusted the root: shoot ratio to testa extract with salinity. Strategic maintainance of steady state level of anthocyanins and saliinity tolerance was reported in sugarcane [64]. Comparatively cv. Pant 11 decreased the effect of salinity on root: shoot length, fresh and dry weight ratios to testa extract and in cv. Kasturi, the root: shoot ratio adjustment response varied with concentration and time. Present study suggests the presence of testa extract decreased the effect of salinity on shoot and root length, fresh and dry weight in both the cultivers at 10 and 15 days. Relative improvements in germination shoot and root growth was better in cv. Pant 11 in presence of testa extract under salinity.

Acknowledgment

Authors are thankful to Mr Dinesh Sharma, Farmer, Selaqui, Dehradun for the genarosity of providing rice seeds and CPU, Chirala, Andhra Pradesh for cashew nut testa and highly grateful to Professor R.C. Ramola, Dean, FST, ICFAI University, Dehradun, Uttarakhand for providing laboratory facilities.

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