Indian Journal of Agricultural Sciences **91** (3): 426–9, March 2021/Article

Exploring novel QTLs among backcross lines for salinity tolerance in rice

SARITA KUMARI[1], BALWANT SINGH[2], SUMEET KUMAR SINGH[1], DHRITI SATYA[2], SANGEETA SINGH[3], KABITA TRIPATHY[2], KISHOR GAIKWAD[2], VANDNA RAI[2] and NAGENDRA KUMAR SINGH[2]*

ICAR - National Institute of Plant Biotechnology, New Delhi 110 012, India

Received: 14 September 2020; Accepted: 26 October 2020

ABSTRACT

Wild progenitor species of rice (*Oryza rufipogon* Griff./ *Oryza nivara* Sharma et. Shastry) are rich source of genes for both the biotic as well as abiotic stress tolerance. Wild rice accession NKSWR 173 has been identified as highly tolerant to salinity stress at seedling stage and moderately tolerant at reproductive stage after evaluation of more than two hundred wild rice accessions from across India. In a bid to introgress the salt tolerance trait from NKSWR 173 to a high-yielding mega variety of rice IR 64, we screened a segregating BC_1 population for identification of suitable lines for making the second backcross using both controlled phenotyping and QTL flanking DNA markers. Four lines, namely SN 32, SN 33, SN 39 and SN 45 were found highly tolerant to salinity at both seedling and reproductive stage and were backcrossed to IR 64 to generate BC_2F_1 seeds for development of advance introgressed lines. Introgression of novel salinity tolerance genes for both the seedling and reproductive stages in mega variety of rice will be useful in achieving high productivity in salt affected rice areas.

Keywords: Introgression, QTL, Reproductive Stage, Salinity tolerance, Seedling Stage, Wild rice

Salinity is a major issue in rice production, which affects about 30 percent of the cultivated land (Singh *et al.* 2016). Critical salinity levels with EC 6.9 dS/m may cause a yield loss of up to 50% (Radanielson *et al.* 2018). Different breeding approaches are being used to develop salt-tolerant crop varieties with varying level of success (Turan *et al.* 2012). A molecular breeding approach accelerates the mapping and introgression programme more precisely in comparison to conventional linkage mapping (Singh *et al.* 2011). An advanced backcross QTL method has been utilized for introgression of the traits from wild species into cultivated species (Tanksley *et al.* 1996). Rice has 24 different species with 11 genome types reported till now (Jacquemin *et al.* 2013). Indian wild rice; *O. nivara* and *O. rufipogon* are gold mine of genes for biotic and abiotic stress tolerance (Singh *et al.* 2018; Tripathy *et al.* 2018). They have been used as donors for several agronomically important traits (Swamy *et al.* 2014). *Saltol* QTL mapped using mapping population derived from Pokkali/IR 29 crosses are widely being used as donor Marker-assisted introgression programme (Gregorio *et al.* 1997). *Saltol* locus has been transferred from FL-478 to Binadhan-7 (Mondal *et al*. 2013), Pusa 44 and Sarjoo 52 (Krishnamurthy *et al*. 2020) using marker-assisted backcross breeding (MABB) programme. ICAR-NIPB has collected over 800 accessions of *O. nivara/O. rufipogon* wild rice from nine eco-geographical zones of India (Singh *et al.* 2018, Tripathy *et al.* 2018). From a subset of this collection NKSWR 173 was found to be highly tolerant to seedling stage salinity stress of 150 mM NaCl (Mishra *et al.* 2016). QTLs have been identified for high salinity tolerance at seedling stage and reproductive stage (EC 6dS/m) using a BC_1 mapping population derived from cross between elite high yielding cultivar IR 64 and NKSWR 173 (unpublished results). The present study was aimed at selecting a suitable backcross line using SNP markers for further backcrossing with IR 64 to ensure the transfer of QTLs for both seedling and reproductive stage salt tolerance.

MATERIALS AND METHODS

Phenotypic Evaluation of the Salinity Tolerant Backcross Lines: BC_1F_2 families were screened for salinity tolerance at seedling stage following randomized block design at National Phytotron Facility, New Delhi (Gregorio *et al.* 1997). The tolerant individuals with SES score 1 or 3 were transplanted in the field (ICAR-IARI, New Delhi-2017). Among the 36 lines that were transferred to the field in salinity micro plots, only 31 could establish successfully. These 31 families were evaluated for yield related traits including plant height (cm), tiller number, panicle length (cm), panicle branches (numbers), seeds per

[1]Rajendra Prasad Central Agricultural University, Pusa; [2]ICAR-National Institute of Plant Biotechnology, New Delhi; [3]National Institute of Plant Genome Research. *Corresponding author's e-mail: nksingh4@gmail.com.

panicle (numbers), and test weight (g). Segregation pattern of different backcross families for different agronomic traits were analyzed. The backcross lines were also evaluated phenotypically for the presence of undesirable wild traits; seed shattering, long awns and chaffiness.

Genotyping for the backcross lines for presence of QTLs: In order to screen the back cross families for the presence of positive alleles of QTLs for salinity tolerance traits at both the seedling and reproductive stages, the BC_1F_1 were analyzed for the peak as well as flanking SNP markers. A list of peak and flanking markers for the mapped QTLs in NKSWR 173 is given in Table 1 (unpublished).

Analysis of the backcross lines for percent RPG recovery: BC_1F_2 families were selected based on phenotype and genotype data, were also evaluated for recurrent parent genome recovery (RPG) using GGT2 software (Berloo *et al.* 2008). All the polymorphic markers between the parents, except the markers used for foreground selections of desired QTLs were used for background genome recovery of tolerant and transferred backcross families.

Development of advanced backcross lines: BC_1F_2 plants selected based on phenotype, QTL flanking markers, and agronomically essential traits were again backcrossed to IR 64 to produce BC_2F_1 seeds. Seeds of BC_2F_1 plants were harvested for growing BC_2F_2 families and kept for evaluation in next season to develop advanced introgressed lines (AILs) during 2020.

RESULTS AND DISCUSSION

Advance backcross quantitative trait loci (AB-QTL) technique is the fastest and easiest way for simultaneous mapping and introgression of useful agronomic traits from a wild unadapted donor parent into the commercial cultivars (Tanksley and Nelson 1996). For precise introgression of the trait, backcross lines must be evaluated for the concerned trait phenotype, genotype and recurrent parent genome recovery. The segregating backcross lines at BC_1 need to be characterized for the identification of a suitable donor for further backcrossing to accumulate favorable alleles for most of the QTL with minimum linkage drag. The backcross inbred lines (BILs) are also analyzed for molecular dissection of the QTL regions (Puram *et al.* 2017). *O. nivara* and *O. rufipogon* have already been used as donors for yield enhancement of PR 114 and Pusa 44 (Gaikwad *et al.* 2014). *Saltol*, an important QTL, for seedling stage salinity tolerance, has also been analyzed in backcross population to dissect the QTL region (Alam *et al.* 2011). The present study was essential to analyse the backcross lines before further backcrossing to ensure that the advance introgression lines (AILs) have all the important QTLs for both seedling and reproductive stage salt tolerance among the segregating BC_1 families. The backcross families was mapped for high salinity tolerance at seedling stage with 150 mM of salt, while moderate salt tolerance at reproductive stage with EC ~ 6.0 165 dS/m (unpublished and communicated data). Among the 74 BC_1F_2 families, 36 were found phenotypically highly tolerant at the seedling stage. These 36 families were analyzed for the presence of mapped QTLs responsible for salinity tolerance at both the stages and are discussed below. Our aim was to identify the backcross lines having QTLs for salinity tolerance for both the seedling as well as reproductive stages from the desired source NKSWR 173. The QTLs for seedling stage; *qSES1.1* and *qSES 3.2* were having high tolerance, whereas those for reproductive stage; *qSTY11.1* was having moderate tolerance to salinity (unpublished and communicated data) (Table 1). Therefore, backcross lines were phenotypically screened for salinity tolerance at seedling stage and tolerant lines were genotypicaly screened for the presence of QTLs for both the stages.

Presence of QTLs in the backcross lines for seedling stage salinity tolerance: Thirty-six BC_1F_1families with seedling stage salinity tolerance were analyzed for the presence of peak as well as flanking markers for the QTL for seedlings stage tolerance. Fifteen families (SN 15, 19, 32, 33, 36, 37, 39, 43, 45, 49, 51, 52, 53, 55 and 75) were identified which were having QTLs from the donor parent NKSWR173 based on peak markers only. However, among these families, ten families (SN 15, 32, 36, 37, 39, 43, 49, 51, 52, & 53) were identified based on flanking markers also (Fig 1). These lines ensured the complete transfer of QTL loci from donor parent.

Reproductive stage salinity tolerance in the backcross lines: The presence of *qSTY11.1* QTL for reproductive

Table 1 Peak and flanking markers for the seedling and reproductive stage salinity tolerance QTLs used for introgression from wild rice accession NKSWR 173 in mega variety of rice IR 64.

Trait	QTL	Peak SNP Marker	Peak position (cM)	Flanking Markers	Marker interval (cM)	Source
QTL for seedling stage salinity tolerance	*qSES1.1*	CSCWR-Os01g48720	123.67	SCR100-Os01g47350 - SCR200-Os01g49670	116.4 - 126.6	NKSWR 173
	qSES3.2	CSCWR-Os03g07870	30.23	SCR100-Os03g07480 - CSCWR-Os03g08999	25.3 - 33.3	NKSWR 173
QTL for reproductive stage salinity tolerance	*qSTY11.1*	SCR200-Os11g32720	44.66	SCR200-Os11g32360 -SCR200-Os11g34150	40.1 - 46.5	NKSWR 173

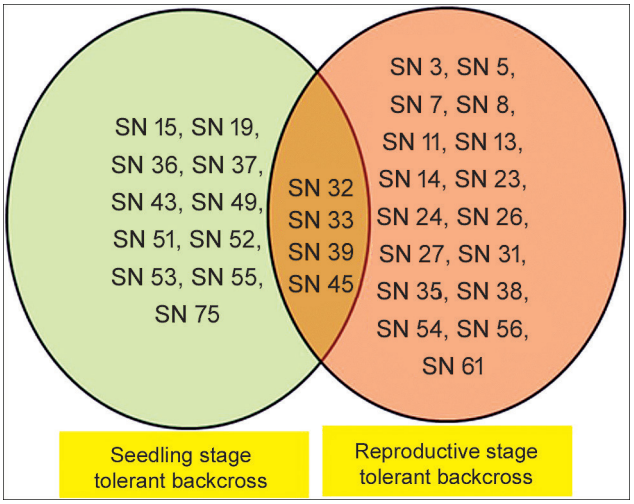

Fig 1 Distribution of BC_1F_1 individual's selected for both the seedling as well as reproductive stage salinity tolerance.

families with seedling and reproductive stage salinity tolerance were analyzed for recurrent parent genome recovery using genotyping data from 50K SNP chip. The recurrent parent genome recovery among backcross lines were ranged from 34.3–98.9%. The recurrent parent genome recovery among salinity tolerant lines at seedling stage was in the range of 34.3–58.6 % (Fig 2). That indicates the need of additional backcrosses in order to get the desired recovery percentage.

Agronomic performance of the BC_1 introgression line hybrids: The agronomic performance of different lines along with their parents were analyzed using Un-weighted Pair-Group Method with an Arithmetic average (UPGMA) clustering analysis for the agronomically important traits were; panicle length, number of branches per panicle, number of tillers per plant and test weight. Two major clusters were obtained in which two parents were separately placed (Fig 3). Cluster I (CI) was formed with the recurrent

Fig 2 Distribution of donor and recipient genome segments in selected BC_1F_1 plants based on background genome analysis. Green = Recipient parent; Red = Donor parent; Pink = Heterozygous. RPG (%) = Recovery percentage of Recipient parent genome.

stage salinity tolerance (grain yield under salt stress) was analyzed in all the thirty-six backcross lines that were phenotypically tolerant at seedling stage. The source of *qSTY11.1* was NKSWR 173. Among backcross families, twenty one families (SN 3, 5, 7, 8, 11. 13, 14, 23, 24, 26, 27, 31, 32, 33, 35, 38, 39, 45, 54, 56 and 61) were identified which were having QTLs based on peak markers. Among these, seventeen backcross lines (SN 5, 7, 8, 13, 14, 23, 24, 26, 27, 32, 33, 35, 38, 39, 45, 54, and 56) having flanking markers genotype also. However, four backcross lines (SN 32, 33, 39 and 45) were found to have all the QTLs from the donor NKSWR 173 that was responsible for salinity tolerance at both the stages (Fig 1).

Recipient parent genome recovery in the backcross lines: Selected BC_1F_1

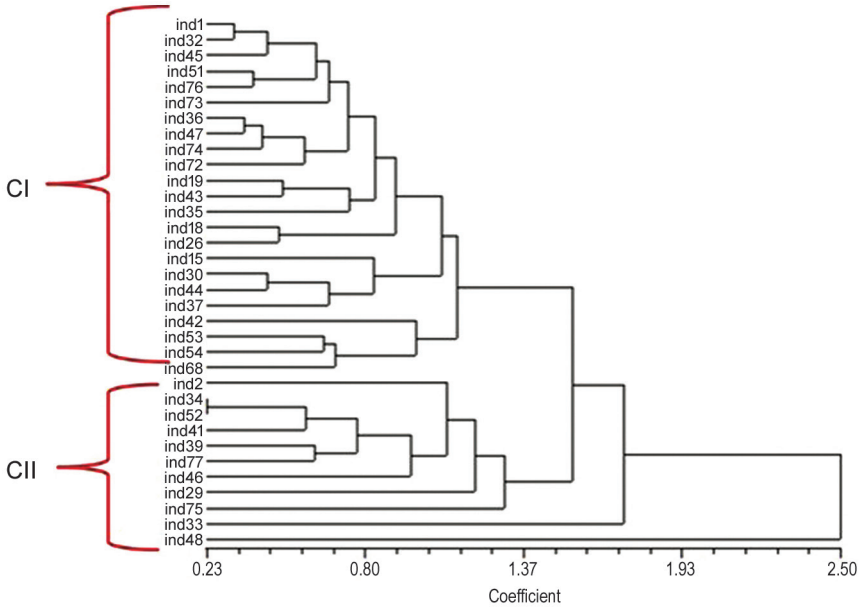

Fig 3 Cluster analysis of all the tolerance BC1F1 families and their parents based on agronomically important traits; Ind 1(IR 64) & Ind 2 (NKSWR 173).

parent, *i.e.* IR 64 (Ind I) and 22 BC_1F_1 individuals those are arranged in different sub cluster and sub-sub cluster based on similarity coefficient. Cluster II (CII) was formed with the donor parent, *i.e.* NKSWR 173 (Ind II) and eight BC_1F_1 individuals. In cluster II donor parent formed a separate sub cluster while other eight individuals formed a separate sub cluster. Ind 33 and Ind 48 formed a separate out group from both these major cluster are showing their extreme phenotypes from parents. It was observed that most of the tolerant individuals of BC_1F_1 families were close to recurrent parent based on yield related traits. Selected salt tolerance lines for both the stages (SN 32, 33, 39 and 45) were also clustered and it was observed that SN 32 and 45 were too closed to IR 64, while SN 39 was highly closed to NKSWR 173. SN 33 was not showed any similarity with either parent, placed them in the out group that showed their extreme phenotypes.

The study was aimed at identifying BC_1 having both seedling stage and reproductive stage salt tolerance based on phenotypic performance as well as QTL peak and flanking markers, so that it can be used for further backcrossing. Among backcross families, ten families were salinity tolerance at the seedling stage, while seventeen were tolerant for the reproductive stage based on peak and flanking markers for QTL. Four backcross families; SN 32, 33, 39, and SN 45 were found tolerant for both the stages with all the QTLs. These lines will be grown to produce BC_2F_2 families in a similar way to identify advance introgression lines with all the QTLs with maximum RPG genome recovery.

ACKNOWLEDGEMENTS

We are grateful for the financial support of ICAR National Professor BP Pal Chair project, ICAR Borlaug Award Project for NKS and CSIR-UGC Fellowship support to SK.

REFERENCES

Alam R, Rahman M S, Seraj Z I, Thomson M J, Ismail A M, Ellen T R and Gregorio G B. 2011. Investigation of seedling-stage salinity tolerance QTLs using backcross lines derived from *Oryza sativa* L. Pokkali. *Plant Breed* **130**: 430–37.

Berloo R V. 2008. GGT 2.0: Versatile software for visualization and analysis of genetic data. *The Journal of heredity* **99**(2): 232–36.

Gaikwad K B, Singh N, Bhatia D, Kaur R, Bains N S, Bharaj T S and Singh K. 2014. Yield-enhancing heterotic QTL transferred from wild species to cultivated rice *Oryza sativa* L. *PLoS One* **9**(6): e96939.

Gregorio G B, Senadhira D and Mendoza R D. 1997. Screening rice for salinity tolerance. International Rice Research Institute discussion paper series, Metro Manila.

Jacquemin J, Bhatia D, Singh K and Wing R A. 2013. The international *Oryza* Map alignment project: Development of a genus-wide comparative genomics platform to help solve the 9 billion-people question. *Current Opinion in Plant Biology* **16**: 1–10.

Krishnamurthy S L, Pundir P, Warraich A S, Rathor S, Lokeshkumar B M, Singh N K and Sharma P C. 2020. Introgressed saltol QTL lines improves the salinity tolerance in rice at seedling stage. *Frontiers in Plant Science* **11**: 833.

Mishra S, Singh B, Panda K, Singh B K, Singh N, Misra P, Rai V and Singh N K. 2016. Association of SNP haplotypes of HKT family genes with salt tolerance in Indian wild rice germplasm. *Rice* **9**: 15.

Mondal U, Khanom M S R, Hassan L and Begum S N. 2013. Foreground selection through SSRs markers for the development of salt tolerant rice variety. *Journal of Bangladesh Agricultural University* **11**(1): 67–72.

Puram V R R, Ontoy J, Linscombe S and Subudhi P K. 2017. Genetic dissection of seedling stage salinity tolerance in rice using introgression lines of a salt tolerant landrace Nona Bokra. *Journal of Heredity* **108**(6): 658–70.

Radanielson A M, Gaydon D S, Li T, Angeles O and Roth C H. 2018. Modeling salinity effect on rice growth and grain yield with *Oryza* v3 and APSIM-*Oryza. European Journal of Agronomy* **100**: 44–55.

Singh B, Singh N, Mishra S, Tripathy K, Singh B P, Rai V, Singh A K and Singh N K. 2018. Morphological and molecular data reveal three distinct populations of Indian wild rice *Oryza rufipogon* Griff.Species Complex. *Frontiers in Plant Science* **9**: 123.

Singh, D, Kumar A and Kumar A S. 2011. Marker assisted selection and crop management for salt tolerance: a review. *African Journal Biotechnology* **10**(66): 14694–98.

Singh N K, Singh B, Mishra S, Singh N, Panda K and Rai V. 2016. Indian Wild Rice: diversity, population structure, trait value and relation with cultivated rice. *Indian Journal of Plant Genetics Resources* **29**(3): 366–68.

Swamy B P M, Kaladhar K, Reddy G A, Viraktamath B C and Sarla N. 2014. Mapping and introgression of QTL for yield and related traits in two backcross populations derived from *Oryza sativa* cv. Swarna and two accessions of *O. nivara. Journal of Genetics* **93**(3): 643–54.

Tanksley S D and Nelson J C. 1996. Advanced Backcross QTL Analysis: a method for the simultaneous discovery and transfer of valuable QTLs from unadapted germplasm into elite breeding lines. *Theoretical and Applied Genetics* **92**: 191–203.

Tanksley S, Grandillo S, Fulton T M, Zamir D, Eshed Y, Petiard V, Lopez J and Beck-Bunn T. 1996. Advanced backcross QTL analysis in a cross between an elite processing line of tomato and its wild relative *L. pimpinellifolium*. *Theoretical and Applied Genetics* **92**: 213–24.

Tripathy K, Singh B, Singh N, Rai V, Mishra G and Singh N K. 2018. A database of wild rice germplasm of *Oryza rufipogon* species complex from different agro-climatic zones of India. *Database* 1–6.

Turan S, Cornish K and Kumar S. 2012. Salinity tolerance in plants: Breeding and genetic engineering. *Australian Journal of Crop Science* **6**(9): 1337–48.