Molecular Characterization And Varietal Identification For Multiple Abiotic Stress Tolerance In Rice (*Oryza sativa* L.)

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Abstract

Coexistence of two or more abiotic stresses is common in most of the rainfed lowland and upland rice growing areas of India and worldwide. Rice production under these conditions is not sustainable. Identification and development of multiple abiotic stress tolerant rice varieties are to be addressed. Here we tried to identify multiple abiotic stress tolerant varieties from a collection of earlier identified varieties for single stress and validated the known SSR markers for stress tolerance. Twenty rice genotypes were evaluated for individual abiotic stress such as drought, salinity and temperature initially and the tolerant three genotypes in each case were further evaluated for combination of stresses various physio-morphological and biochemical parameters were recorded . Among the genotypes evaluated for combination of stresses, PTB-7 was found to be tolerant for drought and salinity, Nagina-22 was tolerant against temperature and salinity. However, the seeds did not germinate in the presence of all three stresses simultaneously.. Twenty rice varieties viz ., Chomala, MO-16, PTB-35, PTB-60, PTB-39, PTB-55, PTB-30, PTB-7, CRdhan307, Apo, Vyttila-3, Vyttila-4, Vyttila-5, Vyttila-6, Vyttila-7, Vyttila-8, Vyttila-9, Vyttila-10, Nagina-22, and NL-44 were further investigated using microsatellite markers to confirm the genotypic level of tolerance to combination of abiotic stresses. Rice genotypes were screened using 30 reported simple sequence repeat (SSR) markers that are linked to drought, salinity and temperature. Molecular marker analysis of rice genotypes also confirmed that RM8904 and RM1287 were associated with salinity tolerance, RM2612, RM6100 and RM5749 were linked to high temperature tolerant trait. Population analysis also revealed that there is five subpopulation among rice genotypes.

Introduction

Rice (*Oryza sativa* L.), a semi aquatic plant with an evolutionary advantage of growing under diverse water regimes (De Datta and Mikkelsen, 1985) starting from flooded conditions to dry land. Globally, the crop is cultivated in about 160.8 million hectares with annual production of more than 725.5 million tons of paddy (USDA, 2019). The food production needs to be increased even from the drought-prone areas with an increase of 40% from the difficult ecosystem to meet the targeted food production by 2025 (Pennisi, 2008). The irrigated and the rainfed rice ecosystem, which forms the major mainstay of food security in Asia has been highly sustainable with the environment having few adverse impacts. Increase in rice production should come from highly vulnerable, less productive drought-prone rainfed lowland and upland rice areas because there is limited scope for expanding irrigated rice area (Khush, 2007). These areas neglected during green revolution period and concentrated on input responsive high yielding varieties. These areas are affected by different types of abiotic stresses like heat, drought, cold, salinity and induce severe cellular damage in plant species, including rice plants. There is a cross talk between abiotic stresses either individually or in combination, they cause morphological, physiological, biochemical, and molecular changes that adversely affect plant growth and productivity, and ultimately yield. (Bitra and Geras, 2013). Abiotic stress like drought, salinity, and low temperature manifest their effect through an osmotic stress, ultimately leading loss in turgor, disorganized membranes and denatured proteins triggering a cascade of events (Krasensky and Jonak, 2012). In addition, increased

salinity of arable land is expected to have devastating global effects, resulting in up to 50% land loss by the middle of twenty-first century.

In rice two reproductive development stages, anthesis and microsporogenesis are very sensitive to high (>33°C) and low (15°C) temperatures (Jagadish *et al.*, 2015). Extreme temperatures affect anther dehiscence, pollination, and pollen germination, leading to spikelet sterility. Water deficit or drought stress has similar effects and exacerbates the problem by reducing transpirational cooling and thereby increasing canopy and tissue temperature (Sheshshayee *et al.*, 2011). Excess salt in soil adversely affects plant growth, development, and productivity when osmotic stress reduces water uptake by roots (Munns and Tester 2008).

Adaptation mechanisms to these environmental cues in plants by appropriate physiological, developmental and biochemical changes through elaborate mechanisms of signal perception, transduction manifest in adaptive responses (Alcazar *et al.*, 2003). However, tolerance or susceptibility to the abiotic stresses is a very complex phenomenon, in part because stress may occur at multiple stages of plant development and often multiple stresses simultaneously affect the plants during their development. Abiotic stress tolerance is quantitative in nature and governed by multiple loci by many genes; therefore, adapting to variable environmental stress is a highly intricate trait (Shinozaki and Shinozaki). Plants survival under environmental stresses depends on the severity of stress and the genetic background of the plant, even though plants constantly adapting these conditions by changing the physiology and metabolism (Pastori and Foyer, 2002). In addition, osmotic adjustment mechanism and redox metabolism help the plants to alter metabolism in such a way that protect plants from severity of abiotic stresses (Bartels and Sunkar, 2005; Valliyodan and Nguyen, 2006; Munns and Tester, 2008). At the molecular level, gene expression is modified upon stress (Chinnusamy *et al.*, 2007) and epigenetic regulation plays an important role in the regulation of gene expression in response to environmental stress (Hauser *et al.*, 2011; Khraiweh *et al.*, 2011).

Earlier studies mainly focused on the effect of individual stress on morpho-physiological, biochemical, molecular and yield parameters in rice (Beena *et al.*, 2012; Silvas *et al.*, 2015; Naresh *et al.*, 2018; Beena *et al.*, 2018a, Beena *et al.*, 2018c). When abiotic stresses happen concomitantly in field condition, tolerant varieties identified of single stress need not necessarily tolerate the combined effect of stresses (Atkinson *et al.* 2013). Hence identification of multiple stress tolerant rice varieties is needed for climatic extremities with increasing population (Khush, 2005). Molecular markers, especially DNA-based markers, have been used extensively for the study of genetic diversity, unambiguous identification of germplasm and their protection. Exploitation of rice germplasm and identification of specific molecular markers linked to different abiotic stresses is highly critical to ensuring sustainable rice production and global food security in the ever-changing climatic conditions. Molecular marker technology is helpful in unraveling the genetic basis of complex traits within rice germplasm to identify major genes/QTLs for use in rice breeding (Beena, 2005; Beena, 2011; Swamy and Kumar, 2013; Swamy *et al.* 2014). With this background this study was undertaken to identify multiple abiotic stress tolerant rice varieties and to validate the reported SSR markers for abiotic stress tolerance.

Material And Methods

Plant material and Germination Assay:

A laboratory experiment was conducted at the Department of Plant Physiology, College of Agriculture, Vellayani, Kerala Agricultural University during 2017-2019. Twenty rice (*Oryza sativa* L.) varieties composed of drought tolerant (Chomala, PTB-60 PTB-55, PTB-30, PTB-7, CR Dhan307, Apo), salinity tolerant (Vyttila-3, Vyttila-4, Vyttila-5, Vyttila-6, Vyttila-7, Vyttila-8, Vyttila-9, Vyttila-10), high temperature tolerant (Nagina-22, NL-44) and three high yielding varieties (PTB-35, PTB-39 and MO-16) were used in this experiment. Seeds of different varieties were collected from Regional Agricultural Research Station, Pattambi and Rice Research Station, Vyttila, Kerala Agricultural University and NRRI, Cuttack, Odisha. Three independent lab studies were conducted for evaluating drought, salinity and high temperature tolerance. Seeds of each varieties were surface sterilized with 70% ethanol solution for 5 minutes. The seeds were then washed three times with sterilized distilled water. Germination assays were performed by paper towel method using 10 seeds per each towel. Each set of paper towel was moistened with 20 ml distilled water or uniform amounts of desired osmotic solutions to mimic drought stress and salinity.

Tolerance evaluation against individual stresses:

Screening of rice genotypes for individual stress tolerance using paper towel method: In this part of study, drought stress was artificially induced by desired strengths of polyethylene glycol 6000 (PEG- 6000; Sigma Chemicals). Polyethylene glycol has been used to simulate water stress effects in plants (Swapna and Shylaraj, 2017). The experiment was laid out in a complete randomized design (CRD) with four levels of drought stress and control with three replications. Distilled water was used as a control (0 MPa) and osmotic potentials -1.0, -3.0, -5.0 and -7.0 bars were created by adding PEG-6000 @ 5, 10, 15 and 20 g per 100 ml distilled water. Four replicates of 50 seeds of each osmotic potential were used to assess the germination percentage. This experiment was carried out in growth chamber at $25\pm0.5^{\circ}\text{C}$ and $80\%\pm1$ of relative humidity.

Salinity stress was artificially induced by desired strengths of NaCl (High Media). The experiment was laid out in a complete randomized design (CRD) with four levels of salinity stress and control with three replications 50 seeds each. Distilled water was used as a control (0 mM NaCl) and osmotic potentials of 100mM NaCl, 150mM NaCl, 200mM NaCl, 250mM NaCl were created by adding @ 5, 10, 15 and 20 g per 100 ml distilled water. This experiment was carried out in growth chamber at $25\pm0.5^{\circ}\text{C}$ and $80\%\pm1$ of relative humidity.

High temperature was induced by keeping the moistened seeds in petri plates covered with two layers of moistened filter papers and kept in incubators at various temperatures 35°C , 40°C , 45°C , 50°C and $80\%\pm1$ of relative humidity. The experiment was laid out in a complete randomized design (CRD) with four levels of high temperature stress and control with three replications of 50 seeds each. From these three independent experiments, the number of germinated seed was recorded at 24 hours interval. The seedling height and seedling dry weights were measured on the 14th day. Seeds were considered germinated when

both plumule and radicle extended to more than 2 mm from the seeds. Seedling vigour index was calculated from germination percentage and seedling height (root length + shoot length). Physiological traits like proline content as per the protocol by Bates *et al.* (1973) for drought, Na^+/K^+ ratio for salinity) by Zasoski and Buraum (1977), cell membrane stability trait by Blum and Ebercon (1981) for high temperature were studied on 14th day of germination.

Further experiment was carried out by selecting the genotypes that are capable of germinating under individual stress at possible highest extent. These selected nine genotypes were evaluated for combination of stresses.

Screening of rice genotypes for combination of stresses using paper towel method:

In this experiment, seeds were exposed to combinations of above mentioned stresses i.e., $D_h \times S_h$, $D_h \times T_h$, $T_h \times S_h$ and $D_h \times S_h \times T_h$. Rice seeds failed to germinate when a combination of all the stress ($D_h \times S_h \times T_h$) and combination of water and high temperature stress ($D_h \times S_h$) was given.

Validation of rice genotypes using microsatellite (SSR) markers:

In this experiment, all the twenty rice genotypes were screened with reported SSR markers for various traits. The documented SSR profiles were carefully examined for the polymorphism in banding pattern between the genotypes. All the 20 genotypes were analyzed for the identification of reported markers linked to stress tolerance such as drought, salinity and temperature. Reported microsatellite markers linked to drought, salinity and temperature were used to screen 20 rice varieties in order to analyze the presence of QTLs linked to stress tolerance in varieties which were selected as multiple abiotic stress tolerant based on phenotypic parameters. The sequence of RM primers used for screening is presented in Table 9.

SSR genotyping: Total genomic DNA of 20 rice genotypes was extracted from young leaves at 15 days old plants. The leaves were excised from each genotype, washed with distilled water to remove dust and any other foreign particles. The leaf samples were placed in small plastic bags individually and kept in container having cool environment, and carried immediately to the laboratory. The leaf sample then stored at -80°C until used for DNA extraction. Total genomic DNA was extracted using the method suggested by Dellaporta *et al.* (1983). The quantity and quality of DNA of each sample was determined by reading the absorbance at 260nm and 280nm in a spectrophotometer (ELICO, SL 21 UV-Vis spectrophotometer). The ratio between the readings at 260nm and 280nm (A_{260}/A_{280}) was used as an estimate of the purity of the DNA samples. Pure preparations of DNA have A_{260}/A_{280} ratio between 1.7 and 1.8 (Sambrook and Russell, 2000). Quality was assessed by using gel electrophoresis with 5µl of crude DNA sample on agarose gel (0.8%). stained with ethidium bromide 0.5 µg/ml DNA. A total of 30 SSR markers were used, out of which nine markers were found to be polymorphic.

The PCR conditions (annealing temperature etc.) for SSRs were optimized through gradient PCR. A total of 20 µl final volume of PCR reaction was made. The DNA concentration for each reaction was 25ng/µl

DNA, 10mM dNTPs, 1 µl forward and reverse primer each, 1 unit of TaqDNA polymerase with 10X reaction buffer. The thermal profile starts with initial denaturation at 94°C for 3 min continued to 35 cycles of denaturation at 94°C for 1 minute, primer annealing 55°C for 1 min, extension 72°C for 1 min and final extension at 72°C for 5 min. After completion of amplification, PCR products were kept in a -20°C deep freeze. An aliquot of 10 µl PCR amplification products were loaded in an agarose gel of 3.5% containing 0.08 µg/ml of ethidium bromide for electrophoresis. The electrophoresis was carried out at 80 volts (2.5 V/cm) in 1X TBE (pH 8.0). Sizes of amplicons were determined by using 100bp DNA ladder. The gel was visualized under UV (312nm) transilluminator and documented in gel documentation system (Syngene G-box documentation system). The documented SSR profiles were carefully examined for the polymorphism in banding pattern between the genotypes.

Population Structure: Population structure of 20 rice genotypes was estimated using a STRUCTURE software V2.3.4 based on Bayesian clustering algorithm (Pritchard *et al*, 2000). In order to conclude the optimum number of subpopulations, values for K= 2 to K = 8 were given using a burn length of 50,000 and run length of 50,000. Five independent runs yielded the reproducible results. The results were imported to STRUCTUREHARVESTER software to calculate exact value of 1K (Earl and Vonholdt, 2012).

Statistical analysis:

The overall effects of treatment and rice genotypes and their interaction were analysed by means of two-way ANOVA with different stress levels and genotypes were taken as fixed factors. Genotypes were treated as fixed factors because we were interested in the response of the specific genotypes used in this experiment. Design of experiment was CRD with treatment levels. Twenty rice genotypes were analysed with three replications each for the treatment levels. The statistical analysis were done using OPSTAT software (Two factorial CRD). Means were separated by least significant difference (LSD).

Results

Screening of rice genotypes for individual stress tolerance using paper towel method:

Evaluation of rice genotypes for seedling vigour index and proline content under varied drought stress condition:

There was significant difference for seedling vigour index among treatments and genotypes under diverse drought induction (Table 1). Among the treatments, control recorded the highest seedling viogur index of 2447. This treatment was followed by the treatment -1bar PEG6000 with an average seedling vigour index of 1746. None of the genotypes were germinated under -7bar PEG6000. Thus it was identified that -5barPEG6000 was the highest tolerating level of drought stress.

Genotypes response to individual stress varied greatly. At the highest tolerating level of drought stress (-5barPEG6000), maximum seedling vigour index was recorded by PTB-7 (998.0) followed by PTB-60 (385.8) and PTB-35 (346.8), whereas 15 genotypes were not germinated under this condition (Table 1). At

-3barPEG6000, highest seedling vigour index was recorded by PTB-7(1904), followed by PTB60 (1682) and PTB35 (1622). At -1bar PEG6000, highest seedling vigour index was recorded by PTB35 (2660), followed by CR Dhan307 (2366), PTB60 (2208), Chomala (2095). There was no significant difference among the genotypes for seedling vigour index under control condition.

There was significant difference for proline content among treatments and genotypes for diverse drought stress condition (Table 2). Among the treatments, -5barPEG 6000 recorded the highest average proline content of 37.87µg/g tissue. Lowest proline content was recorded in control condition with an average of 16.82 µg/g tissue.

At the highest tolerating level of drought stress (-5barPEG6000), highest proline content was recorded by PTB-35 (42.55 µg/g tissue). This genotype was on par with PTB7 (42.23 µg/g tissue) (Table 2). At -3barPEG6000, highest proline content was recorded by PTB-7(37.75 µg/g tissue). This genotype was on par with PTB35 (37.28 µg/g tissue). At -1bar PEG6000, highest proline µg/g tissue content was recorded by PTB7 (36.42 µg/g tissue), followed by PTB60 (35.19 µg/g tissue). This genotype was on par with PTB35 (35.10 µg/g tissue). Under control condition, highest proline content was recorded by PTB30 (17.28 µg/g tissue). This was followed by Vyttila 4(16.82 µg/g tissue). This genotype was on par with PTB55 (15.8 µg/g tissue).

Evaluation of rice genotypes for seedling vigour index and Na^+/K^+ ratio under varied salinity stress condition:

There was significant difference for seedling vigour index among treatments and genotypes under diverse salinity condition (Table 3). Among the treatments, control recorded the highest seedling vigour index of 3017. This treatment was followed by the treatment 100mM NaCl with an average seedling vigour index of 2502. There was a gradual reduction in seedling vigour from 100 mM NaCl (2502) to 250 mM NaCl (257.9).

At the highest tolerating level of salinity stress (250mMNaCl), highest seedling vigour index was recorded by MO-16 (870). This genotype was on par with Vyttila 3(822) and Vyttila 9(790). Chomala and Vyttila 4 were not germinated under 250 mM NaCl (Table 3). At 200mMNaCl, highest seedling vigour index was recorded by Vyttila9 (1674).This genotype was followed by MO-16(1272). At 150 mMNaCl, Vyttila 9 recorded the highest seedling vigour index of 2276. This genotype was followed by MO-16(1737). At 100 mM NaCl, Vyttila 3 recorded the highest seedling vigour index of 3055. This genotype was followed by Nagina-22 (2846). There was no significant difference among the genotypes for seedling vigour index under control condition.

There was significant difference in Na^+/K^+ ratio among the treatments and genotypes for diverse salinity stress condition (Table 4). Among the treatments, 250 mM NaCl recorded the highest average Na^+/K^+ ratio of 5.53. Lowest Na^+/K^+ ratio was recorded in control condition with an average of 0.588.

At the highest tolerating level of salinity stress (250mMNaCl), highest Na^+/K^+ ratio was recorded by PTB-55 (9.5). Lowest Na^+/K^+ ratio was recorded by Vyttila 6 (2.83). This was followed by Nagina-22 and NL-44. Under 200 mM NaCl level, lowest Na^+/K^+ ratio was recorded by Nagina -22(1). This was followed by NL-44(1.16). This genotype was on par with Vyttila -8(1.25). At 150 mM NaCl, lowest Na^+/K^+ ratio was recorded by Vyttila 10(0.87). There is no significant difference among the genotypes for Na^+/K^+ ratio under both 100 mM NaCl and control condition.

Evaluation of rice genotypes for seedling vigour index and cell membrane stability index under high temperature condition:

Seeds were not germinated at temperature 40°C, 45°C and 50°C. There was significant difference between the treatments. Highest seedling vigour index was recorded under control condition (1797) compared to the treatment-35°C (1049). There was significant difference among the genotypes for seedling vigour index under both treatment (35°C) and control condition. Under high temperature condition, Vyttila -3 and Vyttila-5 were not germinated (Table 5). Under both control and high temperature condition, Nagina-22 recorded the highest seedling vigour index of (2807), (2584) respectively. Under high temperature condition Nagina-22 recorded the highest cell membrane stability index of 99.02% (Table 6). This was followed by Vyttila-10, Vyttila-8 and Vyttila-6 and these genotypes were on par with each other.

Screening of rice genotypes for combination of stresses using paper towel method:

Among 20 genotypes, nine genotypes were selected for combination of stresses, of which three are tolerant to high level of salinity stress (Uma, Vyttila-3 and Vyttila-9), three for highest level of drought stress (PTB-7, PTB-60 and PTB-35) and three from highest tolerated temperature stress (Nagina-22, NL-44 and Vyttila-6). The selected genotypes from previously mentioned highest tolerated level of individual stress were subjected to combination of stresses. At highest tolerated level of drought (D_h) and highest tolerated level of salinity stress (S_h) PTB-7 (1615) recorded the highest seedling vigour index, followed by Vyttila-9 (293.6) and the least seedling vigour index was observed in PTB-35 (209.2) (Table 7). At highest tolerated level of Temperature (T_h) and highest tolerated level of salinity (S_h), highest seedling vigour index was recorded by Nagina-22(3.467). This is followed by NL-44 (1.067) and MO-16(1.00) which are on par with each other (Table 8).

Validation of Microsatellite Markers:

Screening of rice genotypes with 30 microsatellite markers revealed that, out of thirty microsatellite markers, eight markers were found to be polymorphic. RM8904 was associated with salinity tolerant trait. In this study, RM8904 showed clear polymorphism between salinity tolerant and susceptible genotypes (Plate 1). This marker clearly separated the genotypes Vyttila-3, Vyttila-4, Vyttila-5, Vyttila-6, Vyttila-7, Vyttila-8, Vyttila-9, Vyttila-10 and Nagina-22 from other genotypes approximately with a band size of 200bp. RM26212 and RM6100 were identified for high temperature tolerant trait in rice. In this study, RM26212 polymorphic between Nagina-22 and other genotypes with a band of size ~ 180bp (Plate 2).

However, RM6100 clearly separated Nagina-22 and NL-44 from other varieties with approximate band size of 150bp (Plate 3). RM1287 was reported for salinity tolerant traits in rice. In this study, RM1287 showed distinct polymorphism PTB-7, Vyttila-3, Vyttila-4, Vyttila-5, Vyttila-6, Vyttila-7, Nagina-22 from other genotypes with appropriate band size of 150 – 220bp (Plate 4). RM5749 was reported for high temperature tolerant trait in rice. This marker showed clear and distinct polymorphism for Nagina-22 and NL-44 from other genotypes with approximate band size of 170bp (Plate 5).

Polymorphism information content: PIC of markers ranged from 0 to 0.69. The primer which showed the highest PIC was RM 26212 (0.69) followed by RM 5749 (0.67).

Population Structure Analysis:

STRUCTURE software was used to analyze genetic structure of different populations and it was run for $K=8$ based on SSR markers banding pattern. K is the number of significant populations in each main group. Results were analyzed using Evanno's method implemented in Structure HARVESTER. The results showed that the maximum delta K was detected at $K=5$ (Fig: 1) therefore the number of sub-populations as obtained from the STRUCTURE analysis of the rice genotypes was observed to be five. The grouping of rice genotypes based on sub-populations is shown in Plate 6.

Five subpopulations obtained by STRUCTURE analysis were, SP1 consists of only one variety NL-44. SP2 consists of two varieties, Nagina-22 and Ptb-7. SP3 consists of varieties, Chomala, PTB-35, PTB-39, Vyttila-3, Vyttila -4, Vyttila-5, CR Dhan 307 and MO-16. SP4 consist of varieties- PTB-60, Vyttila 10, PTB-55, PTB-30 and Vyttila-6. SP5 consist of varieties Vyttila – 9, Vytilla-8, Apo and Vyttila-5 (Plate 7).

Discussion

Exposure of plants to various environmental stresses result in general and specific effects on plant growth and development, reduced productivity, and extensive crop losses worldwide. Drought and salinity together affect more than 10% of arable land, leading to more than 50% decline in the average yields of the major crops of the world (Roychoudhury *et al.*, 2013). Drought-induced crop losses have significant economic impact, which is predicted to increase with global climate change (Marris, 2008; Battisti and Naylor, 2009). High temperature is also flattering rice productivity to a great extent (Beena, 2013). Abiotic stress tolerance is governed by multiple loci and thus is multigenic in nature; therefore, adapting to variable environmental cues is a highly complex phenomenon (Yamaguchi-Shinozaki, K. and Shinozaki, 2006).

Ali *et al.*, (2017) stated that, low productivity of rice in rain fed areas is mostly correlated with decline in yield due to multiple abiotic stresses. Breeding programs are formulated so as to mitigate the effect of any single stress, whereas in field condition multiple stresses occur simultaneously. This provides a platform to work on and to exploit the tolerance level of rice genotypes to multiple abiotic stresses and to validate the molecular markers reported for various abiotic stresses in rice.

Screening of rice genotypes for individual stress tolerance using paper towel method:

In this study, there was significant variation for seedling vigour index and proline content among different levels of drought stress treatments. Seeds were not germinated under -7barPEG6000 and thus -5bar PEG 6000 was identified as the highest tolerating level of drought stress. Under this condition and less range of drought stress also, PTB-7, PTB-60 and PTB-35 were showed higher seedling vigour index. Similar study was conducted by Panda *et al.*, (2019) and reported that among the folk rice genotypes of Odhisha, Nagina-22 showed highest seedling vigour index.

Among the treatments, -5barPEG 6000 recorded the highest average proline content. Lowest proline content was recorded in control condition. Same genotypes recorded the highest proline content under various levels of drought stress. Hsu *et al.*, (2003) reported that drought stress induction by PEG results in proline production in rice and there is 25 fold increase in proline for every 12h under stress. Beena *et al.*, (2012) also reported an increase in proline (89.6%) across the RILs of IR20 x Nootripathu as compared to control. Islam *et al.*, (2018) studied the drought tolerance of 18 genotypes using 0, 5, 10, 15 and 20% PEG6000 and reported that seed germination and relative growth of seedlings decreased under high concentration.

At highest level of salinity stress, 250mM NaCl, MO-16, Vyttila-3 and Vyttila-9 exhibited the highest seedling vigour index. There is gradual reduction in seedling vigour index from control to 250mM NaCl. Zhang *et al.* (2007) reported a similar kind of findings in lucerne crop under salinity stress. High levels of sodium adversely influence the acquisition of pottasium (Munns *et al.*, 2010). There is significant difference in Na^+/K^+ ratio among the treatments and genotypes for diverse salinity stress condition. Among the treatments, 250mMNaCl recorded the highest average Na^+/K^+ ratio of 5.53. Lowest Na^+/K^+ ratio was recorded in control condition with an average of 0.588. Vytilla-6, Nagina-22 and NL-44 recorded lowest Na^+/K^+ ratio, so as to reduce sensitivity of the plants to salinity is due to the failure to prevent Na^+ and Cl^- from transpiration streams (Gorham *et al.*, 1990). Similar results were reported by Bohra and Doerffling, (1993).

In the present study, highest tolerating level of temperature stress was 35⁰C. Seeds were not germinated at different temperature levels of 40°C, 45°C and 50°C. Seedling vigour index was reduced under temperature stress condition. The maximum seedling vigour index was observed in Nagina-22 at 35⁰C. lioh, *et al.* (2014) reported that under temperature regimes seedling vigour index found to be decreasing with increasing temperature in rice, maize and sorghum. Among the varieties, cell membrane stability index was found to be maximum in Nagina-22 under temperature stress condition. Similar results were also reported by Prasad *et al.* (2006) and Beena *et al.*, (2018).

Screening of rice genotypes for combination of stresses using paper towel method:

Highest tolerated level of drought (D_h) and highest tolerated level of salinity stress (S_h) PTB-7 recorded the highest seedling vigour index, followed by Vyttila-9 and the least seedling vigour index was observed

in PTB-35. Combined stresses at their highest tolerable levels resulted in decrease in seedling vigour index, due to reduced water availability for seedling growth and establishment (Babu and Rosaiah, 2017), whereas, at the highest tolerated level of temperature (T_h) and highest tolerated level of salinity (S_h), highest seedling vigour index was recorded by Nagina-22. This is followed by NL-44 and MO-16 which are on par with each other. High temperature stress may cause severe damage to the proteins, disturb their synthesis, inactivate major enzymes and damage membranes. Heat stress could also have major effects on the process of cell divisions (Smertenko *et al.*, 1997). Mokhberdoran *et al.*, (2009) reported that rice cultivar Kalat at seedling stage stated that the deleterious effects of NaCl and PEG were more pronounced with increase in temperature. Metabolic adjustments in response to unfavourable conditions are dynamic and multifaceted and not only depend on the type and strength of the stress, but also on the type of genotypes used for the study.

Simple sequence repeats (SSR) are the DNA markers of choice for rice genetic assessment (*Oryza sativa* L.) owing to their abundance, elevated polymorphism, co-dominance and easy agarose gel electrophoresis assays (Singh *et al.* 2010). In the present investigation microsatellite markers or SSR markers (Simple Sequence Repeats) were used to characterize and to assess abiotic stress tolerance among 20 rice genotypes. Beena, 2011 and Prince *et al.*, 2015 also reported that identification of trait linked molecular markers can improve rice breeding programme. Our results were supported by other studies. RM 6100 is associated with heat tolerance at flowering stage (Bharathkumar *et al.* 2014) and is located in chromosome 10. Buu *et al.* (2014) reported markers RM 7076, RM 3586, 26212 and RM 5749 were polymorphic for heat tolerance. Vu *et al.* 2012 reported SSR markers RM 1287, RM 8094, RM 3412, RM 493 and RM 140 were linked to the *Salto*/QTL on chromosome 1. The microsatellite marker, RM 8094 found in *Salto* is considered as the superior marker for genetic diversity analysis. Rice genotypes with the Pokkali band type for locus RM 8094 marker were either highly tolerant or tolerant to salinity stress at the seedling stage (Nejad *et al.* 2008).

RM8085 was mapped on chromosome 1 and is linked to leaf rolling and leaf drying under drought stress (Salunkhe *et al.* 2011). The QTL on chromosome 9 is associated with spikelet fertility under stress and root and shoot traits (Yue *et al.* 2006). Major QTL on chromosomes 4 (Ctb1) and 8 (qCTB8) for cold tolerance at the booting stage were identified in a tropical japonica cultivar, Silewah, and markers have been used for introducing the tolerance gene (Ctb1) into japonica cultivars (Kuroki *et al.* 2007).

Five subpopulations obtained by STRUCTURE analysis were, SP1 consists of only one variety NL-44. It is NERICA Line from Africa, which is entirely different from *indica* varieties. SP2 consists of two varieties, Nagina-22 and Ptb-7 which have the common traits of drought and high temperature tolerance from previous studies (Beena *et al.*, 2018c; Rejeth *et al.*, 2020). In this study these two varieties showed salinity tolerance also. SP3 consists of varieties, Chomala, Ptb-35, Ptb-39, Vyttila-3, Vyttila -4, Vyttila-5, CR Dhan 307 and MO-16. In this group, except Chomala, all varieties are high yielding. SP4 consist of varieties- Ptb-60, Vyttila 10, Ptb-55, Ptb-30 and Vyttila-6. Among these, PTB60, PTB55 and PTB 30 are drought tolerant and Vyttila 10 and Vyttila 6 are salinity tolerant genotypes. SP5 consist of varieties Vyttila – 9, Vyttila-8, Apo and Vyttila-5. SP4 and SP5 characterized by drought tolerant (Ptb-60, Ptb-55, Ptb-30, Apo)

and saline tolerant varieties (Vyttila-5, 6, 8, 9, 10). This study also reveals the similar tolerance pattern or cross talk between salinity and drought. That may be the reason why saline and drought tolerant genotypes grouped in the same sub-population. Molecular and genomic studies have shown that several genes with various functions are induced by salinity, drought, and cold stresses, and that various transcription factors are involved in the regulation of stress-inducible genes. There are multiple stress perception and signaling pathways, some of which are specific, but others may crosstalk at various steps. Recently, progress has been made in identifying components of signaling pathways involved in salt, drought, and cold stresses (Mishra *et al.*, 2016).

Conclusion

Under field conditions drought and heat stress occur in combination. Simultaneous occurrence of multiple stresses, increase the deleterious effect, such that the effect considerably exceeds the simple additive effects of the action alone. It has been suggested that variability in temperature extremes and water deficit events will be more critical in future climates. The present study based on phenotypic and genotypic analysis, the variety N-22 was chosen the best tolerant variety for the combined stress salinity and temperature. PTB-7 was selected as the most tolerant variety for combined drought and salinity condition. Among the markers, RM6100, RM5749 and RM26212 were validated as markers linked to high temperature tolerance. RM8094 and RM1287 were validated as markers linked to salinity tolerance in rice. These markers can differentiate the tolerant genotypes from susceptible ones. Population structure analysis revealed the genetic relatedness and grouped these varieties into five subgroups. Natural stress tolerance is a very complex process involving numerous metabolites and metabolic pathways. Analyses of metabolic adjustments of plants with different levels of stress tolerance is very important.

Declarations

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Conflict of interest

All authors don't have any conflict of interest regarding this article.

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Tables

Table No 1. Variation in seedling vigour index of rice genotypes under different levels of drought stress

Sl. No.	Variety	-1 bar PEG6000	-3 bar PEG 6000	-5bar PEG6000	Control
1	Chomala	2095 (3.317)ab	0.000 (0.000)h	0.000 (0.000)e	2213 (3.363)
2	MO-16	1921 (3.279)abcd	431.0 (2.621)de	0.000 (0.000)e	2070 (3.341)
3	PTB-35	2660 (3.421)a	1622 (3.209)ab	346.8 (2.541)b	2822 (3.471)
4	PTB-60	2208 (3.337)ab	1682 (3.225)ab	385.8 (2.587)b	2392 (3.402)
5	PTB-39	1240 (3.088)d	232.0 (2.368)f	32.40 (1.500)d	1708 (3.331)
6	PTB-55	1950 (3.287)abc	480.0 (2.683)d	0.000 (0.000)e	2152 (3.366)
7	PTB-30	1312 (3.113)cd	428.0 (2.633)de	124.8 (2.092)c	1819 (3.362)
8	PTB-7	1867 (3.264)abcd	1904 (3.279)a	998.0 (3.000)a	2559 (3.505)
9	CR Dhan307	2366 (3.369)ab	881.0 (2.946)c	0.000 (0.000)e	2464 (3.403)
10	Apo	1647 (3.213)bcd	0.000 (0.000)h	0.000 (0.000)e	1772 (3.272)
11	Vyttila-3	1908 (3.272)abcd	140.0 (2.103)g	0.000 (0.000)e	2344 (3.439)
12	Vyttila-4	1719 (3.228)abcd	296.0 (2.473)ef	0.000 (0.000)e	2563 (3.404)
13	Vyttila-5	1806 (3.251)abcd	397.0 (2.600)de	0.000 (0.000)e	2491 (3.392)
14	Vyttila-6	1797 (3.250)abcd	206.0 (2.317)f	0.000 (0.000)e	2653 (3.420)
15	Vyttila-7	1791 (3.249)abcd	438.0 (2.642)d	0.000 (0.000)e	2380 (3.372)
16	Vyttila-8	1836 (3.261)abcd	0.000 (0.000)h	0.000 (0.000)e	2317 (3.356)
17	Vyttila-9	1821 (3.256)abcd	1166 (3.067)bc	0.000 (0.000)e	2482 (3.390)
18	Vyttila-10	1996 (3.296)abc	1161 (3.065)bc	0.000 (0.000)e	2604 (3.410)
19	Nagina-22	712.2 (2.848)e	0.000 (0.000)h	0.000 (0.000)e	1926 (3.280)
20	NL-44	275.2 (2.434)f	0.000 (0.000)h	0.000 (0.000)e	2073 (3.311)
	Mean	1746.00 (3.202)	674.35 (2.061)	377.56 (0.586)	2447.00 (3.379)
	S.E. ±	2.927	3.804	9.073	2.918
	C.D. (0.05)	0.195	0.164	0.111	NS

Table 2: Variation in proline content ($\mu\text{g/g}$ tissue) of rice genotypes under different levels drought stress

Sl. No.	Variety	-1 bar PEG6000	-3 bar PEG6000	-5 bar PEG6000	Control
1	Chomala	20.04 (1.323)ef	0.000 (0.000)j	0.000 (0.000)d	6.320 (0.864)i
2	MO-16	22.91 (1.378)de	27.15 (1.449)ef	0.000 (0.000)d	6.691 (0.885)i
3	PTB-35	35.10 (1.557)ab	37.28 (1.583)a	42.55 (1.639)a	12.67 (1.135)defgh
4	PTB-60	35.19 (1.558)ab	36.36 (1.572)ab	38.34 (1.592)b	11.62 (1.101)h
5	PTB-39	27.95 (1.461)c	27.99 (1.462)de	34.39 (1.549)c	14.55 (1.191)bcd
6	PTB-55	29.50 (1.484)bc	29.91 (1.490)cde	0.000 (0.000)d	15.80 (1.225)ab
7	PTB-30	29.57 (1.472)c	23.90 (1.396)fg	31.87 (1.517)c	17.28 (1.261)a
8	PTB-7	36.42 (1.573)a	37.75 (1.588)a	42.23 (1.646)a	6.022 (0.846)i
9	CR Dhan307	30.94 (1.504)abc	32.66 (1.527)bc	0.000 (0.000)d	13.47 (1.160)cdefg
10	Apo	23.03 (1.380)de	0.000 (0.000)j	0.000 (0.000)d	11.99 (1.113)gh
11	Vyttila-3	28.14 (1.465)c	29.33 (1.482)cde	0.000 (0.000)d	12.35 (1.125)fgh
12	Vyttila-4	17.27 (1.261)f	18.78 (1.296)i	0.000 (0.000)d	16.82 (1.240)ab
13	Vyttila-5	21.62 (1.355)e	22.91 (1.378)gh	0.000 (0.000)d	18.36 (1.207)abc
14	Vyttila-6	27.32 (1.452)cd	31.83 (1.511)cd	0.000 (0.000)d	20.76 (1.182)bcdef
15	Vyttila-7	19.11 (1.303)ef	20.65 (1.335)hi	0.000 (0.000)d	16.69 (1.184)bcde
16	Vyttila-8	20.47 (1.348)ef	0.000 (0.000)j	0.000 (0.000)d	16.93 (1.157)cdefgh
17	Vyttila-9	21.32 (1.332)e	23.03 (1.380)gh	0.000 (0.000)d	18.23 (1.208)abc
18	Vyttila-10	19.99 (1.322)ef	19.99 (1.3222)i	0.000 (0.000)d	16.42 (1.141)defgh
19	Nagina-22	19.94 (1.321)ef	0.000 (0.000)j	0.000 (0.000)d	16.01 (1.116)gh
20	NL-44	19.14 (1.304)ef	0.000 (0.000)j	0.000 (0.000)d	15.86 (1.133)efgh
	Mean	25.25	27.96	37.87	16.82
	S.E.±	2.714	2.375	4.088	2.471
	C.D.(0.05)	0.080	0.054	0.034	0.058

Table No. 3. Variation in seedling vigour index of rice genotypes under different levels of salinity

Sl. No.	Variety	100mM NaCl	150mM NaCl	200mM NaCl	250mM NaCl	Control
1	Chomala	1932 (3.284)g	243.4 (2.382)m	226.6 (2.791)jk	0.000 (0.000)n	2741 (3.438)
2	MO-16	2769 (3.442) abc	1737 (3.240)b	1272 (2.946)b	870 (2.940)a	3111 (3.493)
3	PTB-35	2509 (3.399)cdef	1,001 (3.000)d	693.6 (2.721)d	311 (2.494)c	2985 (3.475)
4	PTB-60	2723 (3.435) abcd	493.6 (2.693)g	364.0 (2.463)f	145 (2.166)f	3,077 (3.488)
5	PTB-39	2564 (3.409) bcdef	238.8 (2.378)m	230.4 (2.575)hij	63.0 (1.806)i	3134 (3.496)
6	PTB-55	2440 (3.387)def	833.0 (2.921)e	670.0 (2.867)d	139 (2.149)f	2961 (3.471)
7	PTB-30	2706 (3.432) abcde	1,019 (3.008)d	858.0 (2.877)c	322 (2.509)c	3211 (3.507)
8	PTB-7	2380 (3.376)f	827.0 (2.917)e	549.0 (2.739)de	171 (2.235)e	3040 (3.483)
9	CRdhan307	2562 (3.408) bcdef	624.8 (2.796)f	511.2 (2.708)e	360 (2.557)b	2647 (3.423)
10	Apo	1258 (3.096)h	166.0 (2.220)n	101.0 (2.004)m	239 (2.380)d	2103 (3.323)
11	Vyttila-3	3055 (3.485) a	1,133 (3.054)c	999.0 (2.751)c	822 (2.915)a	3039 (3.483)
12	Vyttila-4	2395 (3.379)ef	445.0 (2.648)h	329.0 (2.472)fg	0.000 (0.000)n	2685 (3.429)
13	Vyttila-5	2775 (3.443) abc	316.2 (2.500)l	271.8 (2.296)ghi	64.8 (1.818)i	3047 (3.484)
14	Vyttila-6	2558 (3.407)bcdef	172.8 (2.238)n	147.6 (2.337)l	114 (2.060)g	2752 (3.440)
15	Vyttila-7	2481 (3.394)cdef	355.8 (2.551)jk	319.2 (2.505)fg	10.4 (1.056)m	3514 (3.546)
16	Vyttila-8	2575 (3.410) bcdef	369.0 (2.567)j	295.2 (2.470)fgh	86.4 (1.941)h	3563 (3.552)
17	Vyttila-9	2413 (3.382)def	2276 (3.357)a	1674 (3.223)a	790 (1.553)a	3447 (3.537)
18	Vyttila-10	2452 (3.389)cdef	421.2 (2.624)hi	205.8 (2.313)ijk	34.8 (1.553)l	2996 (3.476)
19	Nagina-22	2846 (3.454) ab	383.4 (2.584)ij	270.6 (2.432)gh	46.8 (1.679)k	3236 (3.510)
20	NL-44	2663 (3.425) bcdef	322.8 (2.509)kl	189.6 (2.277)k	53.4 (1.735)j	3052 (3.485)
	Mean	2,502 (3.392)	668.9 (2.709)	508.8 (2.611)	257.9 (1.945)	3,017 (3.477)
	S.E.±	0.758	0.772	1.791	1.179	
	C.D.(0.05)	0.054	0.044	0.098	0.048	NS

Table 4: Variation in Na⁺/K⁺ ratio of rice genotypes under different levels of salinity

Sl. No.	Variety	100mM NaCl	150mM NaCl	200mM NaCl	250mM NaCl	Control
1	Chomala	0.33 (0.124)	2.00 (0.477)bcde	3.50 (0.653)de	0.000 (0.000)i	0.16 (0.064)
2	MO-16	0.33 (0.124)	2.75 (0.574)bc	3.16 (0.619)def	5.25 (0.796)de	0.29 (0.111)
3	PTB-35	0.50 (0.176)	3.00 (0.602)b	6.00 (0.845)a	7.50 (0.929)bc	0.22 (0.086)
4	PTB-60	0.50 (0.176)	2.25 (0.512)bcd	4.50 (0.740)abcd	7.25 (0.916)bc	0.41 (0.149)
5	PTB-39	0.29 (0.111)	1.00 (0.301)de	4.16 (0.713)bcd	8.00 (0.954)abc	0.33 (0.124)
6	PTB-55	0.75 (0.243)	2.25 (0.512)bcd	5.00 (0.778)abc	9.50 (1.021)a	0.41 (0.149)
7	PTB-30	0.41 (0.149)	1.917 (0.465)bcde	3.75 (0.677)cd	8.50 (0.978)ab	0.25 (0.097)
8	PTB-7	0.50 (0.176)	1.00 (0.301)de	4.25 (0.720)abcd	9.00 (1.000)ab	0.75 (0.243)
9	CRdhan307	1.00 (0.301)	4.00 (0.699)ab	4.16 (0.713)bcd	4.50 (0.740)ef	0.50 (0.176)
10	Apo	0.75 (0.243)	5.75 (0.829)a	4.00 (0.699)cd	6.50 (0.875)cd	0.50 (0.176)
11	Vyttila-3	1.00 (0.301)	1.00 (0.301)de	2.75 (0.574)fg	3.25 (0.628)g	0.50 (0.176)
12	Vyttila-4	0.75 (0.243)	3.50 (0.653)ab	2.0 (0.477)gh	0.000 (0.000)h	1.00 (0.301)
13	Vyttila-5	0.75 (0.243)	2.50 (0.544)bc	5.50 (0.813)ab	6.50 (0.875)cd	1.00 (0.301)
14	Vyttila-6	0.75 (0.243)	3.50 (0.653)ab	2.50 (0.544)efg	2.83 (0.583)h	1.00 (0.301)
15	Vyttila-7	1.00 (0.301)	1.25 (0.352)cde	1.75 (0.439)ghi	3.50 (0.653)fg	1.00 (0.301)
16	Vyttila-8	0.75 (0.243)	1.25 (0.352)cde	1.25 (0.352)ij	3.50 (0.653)fg	1.00 (0.301)
17	Vyttila-9	1.00 (0.301)	1.25 (0.352)cde	1.75 (0.439)ghi	3.50 (0.653)fg	1.00 (0.301)
18	Vyttila-10	1.00 (0.301)	0.87 (0.272)e	1.33 (0.367)hij	4.5 (0.740)ef	1.00 (0.301)
19	Nagina-22	1.00 (0.301)	1.25 (0.352)cde	1.00 (0.301)j	3.00 (0.602)g	0.11 (0.045)
20	NL-44	1.00 (0.301)	2.25 (0.512)bcd	1.16 (0.334)ij	3.00 (0.602)g	0.29 (0.111)
	Mean	0.71	2.37	3.171	5.53	0.588
	S.E.±	--	8.289	9.893	5.986	--
	C.D. (0.05)	NS	0.226	0.121	0.089	NS

Table 5: Variation in seedling vigour index of rice genotypes high temperature stress of 35°C

S.No.	Variety	Treatment (35 ⁰ C)	Control
1	Chomala	1430 (3.155)ab	1728 (3.306)def
2	MO-16	927.0 (2.964)ab	1,446 (3.206)ghi
3	PTB-35	469.2 (2.602)bc	1145 (3.112)j
4	PTB-60	860.0 (2.934)ab	1264 (3.066)ij
5	PTB-39	712.8 (2.829)ab	1740 (3.174)def
6	PTB-55	464.0 (2.667)abc	1291 (3.172)hij
7	PTB-30	857.0 (2.933)ab	1548 (3.170)efg
8	PTB-7	1,423 (3.151)ab	2184 (3.253)bc
9	CRdhan307	905.0 (2.957)ab	1505 (3.262)fgh
10	Apo	94.20 (1.917)cd	1145 (3.107)j
11	Vyttila-3	0.000 (0.000)e	2272 (3.216)bc
12	Vyttila-4	1,815 (3.259)ab	1999 (3.351)cd
13	Vyttila-5	0.00 (0.000)e	1803 (3.242)de
14	Vyttila-6	2,224 (3.347)ab	2278 (3.357)bc
15	Vyttila-7	1033 (3.012)ab	1,609 (3.206)efg
16	Vyttila-8	1995 (3.300)ab	2211 (3.344)bc
17	Vyttila-9	723.0 (2.859)ab	1811 (3.258)de
18	Vyttila-10	91.00 (1.131)d	1608 (3.206)efg
19	Nagina-22	2584 (3.412)a	2807 (3.448)a
20	NL-44	2376 (3.376)ab	2555 (3.407)ab
	Mean	1049 (2.590)	1797 (3.240)
	S.E.±	14.612	1.091
	C.D.(0.05)	0.790	0.074

Table 6: Variation in cell membrane stability index (%) of rice genotypes under high temperature stress of 35°C

Sl. No.	Variety	Treatment
1	Chomala	10.65 (0.998)h
2	MO -16	4.722 (0.750)i
3	PTB-35	44.71 (1.660)cde
4	PTB-60	24.19 (1.401)fg
5	PTB-39	77.07 (1.887)ab
6	PTB-55	35.49 (1.561)ef
7	PTB-30	38.28 (1.594)de
8	PTB-7	38.51 (1.597)de
9	CRdhan307	56.69 (1.757)bcd
10	Apo	7.234 (0.916)hi
11	Vyttila-3	0.000 (0.000)j
12	Vyttila-4	15.41 (1.212)g
13	Vyttila-5	0.000 (0.000)j
14	Vyttila-6	64.11 (1.814)abc

15	Vyttila-7	45.17 (1.663)cde
16	Vyttila-8	63.78 (1.811)abc
17	Vyttila-9	37.47 (1.585)def
18	Vyttila-10	66.66 (1.830)abc
19	Nagina-22	99.02 (2.000)a
20	NL-44	58.22 (1.771)bcd
	S.E. ±	6.6
	C.D.(0.05)	0.190

Table 7: Variation in seedling vigour index of selected rice genotypes at highest tolerated level of drought and highest tolerated level of salinity

Sl. No.	Variety	Treatment	Control
1	MO-16	0.000 (0.000)d	2818 (3.445)
2	PTB-35	209.2 (2.321)c	2712 (3.427)
3	PTB-60	0.000 (0.000)d	2799 (3.444)
4	PTB-7	1615 (3.207)a	2716 (3.428)
5	Vyttila-3	0.000 (0.000)d	2699 (3.426)
6	Vyttila-9	293.6 (2.469)b	2810 (3.432)
	Mean	705.93 (2.665)	2759 (3.433)
	S.E. ±	2.256	2.897
	CD(0.05)	0.053	NS

Table 8: Seedling vigour index under combined stress of highest tolerated level of Temperature (T_h) (35°C) and highest tolerated level of salinity (S_h) (250mM NaCl)

Sl. No.	Variety	Treatment	Control
1	MO-16	1.000 (0.242)bc	2322 (3.366)a
2	Vyttila-3	0.000 (0.000)d	2100 (3.322)c
3	Vyttila-6	0.000 (0.000)d	1990 (3.299)d
4	Vyttila-9	0.000 (0.000)d	1806 (3.257)e
5	Nagina-22	3.467 (0.626)a	2222 (3.347)b
6	NL-44	1.067 (0.287)b	2148 (3.332)c
	Mean	1.844 (0.251)	2098 (3.320)
	S.E. $\pm$	7.974	8.654
	CD(0.05)	0.273	0.021

Table 9: List of SSR markers used for screening among rice varieties

Sl. No.	Marker	Forward primer	Reverse primer
1	RM 5749	GTGACCACATCTATATCGCTCG	ATGGCAAGGTTGGATCAGTC
2	RM 26212	GTCGCTCCTCTCCTCCAATCC	GCTCGCTGCTTCTAATCTCTTGC
3	RM 7076	CTCCACCAACAACCTCGTATC	AAGCTATTACACAAGCAGCTC
4	RM 10793	GACTTGCCAACTCCTTCAATTCC	TCGTCGAGTAGCTTCCCTCTCTACC
5	RM 3412	AAAGCAGGTTTTCTCCTCC	CCCATGTGCAATGTGTCTTC
6	RM 493	TAGCTCCAACAGGATCGACC	GTACGTAAACGCGGAAGGTG
7	RM 8094	AAGTTTGTACACATCGTATACA	CGCGACCAGTACTACTACTA
8	RM1287	GTGAAGAAAGCATGGTAAATG	CTCAGCTTGCTTGTGGTTAG
9	RM10843	CACCTCTTCTGCCTCCTATCATGC	GTTTCTTCGCGAAATCGTGTGG
10	RM349	TTGCCATTTCGCGTGGAGGCG	GTCCATCATCCCTATGGTCG
11	RM 6100	TCCTCTACCAGTACCGCACC	GCTGGATCACAGATCATTGC
12	RM3042	CAAAAAGGAATCAATGTGAA	GGCTGTTGAGAGGTAGAGAA
13	RM7039	GCACATTTGCCATTCTACCG	GCCTTCCAGTGAGGTGACTC
14	RM256	GACAGGGAGTGATTGAAGGC	GTTGATTTGCGCAAGGGC
15	RM224	ATCGATCGATCTTCACGAGG	TGCTATAAAAGGCATTTCGGG
16	RM243	GATCTGCAGACTGCAGTTGC	AGCTGCAACGATGTTGTCC
17	RM235	AGAAGCTAGGGCTAACGAAC	TCACCTGGTCAGCCTCTTTC
18	RM112	GGGAGGAGAGGCAAGCGGAGAG	AGCCGGTGCAGTGGACGGTGAC
19	RM241	GAGCCAAATAAGATCGCTGA	TGCAAGCAGCAGATTTAGTG
20	RM527	GGCTCGATCTAGAAAATCCG	TTGCACAGGTTGCGATAGAG
21	RM507	CTTAAGCTCCAGCCGAAATG	CTCACCTCATCATCGCC
22	RM447	CCCTTGTGCTGTCTCCTCTC	ACGGGCTTCTTCTCCTTCTC
23	RM528	GGCATCCAATTTTACCCCTC	AAATGGAGCATGGAGGTCAC
24	RM454	CTCAAGCTTAGCTGCTGCTG	GTGATCAGTGCACCATAGCG
25	RM348	CCGCTACTAATAGCAGAGAG	GGAGCTTTGTTCTTGCGAAC
26	RM256	GACAGGGAGTGATTGAAGGC	GTTGATTTGCGCAAGGGC
27	RM490	ATCTGCACACTGCAAACACC	AGCAAGCAGTGCTTTTCAGAG
28	RM232	CCGGTATCCTTCGATATTGC	CCGACTTTTCCTCCTGACG
29	RM226	AGCTAAGGTCTGGGAGAAACC	AAGTAGGATGGGGCACAAGCTC
30	RM208	TCTGCAAGCCTTGTCTGATG	TAAGTCGATCATTGTGTGGACC

Figures

Lane 1-100bp ladder, 2-Chomala, 3- MO-16, 4-PTB-35, 5-PTB-60, 6-PTB-39, 7-PTB-55, 8-PTB-30, 9-PTB-7, 10-CRdhan307, 11-Apo, 12-Vyttiila-3, 13-Vyttiila-4, 14-Vyttiila-5, 15-Vyttiila-6, 16-Vyttiila-7, 17- Vyttiila-8, 18-Vyttiila-9, 19-N-22, 20-NL-44, 21-Vyttiila-10

Figure 1

Plate 1: Polymorphism pattern of 20 rice genotypes using SSR marker RM8094

Lane 1-100bp ladder, 2-Chomala, 3- MO-16, 4-PTB-35, 5-PTB-60, 6-PTB-39, 7-PTB-55, 8-PTB-30, 9-PTB-7, 10-CRdhan307, 11-Apo, 12-Vyttiila-3, 13-Vyttiila-4, 14-Vyttiila-5, 15-Vyttiila-6, 16-Vyttiila-7, 17- Vyttiila-8, 18-Vyttiila-9, 19-N-22, 20-NL-44, 21-Vyttiila-10

Figure 2

Plate 2: Polymorphism pattern of 20 rice genotypes using SSR marker RM 26212

Plate 3. Amplification pattern of 20 rice varieties obtained by SSR marker RM 6100(Lane 1 – 100bp ladder, Lane 2 – chomala, Lane 3 - MO-16, Lane 4 – PTB 35, Lane 5 -PTB 60, Lane 6 – PTB 39, Lane 7 – PTB 55, Lane 8 – PTB 30, Lane 9 – PTB 7, Lane 10 – CR dhan 307, Lane 11 – Apo, Lane 12 – Vyttila -3, Lane 13 – Vyttila -4, Lane 14 – Vyttila -5, Lane 15 – Vyttila -6, Lane 16 – Vyttila -7, Lane 17 – Vyttila -8, Lane 18 – Vyttila -9, Lane 19 – N-22, Lane 20 – NL -44, Lane 21

Figure 3

Plate 3: Polymorphism pattern of 20 rice genotypes using SSR marker RM6100

Plate 4. Amplification pattern of 20 rice varieties obtained by SSR marker RM 1287 (Lane 1 – 100bp ladder, Lane 2 – chomala, Lane 3 - MO-16, Lane 4 – PTB 35, Lane 5 -PTB 60, Lane 6 – PTB 39, Lane 7 – PTB 55, Lane 8 – PTB 30, Lane 9 – PTB 7, Lane 10 – CR dhan 307, Lane 11 – Apo, Lane 12 – Vyttila -3, Lane 13 – Vyttila -4, Lane 14 – Vyttila -5, Lane 15 – Vyttila -6, Lane 16 – Vyttila -7, Lane 17 – Vyttila -8, Lane 18 – Vyttila -9, Lane 19 – N-22, Lane 20 – NL -44, Lane 21 – Vyttila -10)

Figure 4

Plate 4: Polymorphism pattern of 20 rice genotypes using SSR marker RM1287

Plate 5. Amplification pattern of 20 rice varieties obtained by SSR marker RM 5749 (Lane 1 – 100bp ladder, Lane 2 – chomala, Lane 3 - MO-18, Lane 4 – PTB 35, Lane 5 -PTB 60, Lane 6 – PTB 39, Lane 7 – PTB 55, Lane 8 – PTB 30, Lane 9 – PTB 7, Lane 10 – CR dhan 307, Lane 11 – Apo, Lane 12 – Vyttila -3, Lane 13 – Vyttila -4, Lane 14 – Vyttila -5, Lane 15 – Vyttila -6, Lane 16 – Vyttila -7, Lane 17 – Vyttila -8, Lane 18 – Vyttila -9, Lane 19 – N-22, Lane 20 – NL -44, Lane 21 – Vyttila -10)

Figure 5

Plate 5: Polymorphism pattern of 20 rice genotypes using SSR marker RM5749

Figure 6

Plate 6: Estimates of subpopulations using delta K-values

Figure 7

Plate 7: The summary plot of Q matrix estimates