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# Evaluation for Multiple Abiotic Stress Tolerance in Rice (*Oryza Sativa*) Genotypes

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## Abstract

Abiotic stresses are the major constraints that affect the morphological, physiological and biochemical attributes of plants resulting in reduction of yield. Recently, combined occurrence of stresses has become a greater cause of concern in farmers' fields. Hence, there is a need to identify genotypes that are tolerant to more than one stress. A study on physiological characterization for water and salinity stress was conducted in twenty rice genotypes during *kharif* 2022 at Regional Agricultural Research Station, Maruteru, Andhra Pradesh, India. Results showed that among all the treatments, root length, shoot length, root and shoot dry weight and chlorophyll content were greater under control followed by 1% mannitol, 2% mannitol and salinity stress. Visual scoring for water stress at seedling stage revealed that under mild water stress (1% M) in genotypes *viz.,* CR4423-8, FL 478 and Pantara recorded 75-90% of survival and under severe water stress (2% M) 40-50% survival. Under salinity stress, the score 3 was recorded in FL 478 and 5 in Naveen, Pantara and CR 4423-75. For combined stresses, FL 478 and Pantara were identified as tolerant and can be suggested as donors.

**Key words:** Chlorophyll content, Rice, Salinity, SES, Water stress

## Introduction

In Asian countries, rice is the primary staple food crop on which more than half of the population is dependent. Rice cultivation provides livelihood, serves as a source of calories and when exported provides support to the nation's economy. There is an incessant need for increasing the rice production keeping in view the increasing demand. However, rice production faces several constraints especially in the form of abiotic stresses, such as water stress and salinity, both being the major threats. Under the changing climatic scenario, a plant in its life cycle may face a single stress, combination of stresses at same physiological stage or two different stresses at different stages of the crop (Rosa *et al*., 2022). Hence, a plant tolerant to more than one stress is desirable.

Morphological, physiological and biological traits of plants are severely affected by abiotic stresses. In particular, water stress and salinity affect various aspects of plant growth and physiology. Exposure of crops to abiotic stresses during seedling stage leads to poor crop establishment due to early death of the seedlings. Drought stress decreases turgor pressure, an important physiological mechanism that affects cell growth. It has been reported that onset of water stress at early seedling stage inhibits rice germination and growth of the seedling (Pirdashti *et al*., 2003) and also decrease the leaf chlorophyll content and hence, affecting the metabolism (Chutia, and Borah, 2012). Salinity at early stages causes reduction in water absorption leading to stomatal closure and thereby reduces plant growth in terms of both height and biomass (Yamamoto *et al*., 2011). The growth of the roots is affected as a result of increasing osmotic pressure outside the roots (Yadav, 2020). Salt stress has detrimental effects on plant physiological processes such as photosynthesis and also affects the pigment composition such as chlorophylls, anthocyanins and carotenoids (Panda *et al*., 2013).

To assess the tolerance level of rice genotypes to abiotic stress in the early seedling phase, static hydroponic culture is the best viable approach that is being used frequently to screen the germplasm (Ali *et al*., 2014). With this background, a hydroponic study was designed and setup with a major objective to identify tolerant rice genotypes for both water stress (mild and severe) as well as salinity. The identified genotypes could be used as a donor in further crop improvement programmes.

## Materials and Methods

The experiment comprised of twenty rice genotypes as listed in **(Table 1)** that were tested for their multiple stress tolerance during *kharif* 2022 at Regional Agricultural Research Station, Maruteru. The seeds of genotypes were surface sterilized with 70% ethanol solution for 5 min followed by thorough washing for two-three times with sterilized distilled water. Pre-germinated seeds of each variety were taken and placed in hydroponic setup in 4 sets with 3 replications. The hydroponic setup was laid down in CRD. The plants were initially grown for 2-3 days in fresh water. Later it was shifted to Hoagland's solution containing the essential macro and micro nutrients. At 3-4 leaf stage, stress was imposed by addition of Mannitol for 1% and 2% for mild and severe water stress treatments, respectively. The amount of mannitol was calculated based on the quantity of the nutrient solution taken in the tray (~5 lit). For salinity stress, sodium chloride was added. An EC of 6 was maintained for first 3 days till the plants were acclimatised. The EC was increased to 12 and maintained till the end of the experiment. The nutrient solutions were changed and replaced by fresh solution every 2-3 days by draining the old solution completely followed by rinsing three–four times with fresh solutions so as to avoid increased stress level due to Mannitol and sodium chloride. The pH of the solution was adjusted daily to 5.5. Observations such as shoot length, root length, shoot dry weight, root dry weight and chlorophyll content were recorded at the end of the experiment.

**Table 1: List of genotypes used for phenotyping for water (mild and severe) and salinity stress conditions**

| S. No. | Genotypes |
|---|---|
| 1 | CR4423-8 |
| 2 | IC-256564 |
| 3 | CR4423-10 |
| 4 | FL 478 |
| 5 | CR4111-B-1-4-S-1-Sub-B |
| 6 | Vandana |
| 7 | CR4423-17 |
| 8 | Naveen |
| 9 | CR3483-29-M-4-B-Sub-79-1 |
| 10 | Pantara |
| 11 | CR4423-20 |
| 12 | IR20 |
| 13 | CR4423-75 |
| 14 | CR 4111-B-1-10-S-1-Sub-B |
| 15 | AC 1125A |
| 16 | CR4423-101 |
| 17 | CR4423-111 |
| 18 | AC847A |
| 19 | IC-256508 |
| 20 | IC-256605 |

Shoot and roots were separated at the shoot-root junction and the length of the of both shoot and root was measured. Both were expressed in cm. The samples were oven dried for 48 h at 60 °C and the weight was recorded as shoot and root dry weight and it was expressed in g.

Chlorophyll content in the samples were recorded at the end of the experiment. 25 mg of leaf sample was taken and placed in 80% acetone as per the methodology described by Porra *et al*., (1989). Using a UV-VIS spectrophotometer, absorbance of chlorophyll a and chlorophyll b were measured at 663.2 nm and 646.8 nm respectively and the chlorophyll content was expressed in mg $g^{-1}$ fresh weight (mg g-1 FW). Chlorophyll a content, chlorophyll b content and the total chlorophyll content were calculated according to Lichtenthaler and Wellburn, (1983).

### Statistical analysis

Two-way analysis of variance (ANOVA) was performed using Statistix 8.1 package. Statistical significance of the parameter means was determined by performing Fisher's LSD test to test the statistical significance.

## Results and Discussion

### Shoot and root length

Imposition of stress resulted in the reduction of shoot and root lengths under all the three stress treatments. Under control conditions, the shoot length varied from 11.6 cm in CR 4423-20 to 24.5 cm in AC 847 A. Under mild and severe water stress (1% M and 2% M), shoot length was maximum in Pantara (19.6 cm and 19.2 cm) followed by AC 847 A (17.6 cm and 16.4 cm) and Vandana (17.5 cm and 14.7 cm), respectively. The shoot length was minimum under 1% M in CR4111-B-1-4-S-1-Sub-B (10.1 cm) followed by CR 4423-20 (10.4 cm). Under 2% M, minimum was in CR 4423-8 (9.4 cm) and CR4111-B-1-4-S-1-Sub-B (9.7 cm). Under salinity stress maximum shoot length was in Pantara (20.0 cm), AC 847 A (15.0 cm) and Vandana (12.1 cm). Minimum shoot length was observed in CR4111-B-1-4-S-1-Sub-B (5.3 cm) and CR4423-10 (7.5 cm) **(Table 2)**.

**Table 2: Impact of water stress (1% M and 2% M) and salinity stress on shoot length (cm) and root length (cm) of rice genotypes.**

| Entry | Shoot length (cm) | | | | Root length (cm) | | | |
|---|---|---|---|---|---|---|---|---|
| | Control | 1% Mannitol | 2% Mannitol | NaCl | Control | 1% Mannitol | 2% Mannitol | NaCl |
| CR4423-8 | 18.5 | 16.3 | 9.4 | 9.2 | 7.4 | 7.1 | 4.8 | 3.3 |
| IC-256564 | 19.7 | 14.4 | 12.0 | 10.1 | 4.5 | 4.4 | 4.3 | 2.8 |
| CR4423-10 | 16.7 | 12.7 | 10.9 | 7.5 | 9.6 | 6.2 | 5.2 | 3.6 |
| FL 478 | 14.2 | 14.1 | 12.3 | 10.6 | 7.7 | 7.4 | 7.3 | 6.9 |
| CR4111-B-1-4-S-1-Sub-B | 15.2 | 10.1 | 9.7 | 5.3 | 5.7 | 5.1 | 5.2 | 2.6 |
| Vandana | 23.6 | 17.5 | 14.7 | 12.1 | 6.9 | 5.8 | 5.2 | 5.0 |
| CR4423-17 | 14.4 | 11.0 | 10.0 | 9.2 | 5.5 | 5.3 | 5.0 | 4.9 |
| Naveen | 14.0 | 11.1 | 10.3 | 9.4 | 6.7 | 5.8 | 5.7 | 5.3 |
| CR3483-29-M-4-B-Sub-79-1 | 14.4 | 11.5 | 10.1 | 9.2 | 6.8 | 5.1 | 4.6 | 4.5 |
| Pantara | 21.3 | 19.6 | 19.2 | 20.0 | 8.9 | 8.7 | 7.8 | 8.4 |
| CR4423-20 | 11.6 | 10.4 | 10.1 | 8.8 | 4.9 | 4.7 | 4.4 | 4.3 |
| IR20 | 13.3 | 12.4 | 11.1 | 8.2 | 7.2 | 6.9 | 6.1 | 6.0 |
| CR4423-75 | 13.8 | 13.1 | 12.7 | 11.4 | 7.8 | 7.5 | 6.2 | 5.3 |
| CR 4111-B-1-10-S-1-Sub-B | 13.6 | 12.4 | 12.2 | 11.5 | 8.2 | 7.8 | 7.5 | 4.9 |
| AC 1125A | 19.6 | 16.0 | 12.8 | 9.7 | 6.2 | 5.6 | 5.4 | 4.7 |
| CR4423-101 | 17.6 | 12.0 | 11.6 | 7.9 | 5.1 | 4.8 | 4.7 | 3.8 |
| CR4423-111 | 20.8 | 11.8 | 10.5 | 9.1 | 6.5 | 6.1 | 6.0 | 5.4 |
| AC847A | 24.5 | 17.6 | 16.4 | 15.0 | 7.5 | 7.2 | 6.8 | 6.5 |
| IC-256508 | 21.0 | 15.8 | 13.6 | 8.4 | 6.5 | 6.2 | 5.7 | 4.4 |
| IC-256605 | 23.9 | 12.4 | 12.2 | 11.4 | 5.1 | 4.6 | 4.8 | 4.2 |
| Mean | 17.6 | 13.6 | 12.1 | 10.2 | 6.7 | 6.1 | 5.6 | 4.8 |
| LSD (T) | 0.38 | | | | 0.19 | | | |
| LSD (V) | 0.86 | | | | 0.42 | | | |
| LSD (TxV) | 1.72 | | | | 0.85 | | | |
| CV (%) | 7.9 | | | | 9.1 | | | |

Root length under control varied from 9.6 cm in CR4423-10 to 4.5 cm in IC-256564. Under 1% M, root length was highest in Pantara (8.7 cm) followed by CR 4111-B-1-10-S-1-Sub-B (7.8 cm) and CR 4423-75 (7.5 cm). Lowest was in IC-256564 (4.4 cm) followed by IC-256605 (4.6 cm) and CR 4423-20 (4.7 cm). Under 2% M, highest root length was in Pantara (7.8 cm) followed by CR 4111-B-1-10-S-1-Sub-B (7.5 cm) and FL 478 (7.3 cm) and lowest was recorded in IC-256564 (4.3 cm) followed by CR4423-20 (4.4 cm). Under salinity stress, Pantara had maximum root length of 8.4 cm followed by FL 478 (6.9 cm) and AC 847A (6.5 cm) while minimum was in CR 4111-B-1-4-S-1-Sub-B (2.6 cm) followed by IC-256564 (2.8 cm) (**Table 2**).

It has been reported that water stress at early seedling stage inhibits the growth of the rice seedling as reflected by reduction in the length and dry matter (Madabula *et al*., 2016). Similar observation was recorded in our study as well. Both mild and severe water stress resulted in reduction in 22.7 and 8.9% of mean shoot and root length respectively. Similarly, Negrao *et al*., (2016) reported that imposition of salinity at early stages reduced plant growth in terms of both height and biomass. Inhibition in plant growth is mainly due to ionic toxicity and osmotic stress due to high

**Table 3: Impact of water stress (1% M and 2% M) and salinity stress on shoot dry weight (g) and root dry weight (g) of rice genotypes**

| Entry | Shoot weight (g) | | | | Root weight (g) | | | |
|---|---|---|---|---|---|---|---|---|
| | Control | 1% Mannitol | 2% Mannitol | NaCl | Control | 1% Mannitol | 2% Mannitol | NaCl |
| CR4423-8 | 1.72 | 1.49 | 1.26 | 1.21 | 0.34 | 0.28 | 0.33 | 0.32 |
| IC-256564 | 1.92 | 1.53 | 1.28 | 1.16 | 0.42 | 0.37 | 0.33 | 0.27 |
| CR4423-10 | 1.95 | 1.56 | 1.51 | 1.31 | 0.46 | 0.42 | 0.39 | 0.32 |
| FL 478 | 1.65 | 1.54 | 1.48 | 1.43 | 0.51 | 0.42 | 0.39 | 0.30 |
| CR4111-B-1-4-S-1-Sub-B | 1.58 | 1.29 | 1.25 | 1.22 | 0.53 | 0.42 | 0.33 | 0.30 |
| Vandana | 1.59 | 1.59 | 1.62 | 1.04 | 0.82 | 0.43 | 0.34 | 0.27 |
| CR4423-17 | 1.62 | 1.35 | 1.31 | 0.92 | 0.54 | 0.44 | 0.35 | 0.15 |
| Naveen | 1.59 | 1.29 | 1.18 | 1.30 | 0.51 | 0.49 | 0.44 | 0.40 |
| CR3483-29-M-4-B-Sub-79-1 | 1.33 | 1.00 | 0.95 | 0.87 | 0.40 | 0.35 | 0.27 | 0.26 |
| Pantara | 1.54 | 1.40 | 1.25 | 1.31 | 0.49 | 0.40 | 0.39 | 0.43 |
| CR4423-20 | 1.56 | 1.25 | 1.11 | 0.91 | 0.49 | 0.37 | 0.34 | 0.22 |
| IR20 | 1.53 | 1.39 | 1.35 | 1.10 | 0.62 | 0.44 | 0.31 | 0.20 |
| CR4423-75 | 1.42 | 1.19 | 1.17 | 1.03 | 0.66 | 0.53 | 0.41 | 0.37 |
| CR 4111-B-1-10-S-1-Sub-B | 1.61 | 1.25 | 0.95 | 0.88 | 0.45 | 0.37 | 0.35 | 0.22 |
| AC 1125A | 1.78 | 1.36 | 1.29 | 1.07 | 0.46 | 0.35 | 0.24 | 0.25 |
| CR4423-101 | 1.06 | 0.77 | 0.82 | 0.85 | 0.41 | 0.34 | 0.32 | 0.26 |
| CR4423-111 | 1.54 | 1.19 | 1.06 | 1.17 | 0.59 | 0.46 | 0.35 | 0.16 |
| AC847A | 1.55 | 1.19 | 1.23 | 0.89 | 0.50 | 0.40 | 0.30 | 0.11 |
| IC-256508 | 1.56 | 1.15 | 1.01 | 0.84 | 0.52 | 0.40 | 0.35 | 0.26 |
| IC-256605 | 1.65 | 1.23 | 0.92 | 0.86 | 0.49 | 0.33 | 0.28 | 0.21 |
| Mean | 1.59 | 1.30 | 1.20 | 1.07 | 0.51 | 0.40 | 0.34 | 0.26 |
| LSD (T) | 0.032 | | | | 0.014 | | | |
| LSD (V) | 0.073 | | | | 0.033 | | | |
| LSD (TxV) | 0.146 | | | | 0.066 | | | |
| CV (%) | 7.05 | | | | 7.01 | | | |

salt concentration (Singhal *et al.,* 2022). A reduction in both root and shoot length in rice seedlings when exposed to salinity was also reported by Rasel *et al.,* (2020). These findings are in agreement with the results of this study where a reduction of both root and shoot length in all the tested genotypes was noted.

### Shoot and Root Dry weight

Similarly, there was reduction in both root and shoot weight under both water stresses as well as salinity stress. Shoot weight varied from 1.06 g in CR4423-101 to 1.95 g in CR 4423-10. Maximum shoot weight under 1% M and 2% M was in Vandana (1.59 g and 1.62 g) followed by CR 4423-10 (1.56 g and 1.51 g) and FL 478 (1.54 g and 1.48 g). Minimum was in CR 4423-101 (0.77 g) followed by CR3483-29-M-4-B-Sub-79-1 (1.0 g) and IC-256508 (1.15 g). Under salinity, highest weight was in FL 478 (1.43 g) followed by Pantara and CR 4423-10 (1.31 g each). Lowest was observed in IC-256508 (0.84 g) followed by CR4423-101 (0.85 g) and IC-256605 (0.86 g) (**Table 3**).

Root weight under control varied from 0.82 g in Vandana to 0.34 g in CR 4423-8. Under 1% M, CR4423-75 had maximum root weight of 0.53 g followed by Naveen (0.49 g) and CR 4423-111 (0.46 g). Minimum was in CR4423-8 (0.28 g) followed by IC-256605 (0.33 g) and CR4423-101 (0.34 g). Under 2% M, Naveen recorded highest shoot weight of 0.44 g followed by CR4423-75 (0.41 g) and Pantara (0.39 g). Lowest was in AC 1125 A (0.24 g) (**Table 3**).

Reduction in the growth in terms of biomass was reported by Madabula *et al*., (2016) under water stress and by Rahman *et al*., (2016a) and Negrao *et al*., (2016) under salinity stress in rice seedlings. These findings are in tune with the results of this study where under stress both root and shoot biomass were reduced in all the genotypes tested.

### Chlorophyll content (mg/g FW)

The mean chlorophyll content reduced by 31.4% under 1% M, 42.0% under 2% M and by 62.3% under salinity stress. Under 1% M, less than 20% reduction of total chlorophyll content was observed in Pantara (19.8%) followed by CR4423-8 (19.4%), IC-256564 (19.0%) and FL 478 (13.3%). More than 40% was noted in IR20 (43.0%) and CR4111-B-1-4-S-1-Sub-B (45.5%). Under 2% M stress, less reduction was in FL 478 (23.8%) followed by CR4423-8 (25.7%), Pantara (29.4%) and IC-256564 (30.5%). Whereas more reduction was seen in CR 4111-B-1-10-S-1-Sub-B (60.2%) and CR4423-101 (57.5%). Under salinity stress, reduction in chlorophyll content was less in Pantara (43.1%) followed by IC-256508 (48.6%), FL 478 (49.2%), Naveen (51.6%) and CR4423-75 (51.7%). Higher reduction was in CR4423-101 (87.9%) followed by AC847A (76.4%) and CR4111-B-1-4-S-1-Sub-B (76.3%) (**Figure 1**).

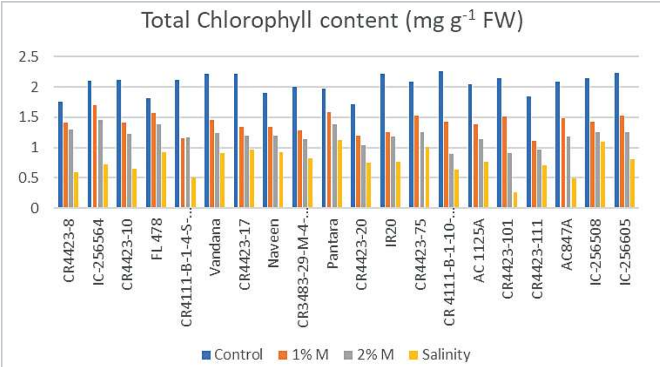


**Figure 1: Impact of water stress (1% M and 2% M) and salinity stress on total chlorophyll content (mg/g FW) of rice genotypes**

Chlorophyll is one of the major components of chloroplast as well as an essential pigment for sustenance of any plant as it is involved in the process of photosynthesis. Abiotic stress greatly affects the pigment composition. Imposition of water stress at early seedling stage is known to affect the chlorophyll content and in turn affect the plant metabolism (Swapna and Shylaraj, 2017). A reduction in chlorophyll content in PEG-induced drought stressed rice seedlings was reported by Dalal and Tripathy, (2012) and Hsu and Kao, (2003). In our study, chlorophyll content was sensitive to both mild and severe water stress compared to normal condition in all the tested genotypes. The main reason being that the imposition of stress leads to production of reactive oxygen species that cause lipid peroxidation as well as damage to the chlorophyll pigment (Hirt and Shinozaki, 2004). It is the increased activity of chlorophyllase enzyme that leads to the degradation of chlorophyll.

Similarly, salinity also causes a reduction in chlorophyll and caretonoid content (Sairam *et al*., 2002). The reduction in total chlorophyll content was more in susceptible genotypes when compared to tolerant (Panda *et al*., 2013). Rahman *et al*., (2016b) in his study on 12-d old rice seedling exposed to 150 mM of salinity stress reported a reduction in the chlorophyll content by 46 and 48% of chlorophyll a and b, respectively. In this study too, tolerant genotypes such as Pantara, FL 478, Naveen and CR4423-75 had less reduction in their chlorophyll content under salinity stress.

**Visual scoring under water and salinity stresses**

Visual scoring for water stress at seedling stage revealed that under mild water stress (1% M) in genotypes *viz.,* CR4423-8, FL 478, Pantara, IR 20, CR4423-75, CR 4111-B-1-10-S-1-Sub-B almost 75-90% of plants survived. Under severe water stress (2% M) almost 40-50% of the seedlings survived in CR4423-8, FL 478, Pantara, CR4423-20 (**Tables 4 and 5**). Under salinity stress the score 3 was recorded in FL 478, and 5 in Naveen, Pantara and CR 4423-75 (**Table 6**).

**Table 4: Visual scoring for water stress (1% Mannitol) at seedling stage**

| Observation | Genotypes |
|---|---|
| Almost all plants dead | CR4423-111, IC-256508, IC-256605 |
| <25% survival | AC 1125A, CR4423-101, AC847A |
| <40% survival | CR4423-17, Naveen |
| 50-60% survival | IC-256564, CR4423-10, CR4111-B-1-4-S-1-Sub-B, Vandana, CR3483-29-M-4-B-Sub-79-1, CR4423-20 |
| 75-90% survival | CR4423-8, FL 478, Pantara, IR 20, CR4423-75, CR 4111-B-1-10-S-1-Sub-B |

**Table 5: Visual scoring for water stress (2% Mannitol) at seedling stage**

| Observation | Genotypes |
|---|---|
| Almost all plants dead | CR4423-17, Naveen, CR3483-29-M-4-B-Sub-79-1, IR20, CR4423-75, CR 4111-B-1-10-S-1-Sub-B, AC 1125A, CR4423-101, CR4423-111, AC847A, IC-256508, IC-256605 |
| <10% survival | CR4423-10 |
| 25%- 40% survival | IC-256564, CR4111-B-1-4-S-1-Sub-B, Vandana, |
| 40%-50% survival | CR4423-8, FL 478, Pantara, CR4423-20 |

**Table 6: Modified standard evaluation score (SES) for salinity stress at seedling stage**

| Score | Observation | Tolerance | Genotypes |
|---|---|---|---|
| 1 | Normal growth, no leaf symptoms | Highly tolerant | - |
| 3 | Nearly normal growth, but leaf tips or few leaves whitish and rolled | Tolerant | FL 478 |
| 5 | Growth severely retarded; most leaves rolled; only a few are elongating | Moderately tolerant | Naveen, Pantara, CR4423-75 |
| 7 | Complete cessation of growth; most leaves dry; some plants dying | Susceptible | CR4111-B-1-4-S-1-Sub-B, CR3483-29-M-4-B-Sub-79-1, CR4423-20, IR20, AC 1125A, CR4423-101 |
| 9 | Almost all plants dead or dying | Highly Susceptible | CR4423-8, IC-256564, CR4423-10, Vandana, CR4423-17, CR 4111-B-1-10-S-1-Sub-B, CR4423-111, AC847A, IC-256508, IC-256605 |

## Conclusion

The data revealed that under mild and severe water stress the genotypes CR 4423-8, FL 478 and Pantara were found to be tolerant. Similarly, under salinity stress, FL 478, Naveen, Pantara and CR 4423-75 were tolerant. For the combined stress (water stress and salinity), FL 478 and Pantara could be identified as promising genotypes. This was also indicated by lesser reduction in the shoot and root lengths, shoot and root dry weight and total chlorophyll content. The above cultures identified for different abiotic stress situations may be used as physiological donors for respective stresses.

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