

Original Research Article

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## Evaluation of *Saltol* QTL Introgression in Rice: A Study on Co-Existence of Salinity Tolerance and Phytoremediation Effect

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### ABSTRACT

Developing salinity tolerant crop varieties will enable crops to grow in saline soils without compromising yield. Improved White Ponni (IWP) is a salt sensitive rice variety but it is widely cultivated for its grain quality and yield. Efforts were made to develop salt tolerant Improved White Ponni (IWP) lines by introgressing *Saltol* QTL from FL478 (Tolerant cultivar) through Marker Assisted Backcross Breeding (MABB). Foreground marker RM3412 was employed to select the positive genotypes harboring *Saltol* QTL. Under stress condition, Na<sup>+</sup>/K<sup>+</sup> ratio ranged from 0.25 (IWP-10-13-3-20-2-2) to 1.36 (IWP). All the NILs viz., IWP-10-13-3-20-2-2 (0.25), IWP-10-13-3-20-2-5 (0.44), IWP-10-13-3-20-2-7 (0.42) and IWP-10-13-3-56-2-7 (0.52) recorded Na<sup>+</sup>/K<sup>+</sup> ratio lesser ratio than IWP (1.36). The Near Isogenic Lines (NILs) showed nearly 60 - 84 per cent enhanced level of salinity tolerance compared to susceptible parent Improved White Ponni. This enhanced tolerance of NILs speculates to investigate the mechanism of salinity tolerance in NILs with a hypothesis of that do salinity tolerance and phytoremediation co-exist as two sides of a coin. The study concluded that NILs with *Saltol* QTL does not had phytoremediation ability but salinity and phytoremediation can coexist if NHX-type Na<sup>+</sup>/H<sup>+</sup> antiporter genes are used to develop salinity tolerant rice genotype.

### Keywords

Rice, Improved White Ponni, *Saltol*, Salinity and Phytoremediation.

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### Introduction

Soils are classified as saline when the electrical conductivity (EC) is 4 dS/m or more which is equivalent to approximately 40 mM NaCl and generates an osmotic pressure of approximately 0.2 MPa. This definition of salinity derives from the EC that significantly reduces the yield of most crops (Pirasteh-Anosheh *et al.*, 2016). Salinity is one of the abiotic stresses limiting rice production globally. In India, total salt affected area is reported to be ~8.1 million ha. In the recent

years, salt affected areas are growing at the rate of 10 per cent every year thus making the soil unsuitable for cultivation and thereby rendering crop productivity to decline (Maji *et al.*, 2010). Salinity contribute to the loss of arable lands due to salt accumulation as a result of excessive use of irrigation water with poor or improper drainage, a fact that is likely to be aggravated by sea level rise in coastal areas caused by climate change (Platten *et al.*, 2013). Management of salinity is energy

intensive agricultural practice hence developing salinity tolerant crop plants is a best strategy to combat salinity. In order to develop salinity tolerant lines of Improved White Ponni (IWP), *Saltol* QTL on chromosome 1 was transferred from FL478 to IWP using Marker Assisted Backcrossing. The Near Isogenic Lines (NILs) showed an enhanced salinity tolerance compared to susceptible parent Improved White Ponni. The salinity tolerance of NILs over the susceptible parent speculates to investigate the mechanism of salinity tolerance in NILs and to study the phytoremediation effect with the help of hydroponics screening under glass house condition.

### **Materials and Methods**

Near Isogenic Lines (BC<sub>2</sub>F<sub>6</sub>) 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 introgressing *salt* QTL from FL478 in Improved White Ponni background. Pokkali was used as a check variety. RM3412 was used for foreground selection of positive plants harboring *Saltol* QTL (Fig. 1). Evaluation of salinity tolerance was carried out under hydroponics using Modified Yoshida Solution in the glass house. The sterilized seeds were placed in petriplates and kept at 27°C for germination.

### **Making floats for seedling growth under hydroponic conditions**

Rectangular thermocole sheets of size 38 × 26 × 1.5 cm were used to make floats for seedling growth. Holes of 1.5 cm diameter were made in the thermocole sheet with spacing of 4.2 x 2.8 cm. A tray of size 38 x 26 with 15 liters of water holding capacity was selected to accommodate seedling floats (Fig. 2). Salinity stress was imposed after well establishment of seedlings (10 days after transfer to seedling floats) under hydroponics culture conditions. The nutrient media was

salinized by dissolving 5.84 g NaCl per litre of the medium to get 100 mM concentration of NaCl. The pH was monitored daily and adjusted to 4.5. Control plants were maintained without salinity stress. Three replications were maintained for control and stress.

### **Estimation of sodium and potassium content in leaves**

Sample for sodium and potassium estimation was collected when the susceptible parent showed salinity stress symptom. Sodium and potassium content of the sample were analysed using Flame Photometer (ELICO, Model CL-361) following triple acid (HNO<sub>3</sub>, H<sub>2</sub>SO<sub>4</sub> and HClO<sub>4</sub> in the ratio of 9:2:1) digestion of the samples (Yoshida *et al.*, 1976). The sodium and potassium content was expressed in mg/g of sample on dry weight basis. Sodium and potassium content of leaves, stem and roots were estimated separately and summed up to arrive the total sodium and potassium content. Sodium/potassium ratio was calculated by dividing the total sodium content by total potassium content.

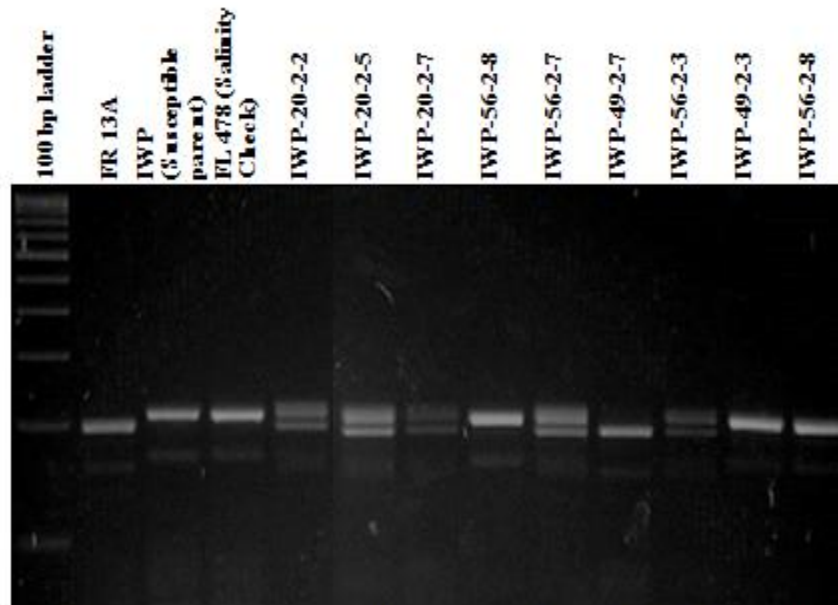
### **Results and Discussion**

The ability of plants to accumulate ions in the plant parts results in the depletion of ions in the growing medium provided if the ions are not replenished by natural or artificial means. This phenomena develops an insight to dissect the salinity tolerance in rice can coexist with phytoremediation. Genetic variability for salinity tolerance was observed in rice and the mechanism of salinity tolerance varies depending up on the nature of gene/s governing the salinity tolerance (Platten *et al.*, 2013). Sodium/potassium ratio was considered to be a reliable parameter used to evaluate salt tolerance ability of rice cultivars (Gregorio *et al.*, 1997).

Under stress condition, total sodium content ranged from 29.97 mg/g (IWP-10-13-3-20-2-2) and 83.13 mg/g (IWP) with a mean of 39.23 mg/g. All the NILs *viz.*, IWP-10-13-3-20-2-2 (29.97 mg/g), IWP-10-13-3-20-2-5 (32.70 mg/g), IWP-10-13-3-20-2-7 (32.95 mg/g) and IWP-10-13-3-56-2-7 (31.74 mg/g) showed lower sodium content than IWP (83.13 mg/g) (Table 1). The lower sodium content in NILs clearly indicates that these plants have sodium exclusion strategy to maintain ion homeostasis rather than sodium sequestration in the vacuole. Similar phenomena were observed in FL478 and Pokkali (Arunroj *et al.*, 2005). This phenomenon is further confirmed by considering the potassium content and also by sodium/ potassium ratio ( $Na^+/K^+$ ). In stress condition,  $Na^+/K^+$  ranged from 0.25 (IWP-10-13-3-20-2-2) to 1.36 (IWP). All the NILs *viz.*, IWP-10-13-3-20-2-2 (0.25), IWP-10-13-3-20-2-5 (0.44), IWP-10-13-3-20-2-7 (0.42) and IWP-10-13-3-56-2-7 (0.52) recorded lesser

ratio than IWP (1.36). Similar pattern of sodium and potassium content was observed in control except for the magnitude of the content.

Among the NILs, the percentage of sodium content is higher in IWP-13-3-30-2-7 with 31.95 % whereas the lowest one is observed in the NIL IWP-13-3-30-2-2 with 28.97 %. The percentage of potassium content is higher in the IWP-13-3-30-2-5 with -0.15 %, the lowest is IWP-13-3-56-2-7 with -0.44 %. Percentage of Sodium/potassium ratio is higher in IWP-13-3-56-2-7 (4.20 %) and the lowest was observed in IWP-13-3-30-2-2 (1.77 %). The percentage of enhanced level of salinity tolerance was observed maximum for the BIL IWP-13-3-30-2-2 with 84 per cent. Other Near Isogenic Lines *viz.*, IWP-13-3-30-2-5, IWP-13-3-30-2-7, IWP-13-3-56-2-7 showed 73%, 75% and 63% enhanced level of salinity tolerance compared to susceptible parent Improved White Ponni respectively.



**Fig. 1** Foreground selection of salinity tolerant BILs using RM3412. FR13A parent is given as these NILs also contains *Sub 1* QTL, but for selecting salinity tolerant BILs FR13A will be ignored only FL478 will be considered. IWP-20-2-2, IWP-20-2-5, IWP-20-2-7 and IWP-56-2-7 was selected for evaluation.

**Fig.2** Screening of parents (NILs) against salinity stress



(a) Before imposition of stress

(b) After imposition of stress (100 mM NaCl)

- |                        |                         |
|------------------------|-------------------------|
| 1) IWP                 | 6) Pokkali              |
| 2) IWP- 10-13-3-20-2-2 | 7) FL478                |
| 3) IWP-10-13-3-20-2-5  | 8) Pokkali              |
| 4) IWP-10-13-3-20-2-7  | 9) FL478                |
| 5) IWP-10-13-3-56-2-7  | 10) IWP- 10-13-3-20-2-2 |

**Table.1** Sodium, potassium and sodium potassium ratio of NILs along with along with tolerant (FL478) and susceptible parent (IWP) under control and salinity stress condition (100mM NaCl) [\* Significant at 0.05 per cent probability]

Genotypes	Sodium content (Na <sup>+</sup> ) (mg/g)		% of Na+ content over control	Potassium content (K <sup>+</sup> ) (mg/g)		Na <sup>+</sup> / K <sup>+</sup> ratio		% of K+ content over control	% of Na <sup>+</sup> / K <sup>+</sup> ratio Content over control
	Control	Stress		Control	Stress	Control	Stress		
<b>IWP-13-3-20-2-2</b>	13.83	29.97*	28.97	161.17*	121.58*	0.09*	0.25*	-0.24	1.77
<b>IWP-13-3-20-2-5</b>	12.21	32.70	31.70	106.98	74.50	0.11	0.44	-0.30	3.00
<b>IWP-13-3-20-2-7</b>	10.22*	32.95	31.95	93.78	79.02	0.11	0.42	-0.15	2.81
<b>IWP-13-3-56-2-7</b>	11.45	31.74*	30.74	110.14	61.35	0.10*	0.52	-0.44	4.20
<b>IWP</b>	11.07*	83.13	82.13	102.97	61.28	0.11	1.36	-0.40	11.36
<b>Pokkali</b>	10.59*	32.55	31.55	100.84	112.93*	0.11	0.29*	0.11	1.63
<b>FL478</b>	11.86	31.55*	30.55	100.61	111.78*	0.12	0.28*	0.11	1.33
<b>Mean</b>	11.61	39.23		110.93	88.92	0.107	0.51		
<b>SE</b>	0.45	7.33		8.60	9.75	0.004	0.15		



The IWP absorbs more sodium from the salinized growing medium but unable to sequester inside the vacuole hence high cytosolic Na<sup>+</sup> impaired the cellular activity. But the salinity tolerant NILs maintains low cytosolic Na<sup>+</sup> due to the fact that *Saltol* affect the Na<sup>+</sup>/K<sup>+</sup> through the sodium transporter *SKCI* the causal gene underlying the *Saltol* QTL (Thomson *et al.*, 2010). *SKCI* was found to encode a sodium transporter that helps control Na<sup>+</sup>/K<sup>+</sup> homeostasis through unloading of Na<sup>+</sup> from the xylem (Ren *et al.*, 2005), which has been suggested to function primarily in roots to reduce the amount of Na<sup>+</sup> ions that are transported to the leaves (Hauser and Horie 2010). Moreover functional annotation of 14 genes within *Saltol* QTL has been reported (Soda *et al.*, 2013). None of the gene related to sodium sequestration process. This clearly indicates that *Saltol* introgressed NILs does not have phytoremediation capacity.

Molecular and functional analyses of rice NHX-type Na<sup>+</sup>/H<sup>+</sup> antiporter genes revealed the important role of this gene in the compartmentalization of Na<sup>+</sup> into vacuoles (Fukuda *et al.*, 2011). With the advent of Next Generation Sequencing technologies the donor which has the highest expression of Na<sup>+</sup>/H<sup>+</sup> antiporter genes can be identified and the same can be utilized to develop salinity tolerant rice genotypes with phytoremediation capacity by molecular breeding. Salinity tolerant genotypes with phytoremediation capacity will be an added advantage. In addition to grain yield, salinity tolerant rice genotypes with phytoremediation capacity ensures the reduced salinity levels in soil with subsequent cultivation of crops there by increasing the percentage of arable land.

Salinity tolerant NILs introgressed with *Saltol* QTL does not have any phytoremediation activity. But, developing plants with Na<sup>+</sup>/H<sup>+</sup> antiporter genes would ensure salinity tolerance along with phytoremediation of

sodium from the soil but this strategy should be further confirmed by experimental procedures.

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