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## Artificial screening of BC<sub>3</sub>F<sub>2</sub> backcross population of rice (*Oryza sativa* L.) for drought and salinity

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#### Abstract

The current study was carried out during 2023 at Department of Rice, CPBG, TNAU, Coimbatore, intending to phenotypically screen the BC<sub>3</sub>F<sub>2</sub> population derived from a cross between CO 52 and APD 19002 against salinity and drought. Salinity screening was done at seedling stage and drought screening was carried out in reproductive stage. Total of 240 BC<sub>3</sub>F<sub>2</sub> plants were utilized from three different high genome recovered plants. These plants are subjected to 12 EC and visual salt injury score was taken based on modified SES score given by IRRRI. 25% plants were survived from the seedling stage salinity stress. These plants are forwarded to screen for reproductive stage drought stress and leaf rolling, leaf drying and senescence score was taken. In that 90% plants were survived. Eleven plants are identified as tolerant for both salinity and drought based on the score. The selected plants may play a crucial role in addressing the challenges posed by climate change and ultimately benefiting both farmers and consumers.

**Keywords:** BC<sub>3</sub>F<sub>2</sub> backcross population, rice, *Oryza sativa* L., drought, salinity

#### Introduction

Rice is life for millions of people and it is a source of carbohydrate, protein, vitamins, minerals and disease fighting phyto-compounds for this reason rice is also known as golden cereal (Pradhan *et al.* 2019) [15]. The world's population is expected to reach 9.6 billion by 2050 and with this, there is an urgent need to increase the rice production to meet out the global demand for food (Leridon, 2020) [11]. Modelling simulations predict that agricultural production will need to double by 2050, particularly for high-demand staple foods like rice, in order to feed the growing population (FAO, 2017) [6]. Abiotic stresses cause significant reduction on crop yield which leads to food insecurity. Drought is one of the most destructive abiotic factor and it is predicted that more than 50% of the world's arable land will be damaged by drought in the year 2050 (Singhal *et al.*, 2016) [20]. Worldwide approximately 23 million ha of rainfed land get affected by drought (Ahmad *et al.*, 2020) [1]. Rice is considered one of the most drought-sensitive plant especially during reproductive stage which leads to dramatic reduction of grain yield because of its small root system, thin cuticular wax and swift stomatal closure (Sahebi *et al.*, 2018, Ji *et al.*, 2012) [17, 9]. The second most devastating abiotic stress is salinity. Globally over 80 million hectares of irrigated land, which is 40% of the total irrigated land have become toxic to plants due to salt accumulation (Xiong *et al.*, 2001) [26]. In India around 6.73 million hectares of land deteriorated by salt and by 2050, the total damage is expected to go up to 16.2 million hectares (CSSRI Vision-2050, 2015). Modern high yielding rice varieties showed typical yield loss of 12% at 3 EC and half of the yield reduction was observed at 6 EC (Linh *et al.*, 2012) [12]. Due to the significant yield losses in rice caused by drought and salt stress, as well as the ongoing climate change issues, there is a need to develop more rice lines that are tolerant to both drought and salt.

Marker-assisted backcross breeding (MABC) is a faster and more precise way to transfer genes that confer salinity and drought tolerance to rice plants. Marker-assisted gene/QTL pyramiding has been shown to be a powerful tool to introduce multiple genes/QTLs for broad-spectrum resistance to abiotic and biotic stresses (Muthu *et al.* 2020) [13]. Phenotypic screening is a fundamental tool in rice breeding and agriculture as it enables the selection of rice varieties with desirable traits, adaptation to local conditions, resistance to stresses and improved yield and quality. This process plays a critical role in ensuring food security, sustainability and the continued success of rice farming worldwide.

Hence, the objective of this study was to screen the BC<sub>3</sub>F<sub>2</sub> plants artificially for both drought and salinity in a combined manner. The attained results based on the salinity and drought score may provide a useful information to identify superior climate resilient plants to cope with

climate change. In future they can then be further evaluated for their superior agronomic performance and quality traits.

### Materials and Methods

A popular high yielding fine grain variety CO 52 was used as a recurrent parent and it is crossed with donor parent called APD 19002 which harbors drought ( $qDTY_{1.1}$  and  $qDTY_{2.1}$ ) and salinity (*Saltol*) tolerant QTLs. Plant number 33-47-13, 33-8-11 and 33-37-10 are identified as a highest genome recovered plants from the previous study and it were used as a material for the current study. These three lines were selfed in third backcross generation to produce BC<sub>3</sub>F<sub>2</sub> population. Salinity screening was done at seedling stage based on the guidelines given by IRRI salinity screening manual. A total of 240 BC<sub>3</sub>F<sub>2</sub> plants were screened in glass house along with check at Department of rice, CPBG, TNAU, Coimbatore. 70 seeds from 33-47-13, 70 seeds from 33-8-11, 100 seeds from 33-37-10, recurrent parent CO 52, donor parent APD 19002, tolerant check Pokkali and FL478 and the susceptible check ADT 45 were pregerminated in Petri plates for 2-3 days. Each pregerminated BC<sub>3</sub>F<sub>2</sub> seeds was placed on each hole in the Styrofoam seedling float. Parents and checks are sowed in each row. The pregerminated seeds were transferred to

Styrofoam seedling float without damaging the radicle along with checks. Styrofoam seedling float were suspended on the tray filled with distilled water and kept for 3 days. After 3 days, distilled water was replaced with Yoshida nutrient solution (Yoshida *et al.*, 1976) [27]. On 14<sup>th</sup> day, EC 6 dSm<sup>-1</sup> was applied by adding 100 mM NaCl. Three days later salinity level was raised to EC 12 dSm<sup>-1</sup>. The solution was renewed at every eight days and the pH was maintained at 5.0. The response of plants against salinity stress at seedling stage was scored as described in the standard evaluation system (SES) developed at International Rice Research Institute, Manila, Philippines (Gregorio *et al.*, 1997) [28].

Those survived plants from seedling stage salinity stress were forwarded to reproductive stage drought stress. Survived plants transferred to pot and maintained as normal plants without any stress. During boot leaf stage irrigation was restricted up to 7 days. Scores were taken at the end day of stress. Leaf rolling, leaf drying, and senescence scores were taken at the end day of stress. Severe leaf rolling and leaf drying were observed at this soil moisture level. At this severe stress, a flash life-saving irrigation was provided, and the excess water was drained out approximately after 24 h. This cycle was constantly repeated until harvest.

Modified standard evaluation score (SES) for seedling stage salinity visual salt injury (Gregorio *et al.*, 1997) [28]

Score	Observation	Tolerance
1	Normal growth, no leaf symptoms	Highly tolerant
3	Nearly normal growth, but leaf tips or few leaves whitish and rolled	Tolerant
5	Growth severely retarded; most leaves rolled; only a few are elongating	Moderately tolerant
7	Complete cessation of growth; most leaves dry; some plants dying	Susceptible
9	Almost all plants dead or drying	Highly susceptible

#### Scale for leaf drying

Scale	Observation
1	Unrolled, turgid
2	Leaf rim starts to roll
3	Leaf has a shape of a V
4	Rolled leaf rim covers the part of the leaf blade
5	Leaf rolled like onion

#### Scale of leaf rolling

Scale	Observation
0	Leaves healthy
1	Leaves starts to fold (shallow V shape)
3	Leaves folding (deep V shape)
5	Leaves fully cupped (U shape)
7	Leaf margin touching (O shape)
9	Leaves tightly rolled

#### Senescence score

Decimal score	Senescence
1	Leaves have natural green colour
3	1/3 <sup>rd</sup> of leaves fully green
5	Intermediate
7	1/3 <sup>rd</sup> of leaves yellowing
9	All leaves yellow or dead

### Results and Discussion

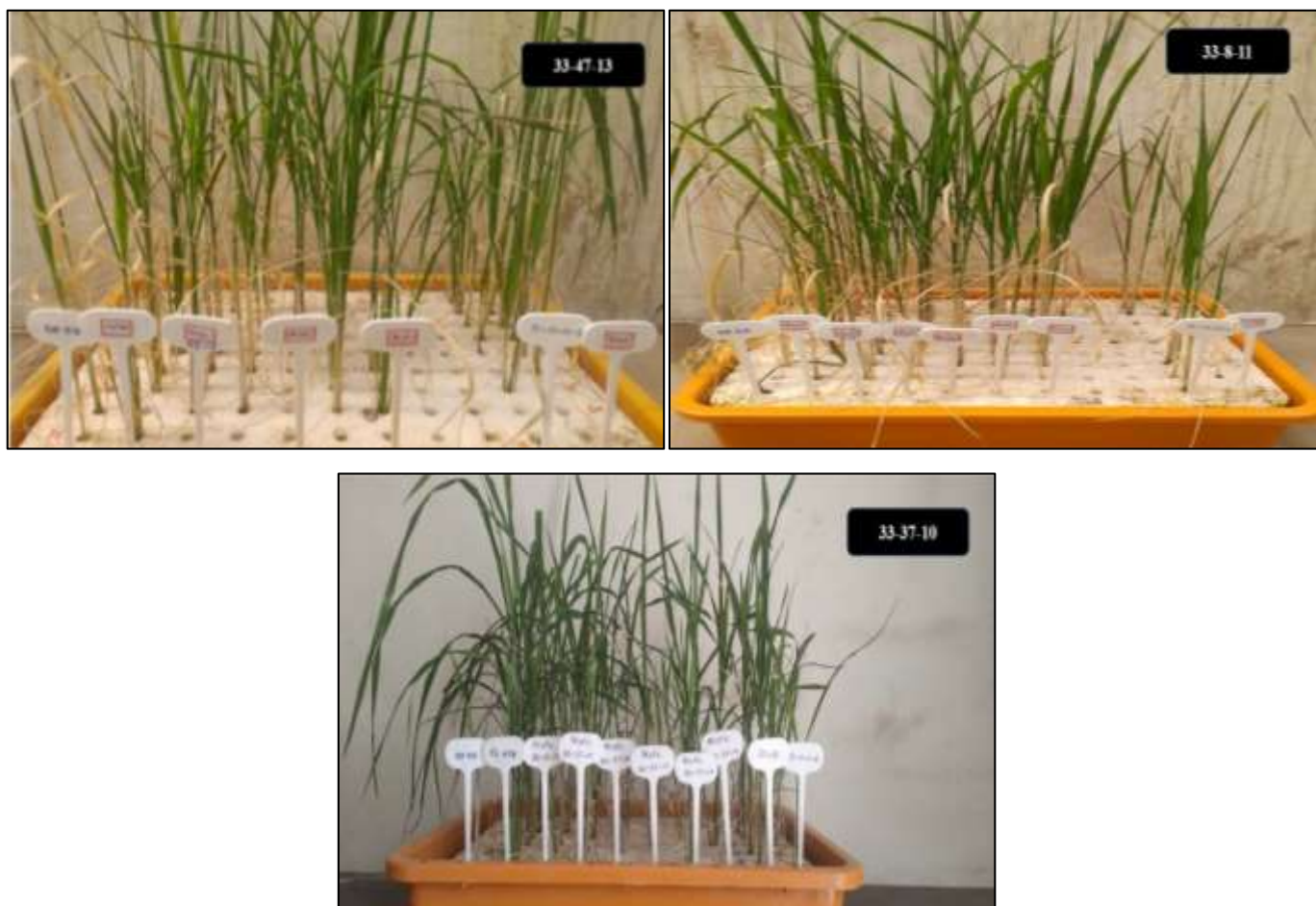
The results from seedling stage salinity screening was given in Table 1. Out of 240 plants, 62 plants were survived at salinity level EC 12 dSm<sup>-1</sup>. The remaining 178 plants are identified as highly susceptible based on the salinity tolerance score 9. Among the 62 plants, thirty plants scored 3, twenty-

nine plants scored 5 and score 7 was observed in three plants. Overall, thirty plants identified as tolerant to salinity, moderately tolerant nature was observed in twenty-nine plants and three plants are susceptible to seedling stage salinity stress. Recurrent parent was highly susceptible to salinity. The donor and the tolerant check Pokkali and FL 478 were found

as tolerant in nature. Similar findings were reported by Tin *et al.* (2021) [24], Tam *et al.* (2019) [22], Thanasilungura *et al.* (2020) [23], Thi Lang *et al.* (2019) [10] and Alshiekheid *et al.* (2023) [2]. Within two to three days of salinization, salt stress symptoms began to appear in the rice seedlings. These symptoms included yellowing and drying of leaves. In some cases, the seedlings died. Other symptoms that were observed included leaf rolling and tip whitening. Salinity stress suppresses leaf growth in rice plants, eventually leading to cessation of growth and premature leaf senescence. Overall, the growth of the seedlings was suppressed under salt stress and only 25% plants were survived at 12 dSm<sup>-1</sup>. From the phenotypic screening, it is clearly visible that the tolerant plants are less sensitive to salinity injury compared to other susceptible plants. Hence, this type of tolerant behavior is the outcome of some physiological, morphological or biochemical mechanism adapted by tolerant plants to withstand salt injury. This theory was also supported by Eti *et al.* (2018) [5].

All the survived 62 plants were transferred to pot for exploiting reproductive stage drought stress. At the end of drought stress screening, 56 plants are survived (Table 2). Leaf rolling, leaf drying and senescence score was taken as drought scores. In leaf rolling, eleven plants are noticed for

score 1 (shallow V shape), twenty-two plants are noted for score 3 (deep V shape), fifteen plants are listed for score 5 (U shape) and seven plants are found in score 7 (O shape). Leaf rolling cause reduction in photosynthetic efficiency leads to reduce the assimilate level. Blum (1988) [29] reported that inability of leaves to meet out transpiration demand cause the leaf rolling as drought symptom. Leaf drying symptoms of score 2 was for thirteen plants, score 3 was noticed in sixteen plants, eighteen plants were scored for 4 and nine plants scored in category 5. In senescence score, eleven plants are under score 1, twenty-one plants were scored 3, 16 plants recognized as an intermediate category and eight plants were scored 7. In parents, the donor parent only was forwarded to drought stress and it was scored 2, 3, 1 for leaf rolling, leaf drying and senescence score respectively. The same results were highlighted by Dwiningsih *et al.* (2021) [4], Garrity *et al.* (1994) [7], Sellammal *et al.* (2014) [18], Pavithra *et al.* (2020) [14], Tu *et al.* (2021) [25] and Bunnag *et al.* (2013) [3]. Susanto *et al.* (2019) [21] reported that leaf rolling and leaf drying are highly associated with ability of plant to recover after drought stress. This trait is still widely used to screen drought tolerant materials of rice. About 90% of survived plants from salinity stress were also withstand the drought during reproductive stage.



**Fig 1:** Seedling stage salinity screening in BC<sub>3</sub>F<sub>2</sub> at 12 EC



**Fig 2:** Phenotypic screening for reproductive stage drought stress in BC<sub>3</sub>F<sub>2</sub>

**Table 1:** Scores obtained from BC<sub>3</sub>F<sub>2</sub> plants for seedling stage salinity stress

Plant no.	33-47-13-1	33-47-13-2	33-47-13-3	33-47-13-4	33-47-13-5	33-47-13-6	33-47-13-7	33-47-13-8	33-47-13-9	33-47-13-10
Score	3	9	5	9	9	9	5	9	5	9
Plant no.	33-47-13-11	33-47-13-12	33-47-13-13	33-47-13-14	33-47-13-15	33-47-13-16	33-47-13-17	33-47-13-18	33-47-13-19	33-47-13-20
Score	9	3	9	9	5	9	7	9	9	9
Plant no.	33-47-13-21	33-47-13-22	33-47-13-23	33-47-13-24	33-47-13-25	33-47-13-26	33-47-13-27	33-47-13-28	33-47-13-29	33-47-13-30
Score	9	9	9	9	9	5	9	5	9	9
Plant no.	33-47-13-31	33-47-13-32	33-47-13-33	33-47-13-34	33-47-13-35	33-47-13-36	33-47-13-37	33-47-13-38	33-47-13-39	33-47-13-40
Score	9	5	9	9	3	9	3	3	9	9
Plant no.	33-47-13-41	33-47-13-42	33-47-13-43	33-47-13-44	33-47-13-45	33-47-13-46	33-47-13-47	33-47-13-48	33-47-13-49	33-47-13-50
Score	9	3	5	9	9	9	9	3	3	9
Plant no.	33-47-13-51	33-47-13-52	33-47-13-53	33-47-13-54	33-47-13-55	33-47-13-56	33-47-13-57	33-47-13-58	33-47-13-59	33-47-13-60
Score	9	3	9	9	9	9	9	3	9	9
Plant no.	33-47-13-61	33-47-13-62	33-47-13-63	33-47-13-64	33-47-13-65	33-47-13-66	33-47-13-67	33-47-13-68	33-47-13-69	33-47-13-70
Score	9	9	9	9	9	9	9	9	9	9
Plant no.	33-8-11-1	33-8-11-2	33-8-11-3	33-8-11-4	33-8-11-5	33-8-11-6	33-8-11-7	33-8-11-8	33-8-11-9	33-8-11-10
Score	3	5	5	9	9	9	3	9	5	9
Plant no.	33-8-11-11	33-8-11-12	33-8-11-13	33-8-11-14	33-8-11-15	33-8-11-16	33-8-11-17	33-8-11-18	33-8-11-19	33-8-11-20
Score	9	9	5	9	9	9	9	3	3	9
Plant no.	33-8-11-21	33-8-11-22	33-8-11-23	33-8-11-24	33-8-11-25	33-8-11-26	33-8-11-27	33-8-11-28	33-8-11-29	33-8-11-30
Score	9	9	3	9	9	9	3	5	3	9
Plant no.	33-8-11-31	33-8-11-32	33-8-11-33	33-8-11-34	33-8-11-35	33-8-11-36	33-8-11-37	33-8-11-38	33-8-11-39	33-8-11-40
Score	9	3	9	9	9	9	9	3	5	9
Plant no.	33-8-11-41	33-8-11-42	33-8-11-43	33-8-11-44	33-8-11-45	33-8-11-46	33-8-11-47	33-8-11-48	33-8-11-49	33-8-11-50
Score	9	3	9	9	5	9	5	3	5	9
Plant no.	33-8-11-51	33-8-11-52	33-8-11-53	33-8-11-54	33-8-11-55	33-8-11-56	33-8-11-57	33-8-11-58	33-8-11-59	33-8-11-60
Score	7	9	9	9	9	9	9	9	9	9
Plant no.	33-8-11-61	33-8-11-62	33-8-11-63	33-8-11-64	33-8-11-65	33-8-11-66	33-8-11-67	33-8-11-68	33-8-11-69	33-8-11-70
Score	9	9	9	9	9	9	9	9	9	9
Plant no.	33-37-10-1	33-37-10-2	33-37-10-3	33-37-10-4	33-37-10-5	33-37-10-6	33-37-10-7	33-37-10-8	33-37-10-9	33-37-10-10
Score	5	5	3	3	9	3	9	9	5	5
Plant no.	33-37-10-11	33-37-10-12	33-37-10-13	33-37-10-14	33-37-10-15	33-37-10-16	33-37-10-17	33-37-10-18	33-37-10-19	33-37-10-20
Score	5	9	9	9	5	3	9	9	9	3
Plant no.	33-37-10-21	33-37-10-22	33-37-10-23	33-37-10-24	33-37-10-25	33-37-10-26	33-37-10-27	33-37-10-28	33-37-10-29	33-37-10-30
Score	9	9	9	9	9	9	9	5	9	9
Plant no.	33-37-10-31	33-37-10-32	33-37-10-33	33-37-10-34	33-37-10-35	33-37-10-36	33-37-10-37	33-37-10-38	33-37-10-39	33-37-10-40
Score	9	9	9	9	9	3	9	9	5	3
Plant no.	33-37-10-41	33-37-10-42	33-37-10-43	33-37-10-44	33-37-10-45	33-37-10-46	33-37-10-47	33-37-10-48	33-37-10-49	33-37-10-50
Score	3	9	9	7	9	9	9	9	5	9
Plant no.	33-37-10-51	33-37-10-52	33-37-10-53	33-37-10-54	33-37-10-55	33-37-10-56	33-37-10-57	33-37-10-58	33-37-10-59	33-37-10-60
Score	9	9	5	9	9	5	9	9	9	9
Plant no.	33-37-10-61	33-37-10-62	33-37-10-63	33-37-10-64	33-37-10-65	33-37-10-66	33-37-10-67	33-37-10-68	33-37-10-69	33-37-10-70
Score	9	9	9	3	9	9	9	9	9	9
Plant no.	33-37-10-71	33-37-10-72	33-37-10-73	33-37-10-74	33-37-10-75	33-37-10-76	33-37-10-77	33-37-10-78	33-37-10-79	33-37-10-80
Score	9	9	9	9	9	9	9	5	9	9
Plant no.	33-37-10-81	33-37-10-82	33-37-10-83	33-37-10-84	33-37-10-85	33-37-10-86	33-37-10-87	33-37-10-88	33-37-10-89	33-37-10-90
Score	9	9	9	9	9	9	9	9	9	9
Plant no.	33-37-10-91	33-37-10-92	33-37-10-93	33-37-10-94	33-37-10-95	33-37-10-96	33-37-10-97	33-37-10-98	33-37-10-99	33-37-10-100
Score	9	9	9	9	9	9	9	9	9	9

**Table 2:** Scores obtained for drought stress at reproductive stage in BC<sub>3</sub>F<sub>2</sub>

S.no	Plant No.	Leaf Drying	Leaf Rolling	Senescence score
1	33-47-13-1	2	1	1
2	33-47-13-3	3	3	3
3	33-47-13-7	3	3	3
4	33-47-13-9	4	5	5
5	33-47-13-12	3	3	3
6	33-47-13-15	3	3	3
7	33-47-13-17	5	7	7
8	33-47-13-26	4	5	5
9	33-47-13-28	4	7	7
10	33-47-13-32	3	5	5
11	33-47-13-35	2	1	1
12	33-47-13-37	3	3	3
13	33-47-13-38	2	3	3
14	33-47-13-42	4	5	5
15	33-47-13-43	4	7	7
16	33-47-13-48	2	1	1
17	33-47-13-49	3	3	3
18	33-47-13-52	2	3	3
19	33-47-13-58	2	1	1
20	33-8-11-1	2	1	1
21	33-8-11-2	3	3	3
22	33-8-11-3	3	3	5
23	33-8-11-7	2	1	1
24	33-8-11-9	3	3	5
25	33-8-11-13	3	3	5
26	33-8-11-18	2	1	1
27	33-8-11-19	3	3	3
28	33-8-1-23	2	1	1
29	33-8-11-27	3	3	3
30	33-8-11-28	3	5	5
31	33-8-11-29	2	1	1
32	33-8-11-32	3	3	3
33	33-8-11-38	2	1	1
34	33-8-11-39	4	5	5
35	33-8-11-42	4	5	7
36	33-8-11-45	4	7	7
37	33-8-11-47	3	3	3
38	33-8-11-48	2	1	1
39	33-8-11-49	4	5	5
40	33-8-11-51	5	7	7
41	33-8-11-1	5	7	9
42	33-37-10-2	5	7	7
43	33-37-10-3	4	3	3
44	33-37-10-4	4	5	3
45	33-37-10-6	4	3	3
46	33-37-10-9	5	9	9
47	33-37-10-10	5	5	5
48	33-37-10-11	5	7	5
49	33-37-10-15	5	7	9
50	33-37-10-16	5	3	3
51	33-37-10-20	4	5	3
52	33-37-10-28	5	5	3
53	33-37-10-36	4	3	5
54	33-37-10-39	5	9	9
55	33-37-10-40	4	5	5
56	33-37-10-41	4	3	3
57	33-37-10-44	5	7	7
58	33-37-10-49	5	3	5
59	33-37-10-53	4	5	5
60	33-37-10-56	5	9	9
61	33-37-10-64	4	5	3
62	33-37-10-78	5	9	9

## Conclusion

The current study was aimed to screen the BC3F2 population derived from a cross between CO 52 a popular high yielding variety from Tamil Nadu and APD 19002 harboring salinity and drought tolerant QTLs. In conclusion, eleven plants (33-47-13-1, 33-47-13-35, 33-47-13-48, 33-47-13-58, 33-8-11-1, 33-8-11-7, 33-8-11-18, 33-8-11-23, 33-8-11-29, 33-8-11-38 and 33-8-11-48) are recognized as tolerant to both seedling stage salinity stress and reproductive stage drought stress. Developing drought and salinity-tolerant rice varieties is essential for ensuring food security, adapting to climate change, conserving water resources, reducing environmental impacts, improving livelihoods, and promoting sustainable agriculture. The selected plants play a crucial role in addressing the challenges posed by climate change and ultimately benefiting both farmers and consumers. In future these plants can be further evaluated for its agronomic and quality traits.

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