

PHYSIOLOGICAL AND BIOCHEMICAL EVALUATION OF BACKCROSS INBRED LINES IN RICE FOR SALINITY AND SUBMERGENCE TOLERANCE

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Abstract- Salinity and submergence tolerant lines in Improved White Ponni genetic background were developed by Marker Assisted Backcrossing by using FL478 and FR13A as the donors. Salinity and submergence tolerant BILs *viz.*, IWP-10-13-3-20-2-2, IWP-10-13-3-20-2-5, IWP-10-13-3-20-2-7 and IWP-10-13-3-56-2-7 were developed by inter-crossing the independent lines to pyramid *SalT* and *Sub1* QTLs in IWP background. The BILs were evaluated for salinity and submergence tolerance independently along with their parents. Biochemical and physiological evaluation were done to select the best salinity/submergence tolerant BILs. Ion concentration (Na⁺/K⁺), H₂O₂ level and antioxidant enzyme activity were determined under salt stress condition (100 mM NaCl). Total sugars and chlorophyll content were determined before and after submergence. Underwater shoot elongation, survival per cent and yield were also recorded during submergence screening. Generally, BILs showed enhanced tolerance to salinity/submergence compared to IWP. Among the BILs, IWP-10-13-3-20-2-2 had salinity tolerant traits such as increased level of antioxidant enzymes, decreased level of H₂O₂, very low Na⁺/K⁺ ratio under salt stress. Similarly, under submergence screening, IWP-10-13-3-20-2-2 had high amount of total sugars and chlorophyll content after submergence. Also IWP-10-13-3-20-2-2 had comparatively lesser underwater shoot elongation and produced high yield. Hence IWP-10-13-3-20-2-2 performed well in both salinity and submergence screening and it can be used as a parent to develop multiple stress tolerance variety by pyramiding QTLs for other abiotic stress.

Keywords- BILs, Biochemical and Physiological Evaluation, Rice, Salinity and Submergence

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Introduction

Rice (*Orzya sativa* L.) is world's second most important cereal and it is the staple food for more than half of the world's population. It is a major source of food for more than 2.7 billion people on a daily basis and is planted in one-tenth of the earth's arable land [1]. Abiotic stress is the major threat to crop production worldwide, reducing average yields of major crops by more than 50 per cent [2]. Abiotic stress conditions cause extensive losses to agricultural production worldwide in the field conditions. Crops are routinely subjected to a combination of different abiotic stresses. In flood prone areas, crops encounter salinity along with submergence stress. Rice is sensitive to a variety of abiotic stresses, including drought, salinity and submergence [3].

Salinity is a second major threat to rice production worldwide and rice is very sensitive to salinity at seedling stage [4]. Salinity affects crop growth and development and it is estimated that salt affected soil reduce yield by 40-50 per cent. Out of the total rice cultivated area in the world, 30 per cent is affected by salinity.

Submergence poses serious threat to rice productivity. It is estimated that 3 million ha of rice area is affected by submergence or flooding stress every year whereas complete crop failure occurs whenever there is a flash flooding or submergence for more than one week to ten days. Most of the rice varieties are susceptible to flooding when they are submerged under water for more than seven days [5].

In recent years, Rice production systems of the world have become increasingly threatened by the effects of climate change [6], as a large portion of the ricegrowing areas are located in especially vulnerable regions. Future farming and food systems will have to be better adapted to a range of abiotic stresses to cope with the direct and indirect consequences of a progressively changing climate. The resilience of rice production systems has to be increased in a two-pronged approach, (i) increasing tolerance to individual stresses and at the same time (ii) achieving multiple stress tolerance. Combining tolerance of multiple stresses is particularly feasible in rice because of the enormous progress made in disentangling the traits associated with tolerance and in developing DNA-based technologies for precise and speedy breeding of more adapted varieties.

In Southern part of India, Cauvery river delta is facing serious problems due to flash flooding during the monsoon period. About 0.3 million ha of paddy area is being affected severely every year due to submergence combined with salinity. Submergence/salinity tolerance rice variety is best suited to grow in these regions, but consumer preference in Tamil Nadu is towards fine grain type rice. Improved White Ponni (IWP) is a ruling variety in Tamil Nadu with duration of 135 days, provides medium slender grain with yield potential of 5.0 t/ha. But IWP is susceptible to salinity and submergence [7]. Hence, developing tolerance to salinity/submergence is very important to increase the area of cultivation in these regions.

The objective of this study was to evaluate the salinity/submergence tolerance ability of backcross inbred lines developed through Marker Assisted Backcrossing to identify multiple stress tolerance fine grain rice genotype.

MaterialsandMethods

Genetic material

Backcross Inbred Lines viz., IWP-10-13-3-20-2-2, IWP-10-13-3-20-2-5, IWP-10-13-3-20-2-7 and IWP-10-13-3-56-2-7 were developed by pyramiding salT QTL from FL478 and sub 1 from FR13A in Improved White Ponni background. The BILs were raised along with parents for evaluation of salinity and submergence independently. Pokkali and Swarna sub1 were raised as a local tolerant check for salinity and submergence respectively. The details of the genetic material used for

the study was given in [Table-1].

Genotype		\$	Salinity scores	Submergence		
	Comment	5 DAS	10 DAS	15 DAS	Seedling Vigor	Tolerance score
IWP-10-13-3-20-2-2	Backcross Inbred Line with salT+Sub 1	1.00	2.08	3.73	1.4	4.3
IWP-10-13-3-20-2-5	Backcross Inbred Line with salT+Sub 1	1.00	2.85	4.46	4.5	5.7
IWP-10-13-3-20-2-7	Backcross Inbred Line with salT+Sub 1	1.00	2.85	3.64	2.7	5.7
IWP-10-13-3-56-2-7	Backcross Inbred Line with salT+Sub 1	1.00	2.54	4.37	4.6	5.0
IWP	Susceptible Parent – Salinity and submergence	3.42	6.85	8.14	2.72	8.3
Pokkali	Local Tolerant Check – Salinity	0.00	0.39	1.13	-NA-	-NA-
Swarna Sub1	Local Tolerant Check – Submergence	-NA-	-NA-	-NA-	-NA-	-NA-
FL478	Tolerant Parent – Salinity	0.00	0.62	1.24	-NA-	-NA-
FR13A	Tolerant Parent – Submergence	-NA-	-NA-	-NA-	1.47	2.3

DAS – Days after stress

NA – Not applicable

Screening for salinity

The screening experiment was carried out under hydroponics system using Modified Yoshida Solution [8] in controlled green house condition. The experiment was carried out in completely randomized design with three replications. Ten days old seedlings were transferred to the seedling float and the seedlings were kept in the nutrient medium. Salinity stress was imposed after well establishment of seedling under hydroponics culture conditions. The nutrient media was salinized by dissolving 5.84 g NaCl per liter of the medium to get 100 mM concentration of NaCl. The pH was monitored daily and adjusted to 4.5. The plants were observed for salt stress symptoms after salinization of the nutrient media. Genotypes which are highly sensitive to salt stress produce symptoms first among all other genotypes. The modified standard evaluation score was used to score the visual symptoms of salt toxicity. Antioxidant enzymes peroxidase, catalase and ROS content H_2O_2 was determined according to the method [9]. Ion accumulation in plant tissue was determined according to the method [8].

Screening for submergence

The screening experiment for submergence was carried out in submergence tank with a dimension of $3m \times 1m \times 0.7 m$ (lbh). Plants were raised in pots and kept in the tank for submergence screening. Experiment was carried out in completely randomized design with three replications. Submergence was imposed at 30 days of sowing and extended up to 45 days. After 45^{th} day water was drained and the plants were allowed to recover for 15 days. Seedling vigor was recorded at 10 days after sowing. Seedling vigor was scored based on standard evaluation of rice [10]. The height of the plant was measured from the ground level to the tip of the highest plant and expressed in centimeter. The plant height was measured at three different intervals, *viz.*, 30 DAS, 45 DAS and maturity.

After imposing stress the plants were allowed to recover for 15 days and comparative survival (%) was calculated based on the formula given below.

$$Comparative survival(\%) = \frac{Total number of plants that survived}{Total number of plants} X 100$$

The comparative survival per cent was converted into submergence tolerance using the scale given in SES [10]. SPAD reading is a measure of chlorophyll content in leaves [11]. Chlorophyll content was determined using SPAD meter (SPAD-502, Minolta Co., Japan). SPAD reading was taken before and after submergence. Total sugar accumulation in leaves was estimated before and after submergence. Total sugar was estimated using anthrone method [12]. The primary panicle and secondary panicle weighed was subjected to threshing. Only the filled grains were retained. Grains from primary panicle and secondary panicle were collected separately and weighed. The sum of primary panicle and secondary panicle grain weight constitute the yield per plant and expressed in grams. Hundred grains were counted and weighed.

Statistical analysis

All calculations and data analysis were performed using the SPSS 20.0 for Windows software package.

Results and Discussion Salinity tolerance

Salinity score under salinity stress revealed the differences in salinity tolerance within the BILs. The salinity tolerance scores calculated for BILs ranged from 3.64 to 4.46. The tolerant parent FL478 recorded 1.24 and the susceptible parent IWP recorded 8.14. The local tolerant check Pokkali recorded 1.13. All BILs showed enhanced salinity tolerance compared to susceptible parent IWP [Table-1]. Two BILs IWP 10-13-3-20-2-7, IWP 10-13-3-20-2-2 exhibited high degrees of salinity tolerance with the salinity tolerance scores of 3.64, 3.73 respectively and IWP-10-13-3-56-2-7, IWP-10-13-3-20-2-5 were moderately tolerant to salinity, exhibiting the salinity tolerance scores of 4.37, 4.46 respectively. The highest score was recorded by the salt susceptible variety IR 29 while CSR 27 and Ezhome-2 scored the lowest value, indicating their tolerance. [13] opined that this scoring discriminated the susceptible from the tolerant and the moderately tolerant genotypes. Salt tolerance varies considerably across rice genotypes, it is due to different levels of ion homeostasis strategies rice genotypes have evolved to cope with excess Na⁺ [14]. Tolerant genotypes of maintenance low Na⁺/K⁺ ratios, through exclusion, compartmentation and partitioning of Na⁺ in shoots or roots [14]. The Na+/K+ ratios of rice seedlings were noted to increase among all genotypes compared to control under salt stress [Table-2]. IWP-10-13-3-20-2-2 recorded the lowest Na+/K+ ratio of 0.246 compared to tolerant parent FL478 under salt stress. The tolerant genotypes pokkali, FL478 recorded Na+/K+ ratio of 0.288, 0.282 respectively and are on par with each other. The susceptible genotype IWP recorded the highest Na+/K+ ratio of 1.357. The BILs IWP-10-13-3-20-2-7, IWP-10-13-3-20-2-5 and IWP-10-13-3-56-2-7 recorded Na⁺/K⁺ ratio of 0.417, 0.439 and 0.517 respectively. The lower level of Na+/K+ in BILs suggest that BILs had superior salt tolerance ability compared to IWP, particularly IWP-20-2-2 which recorded lowest Na+/K+ ratio than the salt tolerant parent FL478. The varieties TRY(R) 2, Ezhome-1 and CSR 27 maintained comparatively lesser sodium content in their shoot. This is in accordance with [15] who reported that the salt tolerant varieties of rice maintain low concentration of sodium in their leaves than those of the salt sensitive lines when exposed to salt stress. So selection within varieties or lines with low sodium transport has been made in rice [16]. Salinity induces excessive production of ROS like H2O2 owing to ion imbalance and hyper-osmotic stresses, which cause damage to plant tissues. ROS accumulation leads to lipid oxidation thus detrimentally affecting the membrane integrity [17]. It was evident that levels of H₂O₂ production in IWP are higher (37.61) under salt stress, as presented in [Table-2], by contrast, lower H₂O₂ production was noted in the salt tolerant genotypes FL478 and Pokkali (17.43 and 18.85 respectively). The BILs recorded H₂O₂ levels ranging from 19.03 to 24.91 under salt stress. The BILs IWP-10-13-3-20-2-2 and IWP-10-13-3-20-2-2 recorded H₂O₂ level of 19.03 and 21.35, which is lower, compared to other BILs. Antioxidant enzymes play an important role in plant adaptation to stress conditions [18]. Antioxidant enzymes such as peroxidase and catalase scavenge H₂O₂ in tolerant genotypes. The levels of peroxidase and catalase were higher in salt tolerant genotypes FL478 and Pokkali. The salt tolerant parent FL478 recorded the peroxidase activity of 26.36 and catalase activity of 125.90 under salt stress. The BILs IWP-10-13-3-20-2-2, IWP-10-13-3-20-2-2 recorded the peroxidase activity of 24.94, 22.64

International Journal of Agriculture Sciences ISSN: 0975-3710&E-ISSN: 0975-9107, Volume 8, Issue 17, 2016 respectively and catalase activity of 120.05 and 117.18 respectively. Peroxidase activity of IWP-10-13-3-20-2-2 is on par with IWP-10-13-3-20-2-5. Thus among the BILs, IWP-20-2-2 had significantly higher activity of peroxidase and catalase enzyme and considered to be salt tolerant. Pearson's correlation coefficients among salinity tolerance score, Ion concentrations, H_2O_2 level and antioxidant enzyme activity were listed in [Table-3]. The salinity tolerance scores displayed negative correlations to the POX, CAT, potassium (r=-0.956, p< 0.01; r= -0.924, p< 0.01; r= -0.736, p< 0.05

respectively) and positive correlation with H_2O_2 , sodium, Na⁺/ K⁺ ratio(r=0.937, p< 0.01; 0.807, p< 0.05; 0.884, p< 0.01 respectively). The Na⁺/ K⁺ ratio had significantly negative correlations with peroxide activity (r=-0.978, p< 0.01), catalase activity, (r= -0.879, p< 0.01) and positive correlation with H_2O_2 (r=0.979, p< 0.01), Sodium content (r=0.972, p< 0.01). Moreover, H_2O_2 had a significantly, negative correlation with the POX (r=0.985, p<0.01) and catalase activity (r=0.936, p<0.01). Similarly, the POX activity showed a positive correlation with the CAT activity (r=0.926, p<0.01).

		Table-2	Comparison	of physiologic	al and bioch	nemical chai	racters of B	ILs exposed	to 100mM N	laCl			
Canadama	Peroxidase		Cata	Catalase		H ₂ O ₂		Sodium (Na⁺)		Potassium (K+)		Na+/K+ ratio	
Genotype	C	S	С	S	С	S	С	S	C	S	С	S	
IWP-10-13-3-20-2-2	22.515°	24.935⁰	98.734 ^f	120.0510	16.739 ^f	19.028 ^f	13.833ª	29.968 ^f	161.173ª	121.583ª	0.086 ^d	0.246 ^f	
IWP-10-13-3-20-2-5	20.765 ^d	22.383d	102.548d	116.381 ^d	17.535°	24.370 ^d	12.212 ^b	32.699°	106.985 ^d	74.498d	0.114 ^b	0.439	
IWP-10-13-3-20-2-7	20.293e	22.637d	102.902	117.183 ^d	18.714 ^d	21.350e	10.221 ^h	32.946°	93.784g	79.019⁰	0.109 ^b	0.417 ^d	
IWP-10-13-3-56-2-7	19.675 ^f	21.211°	99.313°	109.015e	19.244°	24.913⁰	11.454 ^d	31.738de	110.144°	61.352°	0.104°	0.517 ^b	
IWP	8.494 ^h	10.569 ^g	97.103 ^g	102.313 ^f	20.993ª	37.614ª	11.067 ^f	83.135 ^b	102.973 ^e	61.278°	0.107 ^b	1.357ª	
Pokkali	25.526ª	27.359ª	105.025ª	122.007	14.775 ^h	18.846 ^f	10.592 ^g	32.552 ^{od}	100.843 ^f	112.925	0.105⁰	0.288e	
FL478	24.919 ^b	26.362 ^b	103.115	125.897ª	16.553g	17.4349	11.857°	31.552e	100.610 ^f	111.775	0.118ª	0.282e	
Mean	18.978	20.871	99.728	114.341	18.091	24.839	11.559	44.872	110.975	85.495	0.106	0.615	
SEd	0.097	0.159	0.076	0.685	0.030	0.172	0.016	0.424	0.310	0.649	0.003	0.007	
CD(0.01)	0.289	0.474	0.225	2.038	0.090	0.513	0.048	1.261	0.924	1.932	0.008	0.021	

Table-3 Pearson's correlation coefficients among salinity tolerance score, Ion concentrations, H₂O₂ level and antioxidant enzyme activity of rice genotypes exposed to 100mM NaCl

	SES Score	POX	CAT	H ₂ O ₂	Sodium (Na ⁺)	Potassium (K ⁺)	Na ⁺ / K ⁺ ratio
SES Score	1						
POX	-0.956**	1					
CAT	-0.924**	0.926**	1				
H_2O_2	0.937**	-0.985**	-0.936**	1			
Sodium (Na*)	0.807*	-0.920**	-0.761*	0.918**	1		
Potassium (K*)	-0.736	0.750	0.848*	-0.766*	-0.496	1	
Na+/ K+ ratio	0.884**	-0.978**	-0.879**	0.979**	0.972**	-0.680	1
** • • • •		0.04.1 1.00					

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Submergence tolerance

Survival per cent of BILs along with parents and local check variety was significantly negatively correlated ($r^2 = 0.908$) with underwater shoot elongation [Fig-1]. Shoot elongation occurs at the cost of hydrocarbons and metabolic energy for maintenance processes.



Fig-1 Regression Curve between under water shoot elongation during submergence and survival in rice Underwater shoot elongation ranged from 0.6 to 2.2 cm/day and more elongation being in susceptible parent IWP. Submergence tolerant parent FR13A had shoot elongation of 1.0 cm/day whereas the tolerant check Swarna sub1 recorded shoot elongation of 0.6 cm/day. The BIL IWP-10-13-3-20-2-2 recorded the lesser shoot elongation of 1.4 cm/day among BILs. The BILs IWP-10-13-20-2-2 and IWP-10-13-3-56-2-7 recorded shoot elongation of 1.7 cm/day. The BILs recorded relatively lesser elongation and consequently better survival [Table-4]. The importance of slow growth during submergence was suggested to be beneficial which prevents damage due to lodging once the flood water recedes after flash flood. Sugars are the prime substrate for energy production and have strong relevance to submergence tolerance. Genotypes with higher carbohydrate before and after submergence had better survival. The tolerant genotype FR13A had significantly higher sugars during before and after submergence (70.9 and 55.4 respectively) followed by IWP-10-13-3-20-2-2 (64.8 and 42.8 respectively). The per cent decrease of sugar after submergence is very low in FR13A (21.9) followed by Swarna sub1 (31.9). Among the BILs, IWP-10-13-3-20-2-2 had lower per cent decrease of total sugars (34.0 %). The susceptible parent IWP had lower total sugars and higher per cent decrease of sugars [Table-4].

	Table -4 Effect of submergence on physiological and biochemical characters in rice											
Canatura	Plant Height (cm)		Shoot elongation	SPAD		Per cent	Total sugars (mg/g)		Per cent	Survival	Yield/Plant	100 g
Genotype	BS	AS	(cm/day)	BS	AS	decrease	BS	AS	decrease	(%)	(g)	wt(g)
IWP-10-13-3-20-2-2	53.3 ^d	78.5⁰	1.7	32.8d	30.1°	8.2	64.8 ^b	42.8 ^b	34.0	85.6°	14.0°	1.92 ^d
IWP-10-13-3-20-2-5	57.5ª	85.5 ^b	1.9	35.4 ^b	32.0 ^b	9.6	65.1 ^b	40.1°	38.4	74.1e	10.6 ^f	2.11°
IWP-10-13-3-20-2-7	56.4 ^b	76.7d	1.4	34.0°	31.0 ^d	8.8	62.4d	36.9e	40.9	83.0 ^d	13.7 ^d	1.81 ^f
IWP-10-13-3-56-2-7	53.0 ^d	78.2°	1.7	34.4°	31.5⁰	8.4	62.4d	38.3 ^d	38.6	86.6°	11.3º	1.88e
Swarna Sub1	41.9e	51.6 ^f	0.6	37.1ª	34.7ª	6.5	63.6°	43.3 ^b	31.9	89.3 ^b	15.7 ^₅	2.16 ^b
IWP	55.7℃	88.5ª	2.2	34.0°	30.4e	10.6	58.3e	27.4 ^f	53.0	41.7 ^f	8.3 ^g	1.79 ^f
FR13A	56.3℃	70.6e	1.0	35.7⁵	34.5ª	3.4	70.9ª	55.4ª	21.9	95.8ª	17.4ª	2.67ª
Mean	53.45	75.62		34.77	32.03		63.92	40.61		79.44	13.00	2.05
SEd	0.34	0.49		0.22	0.20		0.41	0.29		0.58	0.10	0.01
CD(0.01)	1.05	1.50		0.67	0.62		1.25	0.90		1.76	0.30	0.04

International Journal of Agriculture Sciences ISSN: 0975-3710&E-ISSN: 0975-9107, Volume 8, Issue 17, 2016 After de-submergence tolerant varieties maintained higher sugars which enabled them to recover faster than susceptible. A strong negative correlation was observed between per cent decrease in sugar level and survival percent indicating the importance of sugars in submergence tolerance of rice. Lower survival per cent of susceptible parent IWP is therefore due to starvation and lower energy supply for maintenance and repair processes of membrane integrity during submergence and recovery phase. Thus, higher amount of carbohydrate in plants prior to submergence and its slower depletion is often positively correlated with the level of submergence tolerance [19, 20].



Fig-2a Comparison of yield with shoot elongation under submergence

Swarna *sub1* had significantly higher chlorophyll content followed by FR13A before submergence but after submergence the chlorophyll content of Swarna *sub1* and FR13A are on par with each other with percent decrease of chlorophyll content of 6.5 and 3.5 respectively [Table-4].



Fig-2b Comparison of yield with percentage decrease of total sugars after submergence

Among the BILs IWP-10-13-3-20-2-5 and IWP-10-13-3-56-2-7 recorded higher chlorophyll content before and after submergence but the per cent decrease of chlorophyll content is low for IWP-13-3-20-2-2 (8.2). The susceptible parent IWP had highest percent decrease of chlorophyll (10.6). The loss of chlorophyll in leaves after submergence is caused by caused by ethylene [21], which triggers the enzyme activity of chlorophyllase, the first enzyme involved in chlorophyll breakdown. Tolerant genotypes maintained more chlorophyll than the susceptible genotypes.

	Seedling vigor	SubTol scale	Shoot elongation	SPAD (Per cent decrease)	Total Sugar (Per cent decrease)	Survival %	Yield/Plant	100gwt
Seedling vigor	1							
SubTol	0.461	1						
Shoot elongation	0.587	0.791*	1					
SPAD (Per cent decrease)	0.557	0.915**	0.792*	1				
Total Sugar (Per cent decrease)	0.441	0.989**	0.746*	0.923**	1			
Survival %	-0.255	-0.924**	-0.737*	-0.748*	-0.887**	1		
Yield/Plant	-0.682	-0.925**	-0.883**	-0.906**	-0.908**	0.849*	1	
100gwt	-0.370	-0.776*	-0.595	-0.887**	-0.845*	0.539	0.706	1

**. Correlation is significant at the 0.01 level (2-tailed).

Survival per cent had significant negative correlations with percent decrease of SPAD value suggesting he tolerant genotype have low chlorophyll degradation compared to susceptible genotype. Among the BILs IWP-10-13-3-20-2-2 recorded highest grain yield of 14.0 g/plant followed by IWP-10-13-3-20-2-2, which recorded 13.7 g/plant. Yield had non-significant positive correlation with 100g weight [Table-5]. The genotypes which had lower underwater shoot elongation rate and lower per cent decrease of total sugars yielded more [Fig-2]. Tolerant genotypes spend less energy during submergence and have high amount of total sugars, which helps the plant review more quickly and to accumulate higher dry matter after submergence, which gets reflected in the yield [22].

Conclusion

Independent evaluation of BILs to salinity and submergence showed that BILs performed good under stress conditions compared to susceptible parent. Among the BILs, IWP-20-2-2 had increased level of antioxidant enzymes, decreased level of H₂O₂, very low Na⁺/K⁺ ratio under salt stress. Similarly, IWP-13-3-20-2-2 had high amount of total sugars and chlorophyll content after submergence. Also IWP-13-3-20-2-2 had comparatively lesser underwater shoot elongation and produced high yield. In salinity and submergence IWP--13-3-20-2-2 performed well and it can be used as a parent to develop multiple stress tolerance variety by pyramiding QTLs for other abiotic stress. Also it can be forwarded to as a salinity/submergence tolerant fine grain rice variety suitable for Cauvery delta regions of Tamil Nadu.

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Conflict of Interest: None declared

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