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Screening of weedy rice biotypes for water deficit and salt stress tolerance

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ABSTRACT

Water deficit and Salt stress are major abiotic stresses limiting sustainable rice production worldwide. Breeding rice for salinity and drought stress requires screening of germplasm for tolerance to the abiotic stresses as a preliminary and indispensable step. Weedy rice, a natural hybrid of cultivated and wild rice, is expected to bear tolerance to abiotic stresses. Thus, in the present study, nine weedy rice accessions (Oryza sativa f. spontanea L.), one wild rice species (Oryza nivara) along with three check cultivars were evaluated for tolerance to PEG induced drought and salt stress at the seedling stage. Significant variability was observed for evaluated traits among the weedy rice biotypes studied. Under water deficit condition, higher germination (90%) and the seed vigor index (324) was observed with weedy rice accession WR-23 while, the drought tolerant cultivar (Indira Barani Dhan-1) germinated fully (100%). Under salt stress condition, the higher germination was observed in weedy rice accession WR-15 along with the maximum seed vigor index (350) and salt tolerant cultivar CSR-10 germinated 100%. Results of this study may be useful for identification of germplasm for improvement of cultivated rice varieties for water deficit and salt tolerance.

Key words: Germination, salt stress, seed vigor index, water deficit, weedy rice, wild rice

INTRODUCTION

Global agricultural productivity is subjected to increasing environmental constraints in the form of abiotic stresses that adversely affected plants growth and development also reduced crop yield more than 50% (Muscolo *et al.* 2014). However, significant success in developing stress tolerant rice cultivars has not yet been achieved. Abiotic stress is the main factor negatively affecting rice crop growth and productivity, leading to serious losses in yield. Different climatic factors affecting rice production including abiotic stresses such as temperature, carbon dioxide, precipitation and rise in sea levels *etc*. When rice crop subjected to water deficit and salinity stress, numerous changes occur at the plant physiology and metabolism ultimately decreasing crop yields and grain quality in comparison with crops grown under ideal conditions. Erratic precipitation due to climate change can cause water deficit at some places and flooding at others. Increase in sea level and hence, increase in salinity is also expected. In India, 13.6 million hectors area major challenge in agriculture to identify at suitable traits that would support plant breeders in conspicuous selection programs.is affected by drought and 6.73 million hectors area is affected by salinity. About 20% of the cultivated land worldwide is adversely affected by high salt concentration which inhibits plant growth and yield. Therefore, physiological and biochemical approaches have a great importance in order to understand the complex responses of plants to water deficiency and develop rapidly new varieties (Maralian *et al.* 2010). Understanding the molecular and biochemical mechanisms involved in rice plant for drought and salt stress tolerance is still a major challenge in agriculture to identify at suitable traits that would support plant breeders in conspicuous selection programs. Weedy rice (*Oryza sativa f. spontanea L.*), a natural hybrid of cultivated and wild rice has been recognized as one of the most problematic weed in rice production system and is widely distributed in almost all rice growing regions worldwide (Rathore *et al.* 2013, Mishra *et al.* 2017). The weedy rice characteristics are easy grain shattering, long seed dormancy, high seed persistence in the soil and greater nitrogen-use efficiency for biomass production than cultivated rice (Rathore *et al.* 2017, Ghosh *et al.* 2017). Traits such as varied color pericarp of grain; higher survival rates, vigorous growth, higher tillering, and greater plant height are some useful characteristics for crop improvement in rice breeding. Weedy plants can

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also adapt to a wide range of environmental conditions and they show wide variability of anatomical, biological and physiological features. In recent years, weedy rice has been considered to be an important germplasm resource for rice improvement, because it has many useful genes for cold tolerance, high salinity tolerance and water deficit tolerance (Bevilacqua *et al.* 2015). Therefore, screening of weedy rice biotypes for drought and salt stress tolerance based on morphological and physiological traits, further detecting and transferring useful genes from weedy rice to cultivated rice varieties may play an important role in enhancing tolerance to drought and salt stress. The present investigation was undertaken to record the response of weedy rice biotypes towards water deficit and salt stress condition, in order to select the best accessions that are resistance/tolerant to the stress condition.

MATERIALS AND METHODS

The experiment was conducted to screen germplasm of weedy rice against water deficit and salt stress in (controlled conditions) with 35/27 ^{o}C (day/night) temperature at ICAR-Directorate of Weed Research, Jabalpur. Nine biotypes of weedy rice collected from different regions of Madhya Pradesh, three check cultivars of rice and one wild rice (*Oryza nivara*) were used for the study (Table 1).

Table1: Detail of germplasm used in the study

Germplasm	Type
WR-2	Weedy rice
WR-15	Weedy rice
WR-23	Weedy rice
WR-56	Weedy rice
WR-69	Weedy rice
WR-27	Weedy rice
WR-18	Weedy rice
WR-12	Weedy rice
WR-30	Weedy rice
WIR-5	Wild rice (*Oryza nivara*)
IR-64	Drought and salt susceptible rice variety
Indira Barani Dhan-1	Drought tolerant rice variety
CSR-10	Salt tolerant rice variety

Seeds were surface sterilized with 0.01% Tween-20 for 10 min and then washed twice with distilled water and chaffy seeds were removed. Twenty seeds of each accession were placed at one cm at depth in plastic pots (20 cm height and 20 cm diameter) containing autoclaved soil (vertisol) mixed with vermicompost in 3:1 ratio. The experiment was set up in completely randomized design (CRD) with three replications. Water deficit treatment was applied by 200 ml 20% polyethylene glycol- 6000 (w/v) to each pot at the time of sowing. Similarly, 200 ml of 200 mM NaCl solution was applied for salt stress. After sowing, number of germinated seeds was counted daily up to 30 days. Germination percentage was calculated on the basis of number of normal seedlings (Raun *et al.* 2002). Length and dry weight of root and shoot were recorded for each accession after 30 days of sowing.

Germination indices calculation equation

$$\text{Germination \%} = \frac{\text{Number of germinated seeds}}{\text{Total number of seed}} \text{ X } 100$$

$$\text{Germination index (GI)} = \frac{n1}{1} + \frac{n2}{2} + \cdots \frac{nx}{x}$$

Where:
n_1 …….. nx are the number of seed germinated on day 1 to day x
1 ……..... x are the number of days

Seed vigor index (SVI) was calculated according to Abdul and Anderson (1970) as follows

$$\text{Seed vigor index} = \frac{\begin{bmatrix}\text{germination percentage } \times \\ (\text{root length} + \text{shoot length})\end{bmatrix}}{100}$$

At 30 days after sowing, three seedlings were selected randomly from each pot for measurement of length of root and shoot using mm scale. Dry weight of root and shoot were also recorded in same plants using precision electronic balance. For dry weight, samples were oven dried at 60 ^{o}C till the constant weight achieved. SAS (version 9.4) software was used for all statistical analysis. Difference among the various treatment combinations were calculated by using two ways ANOVA and compared using Tukey's Honest Significant Difference (HSD) test at 5% level of significance.

RESULTS AND DISCUSSION

Effects of water deficit and salt stress on germination % and index

Under control conditions, 100% germination was observed in eight accessions of weedy rice and three cultivars i.e. *IR-64, CSR-10* and *Indira Barani Dhan-1* whereas, only 91% germination was observed in *WR-69* accession. On the other hand, only 66% germination was noted in wild rice. The germination index (GI) in cultivated rice varieties was noted from 42 to 45, while in weedy rice accessions, GI ranged between 33.0 to 41.6. On the other hand, GI in wild rice was noted as 27.7. Under water deficit, the germination percentage declined by 64, 47 and 80% in *WR*-2*,* *WR*-69 and *WR*-12 weedy rice accessions, respectively (Table 2). At the same in case of Wild rice it was 52%. The higher germination % was observed in weedy rice accessions *WR*-23 (90%) followed by *WR*-27(75%) and *WR*-30(81%) under drought stress. At the same time, drought tolerant cultivar Indira Barani Dhan-1 was germinated fully. Conversely, stress sensitive cultivar *IR64* and salt tolerant cultivar *CSR-10* were germinated 26.7 and 20%, respectively. Significant variation was seen in the GI under drought stress condition among different weedy rice accessions and it ranged from 5.8 to 27.6. On the other hand, wild one it was 8.5 and rice cultivar ranged from 5.5 to 37.2.The germination% and index of different weedy rice accessions was significantly lower than that of cultivated cultivar (*Indira B. Dhan-1*). Seed germination is first critical and the most sensitive stage in the lifecycle of crop plants and the seeds exposed to unfavorable environmental conditions like water deficit may have to compromise the seedlings establishment (Albuquerque and Carvalho, 2003). Result showed significant reduction in some accessions of early growth and rate of germination at 20% PEG concentration which may be due to the metabolic disorder induced by stress. Vibhuti *et al.* (2015) reported that water deficit adversely affected the seed germination and seedling growth.

Table 2: Germination attribute of selected germplasm of weedy rice and rice cultivar under water deficit and salt stress condition

Germplasm	Germination %			Germination index		
	Control	Water deficit	Salinity	Control	Water deficit	Salinity
WR-2	100[a]	36.7[e]	35.0[dc]	41.5[bcd]	9.71[e]	10.8[bc]
WR-15	100[a]	0.00[h]	100[a]	41.6[bcd]	0.00[g]	31.3[a]
WR-23	100[a]	90.0[b]	0.00[e]	33.0[g]	27.6[b]	0.00[d]
WR-56	100[a]	0.00[h]	100[a]	36.8[ef]	0.00[g]	31.3[a]
WR-69	91.7[b]	48.3[d]	51.7[b]	36.9[ef]	15.0[d]	14.5[b]
WR-27	100[a]	75.0[c]	0.00[e]	35.6[fg]	21.5[c]	0.00[d]
WR-18	100[a]	0.00[h]	100[a]	39.6[cde]	0.00[g]	32.0[a]
WR-12	100[a]	20.0[g]	50.0[b]	37.9[def]	5.80[ef]	14.6[b]
WR-30	100[a]	81.7[bc]	0.00[e]	35.3[fg]	26.4[b]	0.00[d]
WIR-5	66.7[c]	31.7[ef]	41.7[bc]	27.7[h]	8.52[ef]	12.2[bc]
IR-64	100[a]	26.7[gf]	36.7[dc]	43.6[ab]	7.26[ef]	9.75[c]
Indira B. Dhan-1	100[a]	100[a]	28.3[d]	45.7[a]	37.2[a]	8.69[c]
CSR-10	100[a]	20.0[g]	100[a]	42.9[abc]	5.53[f]	34.0[a]

Means with same latter in the columns do not differ significantly ($p<0.05$)

Among the different weedy accession, *WR-15, WR-18* and *WR-56* and the salt tolerant cultivar *CSR-10* were germinated fully (Table 2). On the other hand, the stress sensitive cultivar *IR-64* was germinated 36.7% and the drought tolerant cultivar *Indira Barani dhan-1* was germinated 28.3%. The rate of germination was decreased with the increment of salt stress; and the rate of germination was varied from 10.0 to 32.0 in weedy rice accessions. The rate of germination of wild one it was 12.2 and rice cultivar varied from 8.6 to 34.0. As compared to other weedy rice accessions, *WR-15, WR-18* and *WR-56* showed higher GI over a period of time under salinity stress. And these accessions performed statistically similar to tolerant counterpart i.e. *CSR 10* under salt stress situation, whereas, performance of weedy rice accessions were inferior to CSR 10 under normal (stress free) condition. However, *WR-15,* *WR-18* and *WR-56* accessions were not found to be drought tolerant based on GI. Under stress free environment, the different germplasm required less time to germinate than that of salt stress condition. The variability in salinity tolerance among rice varieties at germination have also been reported by Hakim *et al.* (2010).

Effects of water deficit and salt stress on shoot and root length

At the controlled condition, the maximum shoot and root length was observed with weedy rice accessions (Table 3). Among the different weedy rice accessions the maximum shoot length (42.5cm) was recorded with *WR-12*.And the minimal one was noticed with*CSR-10*(24.4cm). Whereas, the weedy rice accession *WR-30*was illustrated the maximum root length (5.50 cm), while *WR-69*producedthe lowest root length (2.66cm). Water stress causes the significant reduction of shoot and root growth of germinated seeds. But, there was no definite trend of increment or decrement of shoot and root length of different weedy rice accessions under water deficit condition. The weedy rice accessions *WR-23, WR-27* and *WR-30* showed the higher shoot length 20.8, 19.8 and 18.6cm, respectively under water deficit (Table 3).

Table 3: Shoot and root length of selected germplasm of weedy rice and rice cultivar under water deficit and salt stress condition

Germplasm	Shoot length (cm)			Root length (cm)		
	Control	Water deficit	Salinity	Control	Water deficit	Salinity
WR-2	34.5^{b}	9.16^{e}	13.0^{d}	4.50^{ab}	1.83^{b}	1.73^{b}
WR-15	41.6^{a}	0.00^{h}	24.8^{a}	4.93^{ab}	0.00^{c}	3.50^{a}
WR-23	33.3^{b}	20.8^{a}	0.00^{g}	4.76^{ab}	3.60^{a}	0.00^{c}
WR-56	30.0^{c}	0.00^{h}	20.2^{c}	5.03^{ab}	0.00^{c}	3.06^{a}
WR-69	25.6^{de}	11.5^{d}	6.66^{f}	2.66^{c}	2.00^{b}	1.06^{b}
WR-27	30.2^{c}	19.8^{ab}	0.00^{g}	5.10^{ab}	3.43^{a}	0.00^{c}
WR-18	33.3^{b}	0.00^{h}	22.0^{b}	4.00^{abc}	0.00^{c}	3.10^{a}
WR-12	42.5^{a}	5.63^{f}	12.5^{d}	3.66^{bc}	1.46^{b}	1.00^{b}
WR-30	34.6^{b}	18.6^{c}	0.00^{g}	5.50^{a}	3.16^{a}	0.00^{c}
WIR-5	31.0^{c}	10.3^{de}	10.1^{e}	5.23^{ab}	1.76^{b}	1.66^{b}
IR-64	25.8^{de}	5.00^{fg}	6.00^{f}	5.46^{a}	1.30^{b}	1.00^{b}
Indira B. Dhan-1	26.9^{d}	19.5^{bc}	5.43^{f}	4.06^{abc}	3.10^{a}	1.06^{b}
CSR-10	24.4^{e}	4.23^{g}	20.0^{c}	4.50^{ab}	1.10^{b}	2.83^{a}

Means with same latter in the columns do not differ significantly ($p<0.05$)

In the same way, drought tolerant cultivar Indira Barani dhan-1 had a shoot length of 19.5 cm. Similarly, the weedy rice accession *WR-23* (3.60 cm) followed by *WR-27* (3.43cm) and *WR-30* (3.16 cm) produced significantly higher root length compared to drought tolerant cultivar Indira Barani dhan-1 (3.10 cm). Whereas, the stress sensitive cultivar *IR-64*produced lowest shoot length (1.30 cm) at drought stress condition. The GI of weedy rice accessions *WR-23, WR-27* and *WR-30* were significantly lower than that of cultivated cultivar (*Indira B. Dhan-1*), but at later stage these accessions produced higher seedling growth (shoot and root length) compared to Indira B. Dhan-1. The present study revealed that, shoot and root length was increased with decrease in PEG induced water deficit. One more important aspect for drought stress is root length, because it has direct contact with soil and water absorbance. Mostafavi *et al.* (2011) have also been proposed root length make imperative clue to a plant response under water deficit. Vibhuti *et al.* (2015) reported the similar effect of the water deficit in Narendra 1 rice varieties. The declined crop growth under stress condition is not an unusual phenomena and this have been reported by different workers in different crops (Shinwari *et al.* 2013).

Under salt stress condition, weedy rice accessions *WR-15* (24.8 cm), *WR -18* (22 cm) and *WR -56* (20.2cm) produced significantly higher shoot length compared to salt tolerant cultivar *CSR-10* was (20.0 cm).On the other hand, the same weedy rice accessions *WR-15, WR -18* and *WR -56*showedhigher root length

(3.50, 3.10 and 3.06 cm, respectively) compared to cultivated cultivar i.e. *CSR-10* (2.83 cm).Hakim *et al.* (2010) and Vibhuti *et al.* (2015) observed that shoot and root length was evidently affected by salt stress under high salinity level. Chachar *et al.* (2008) studied the influence of sodium chloride on seed germination and seedling root growth of cotton. They also reported that only seed germination was slightly affected by an increase in salinity, whereas root length, root growth rate were severely affected. In the present study, authors suggested that the weedy rice accessions *WR-15*, *WR -18* and *WR -56* were performed statistically similar to the tolerant counterpart i.e. CSR 10 under salt stress condition.

Effects of water deficit and salt stress on seedling biomass

Dry weight of seedlings were taken from selected germplasm of weedy rice and rice cultivar. At control condition, the maximum shoot dry weight (SDW) 166 mg was recorded with weedy rice accession*WR-15* (Table 4). And the minimum SDW was noticed with *WR-69* (38.3mg). On the other hand, the weedy rice accession *WR-12* was showed the maximum root dry weight (RDW) (36.01mg). Whereas, the weedy rice accession*WR-69* displayed the minimum RDW (5.06 mg).

Table 4: Dry weight of shoot and root biomass of selected germplasm of weedy rice and rice cultivar under water deficit and salinity condition

Germplasm	Shoot dry weight (mg)			Root dry weight (mg)		
	Control	Water deficit	Salinity	Control	Water deficit	Salinity
WR-2	78.3^{G}	24.6^{G}	14.0^{G}	24.04^{C}	3.33^{DE}	1.25^{F}
WR-15	166^{A}	0.00^{K}	37.5^{A}	17.6^{EF}	0.00^{F}	7.50^{A}
WR-23	113^{C}	52.3^{B}	0.00^{J}	26.8^{B}	8.65^{A}	0.00^{G}
WR-56	53.0^{I}	0.00^{K}	34.5^{B}	8.01^{H}	0.00^{F}	6.60^{B}
WR-69	38.3^{J}	18.3^{J}	11.0^{I}	5.01^{I}	3.18^{E}	1.51^{F}
WR-27	104^{D}	56.3^{A}	0.00^{J}	21.6^{D}	6.34^{B}	0.00^{G}
WR-18	94.6^{F}	0.00^{K}	33.0^{C}	21.1^{D}	0.00^{F}	6.25^{B}
WR-12	160^{B}	27.0^{F}	27.0^{D}	36.01^{A}	3.68^{DE}	4.50^{D}
WR-30	111^{C}	47.3^{C}	0.00^{J}	15.3^{G}	6.00^{B}	0.00^{G}
WIR-5	113^{C}	20.0^{I}	23.0^{F}	17.9^{E}	3.74^{DE}	3.60^{E}
IR-64	79.6^{G}	33.6^{E}	11.5^{HI}	16.4^{FG}	3.69^{DE}	1.60^{F}
Indira B. Dhan-1	100^{E}	46.0^{D}	12.0^{H}	26.9^{B}	4.62^{C}	3.65^{E}
CSR-10	72.0^{H}	22.3^{H}	24.0^{E}	15.2^{G}	4.01^{DC}	5.00^{C}

Means with same latter in the columns do not differ significantly ($p<0.05$)

The maximum SDW was found in weedy rice accessions *WR-23* (52.3 mg) followed by *WR-30* (47.3 mg) and *WR-27* 56.3mg (Table 4). The cultivated variety (*Indira Barani dhan-1*) was produced 46mg of SDW. On the other hand, the maximum RDW were also observed in same weedy rice accessions *WR-23*(8.65 mg) followed by *WR-30* (6.00 mg) and *WR-27* (6.34mg). Whereas, RDW of *Indira Barani dhan-1* was (4.62 mg). The weedy rice accession '*WR-69*' showed minimum SDW was (3.18 mg). Present study revealed that, dry weight of weedy rice accessions decrease greatly under water deficit, but three weedy rice accessions *WR-23, WR-27* and *WR-30* was performed satisfactory under drought stress condition. These results indicate that weedy rice also appears more tolerant to the water deficit. Seedling dry mass with special root organ is a reliable and good selection criteria for drought tolerance (Datta *et al.* 2011). Shoot weight was inversely related to salt stress and was relatively less sensitive as compared to root weight especially at higher salt concentration. Under salt stress situation as compared to the tolerant counterpart (*CSR-10*) the SDW (24.0 mg) was relatively higher with weedy rice accessions *WR-15* (37.5 mg), *WR-56* (34.5 mg) and *WR-18* 33.0 mg (Table 4). On the other hand, the maximum RWD was found in the same weedy rice accessions *WR-15* (7.50mg) followed by *WR-18* (and 6.25 mg) and *WR-5* 6.60mg (Table 4). At the same time, cultivated cultivar (CSR-10) produced 5 mg of RDW. Thus by, according to the above findings and result, weedy rice accessions *WR-15*, *WR-56* and *WR-18* performed statistically similar to tolerant counterpart *CSR-10* under salt stress condition. Similar findings also reported by Zafar *et al.* (2015) in five different genotypes of Basmati rice. Salinity has both osmotic and specific ionic, which adversely affects the seedling growth and ultimately leads to reduced plant biomass (Dioniso and Tobita, 2000). Reduction in seedling growth as a result of salt stress has also been reported by Achakzai *et al.* (2010); Akram *et al.* (2010) in several other crop species.

Effects of water deficit and salt stress on seed vigor index

At the control condition, SVI for check cultivar was from 406 to 546(Figure1). And the weedy rice accessions also had more or similar range of SVI from 245 to 550, whereas, in case of wild rice it was 349. But when exposed to water deficit a significant impact was seen in the vigor index among different weedy rice accessions and it ranged from 31.2 to 324 (Figure1). At the same time it was 54.7 in wild rice, while in rice cultivars, it ranged from 22.8 to 310. However, analysis of variance revealed that weedy rice accessions *WR-23, WR-27* and *WR-30* similar SVI to check cultivar (*Indira B. Dhan-1*). Result of this study implies that plants were comparably vigorous in terms of SVI. Janmohammadi *et al.* (2008) reported that SVI of maize crop was also significantly affected under water deficit and salt stress.

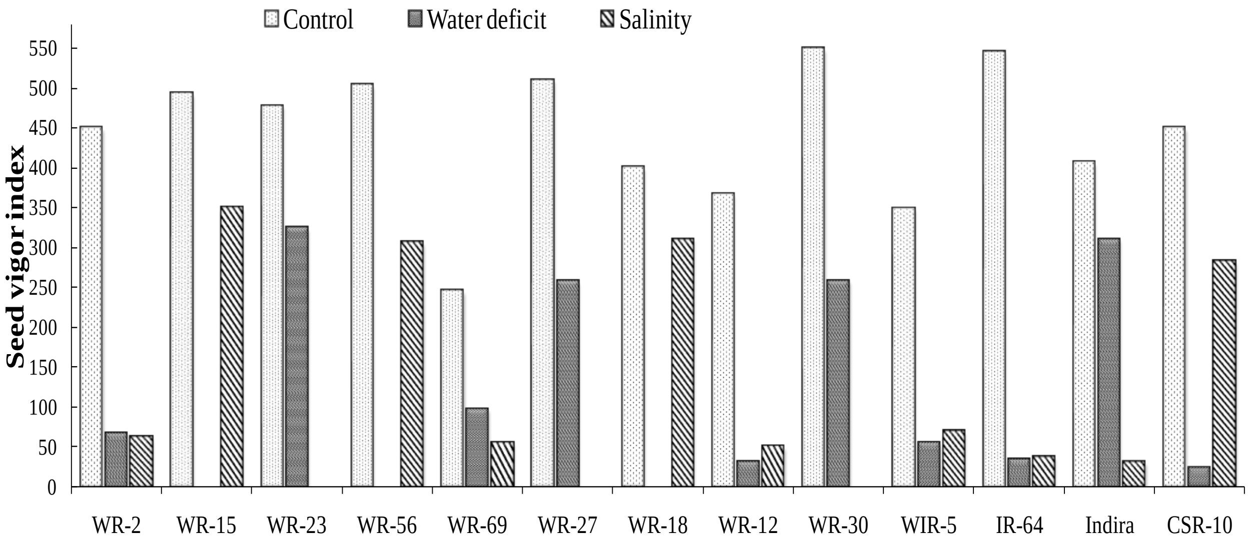

Figure 1**:** Effect of drought and salt stress on seed vigor index on the basis of seedling growth

On the other hand, at the salt stress condition the SVI of wild rice was 69.2 and check cultivar varied from 30 to 283 (Figure1). As compared to other weedy rice accessions, *WR-15, WR-18* and *WR-56* showed higher SVI over a period of time under salinity stress. And these accessions were performed similar to tolerant counterpart i.e. CSR 10 under salt stress situation. Vibhuti *et al.* (2015) reported that seedling growth and seed vigor of crop plant was also affected at salt stress conditions. It has also been reported that salinity suppresses the uptake of essential nutrients like P and K (Nasim *et al.* 2008), which could adversely affect seedlings growth and vigor. Based on the performance of different biotypes of weedy rice and comparison with check cultivars, weedy rice accessions *WR-23, WR-27*and WR-*30* performed well under water deficit conditions, whereas, *WR-15, WR-18* and *WR-56* showed significant degree of salt tolerance. Results of the study suggest that weedy rice biotypes may serve as genetic resource for improvement of rice against water deficit and salt stress tolerance.

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