



Electronic Journal of Plant Breeding

Research Article

Marker assisted recurrent selection for genetic male sterile population improvement in rice

P.J. Suryendra¹, P. Revathi^{2*}, Arun Kumar Singh³, B.C. Viraktamath⁴

Crop Improvement Section, ICAR-Indian Institute of Rice Research, Rajendranagar, Hyderabad 500030, India.

*Senior Scientist (Plant Breeding) Hybrid Rice- Crop Improvement Section, IIRR

E-Mail: Revathi.P@icar.gov.in, revathi.ponnusamy@gmail.com

Abstract

In the present study, by deploying marker assisted recurrent selection (MARS) strategy, the improvement of IR58025B genetic male sterile maintainer population for drought and salinity tolerance was carried out to develop maintainers with drought and salinity tolerance. Individual crosses were performed among sterile plants of IR58025B maintainer population and donors of drought tolerance viz., Vandana (qDTY12.1) and FL478 (Saltol). F₁ plants were confirmed for the presence of target genes/QTLs with the help of SSR markers and raised to produce F₂ seeds. Selected F₂ plants were harvested and seeds from each cross combination were mixed in equal proportions to generate population for the first random mating or recurrent selection cycle. The same process is continued up to 4th cycle and 200 selected plants were subjected to genotyping for drought and salinity with the help of reported SSR markers. About 48 plants were found positive for salinity tolerance, 17 plants were positive for drought and 10 plants were positive for both the traits. These lines were advanced by pedigree method of breeding for deriving maintainers with drought and salinity tolerance.

Keywords

MARS, recurrent selection, Genetic male sterility, Population improvement, SSR markers, maintainers, Hybrid rice

INTRODUCTION

Rice is the most important staple food for more than 60% of world's population (Shaheen *et al* 2017). India is the 2nd largest producer of rice after China with the production of 106 million tonnes. The main goal of breeders is to increase the rice productivity by imparting tolerance against biotic and abiotic stresses. In general, biotic stresses of rice include leaf blight, blast, plant hoppers and leafhoppers; and abiotic stresses include salt, drought, temperature, etc. Among all the abiotic stresses, salinity and drought significantly limit the rice yield compared to biotic stresses (Hossain, 1995; Dey *et al* 1995; Asch *et al* 1997).

Drought stress is a major problem for much of the world's cultivation land (O'Toole and Chang, 1979; Chang *et al* 1982). Yield stability and production in rainfed areas are severely affected by drought stress (Shaheen *et al* 2017). As drought is the most severe abiotic constraint in upland rice cultivation, breeding strategies for production of cultivars with an improved drought tolerance were in quick progress (Bernier *et al* 2008). In addition to drought, rice cultivation is also effected by salinity especially in inland areas due to excessive irrigation with poor quality of water

and improper drainage systems (Ismail *et al* 2010). Some other factors that significantly increase salt affected areas are increased surface evaporation, weathering of rocks, salt water irrigation, over-exploitation of underground water and poor cultural practices (Rekha *et al* 2018). However, rice is sensitive to salinity at early vegetative and reproductive stages and can survive well in standing water. (Ismail *et al* 2007)

Recurrent Selection (RS) has been widely used to increase a desired gene frequency in a population which leads to breakdown of undesirable linkage (Hull, 1945; Fujimski, 1979) in crop systems like maize (Dudley and Lambert, 2004) and rice (Hull, 1945;). It includes three phases - development of base population for selection, evaluation of selected individuals and selection of best individuals followed by their inter-crossing to generate a new population. In general, compared with cross pollinated crops, this approach is difficult to implement in self pollinated crops due to tedious inter-crossing. To overcome this, strategy of genetic male sterility was introduced in rice (Fujimski, 1979), soybean (Werner and Wilcox, 2004) and wheat (Wang *et al* 1996) for

facilitating intercrossing. With this background, the present study was targeted to derive the maintainer lines that are tolerant to salinity and drought with the help of genetic male sterility mediated marker-assisted recurrent selection.

MATERIALS AND METHODS

Plant Materials: The genetic male sterile maintainer population in the back ground of IR58025 B was obtained from IIRRI, Philippines was maintained by IIRR as DRCP-105 population was utilized in the present study. GMS maintainer population was developed by ethyleneimine mediated induced chemical mutation by IIRRI. Drought tolerant genotype Vandana carrying *qDTY12.1* and salinity tolerant genotype FL478 carrying *Salto1* were chosen as donors for transferring drought and salt tolerance to maintainer population.

Execution of the Experiment: From the IR 58025B base population, sterile plants (with white-shriveled anthers) were selected and confirmed by pollen study under microscope by staining with 1 % I-KI (Virmani *et al* 1997). These sterile plants were individually crossed with the drought and salt tolerant donors. F₁s were grown as and evaluated for their heterozygosity and all were found to be fertile.

msms (sterile) X MSMS(fertile)



Selected F₂ plants were harvested and seeds from each individual cross were bulked in the equal quantity to generate first random mating population. At the time of peak anthesis supplementary pollination was given to maximizing out-crossing. After maturity, out crossed seeds on sterile plants were harvested and dried properly. Bulked seeds from sterile plants of first random mating cycle constituted second random mating and the same procedure continued till the fourth cycle. During the fourth random mating cycle, 200 plants were genotyped for drought and salt tolerance. Plants showing homozygous

and heterozygous banding patterns were advance by pedigree method of breeding for further evaluation.

DNA was extracted from fresh rice leaf samples through mini preparation method suggested by (Zheng *et al* 1995). Microsatellite markers were amplified in Thermal Cycler (Eppendorf vapo. protect) using 30ng template DNA, 2.5mM dNTPs each, 10p moles of each primer, 1 U/μl *Taq* polymerase (Genaxy Scientific Limited, India) and 10 X *Taq* buffer A with tris and 15mM MgCl₂ (Bangalore Genei, India) in a volume of 10μl reaction with initial denaturation at 94° C for 5 min, followed by 30 s at 94° C, 30 s at 55°C, and 1 min at 72°C for 35 cycles, and final extension at 72°C for 7 min. The PCR products were separated on 3.5% agarose gel with ethidium bromide staining followed by UV visualization.

Phenotyping for seedling stage salinity tolerance and reproductive stage drought tolerance: Some of the rice lines found to be possessing *Salto1* by marker assisted selection during progeny evaluation were phenotyped for seedling stage salinity tolerance along with the negative and positive controls as reported by Gregorio *et al* 1997. Prominent drought tolerant lines identified by genotyping for drought tolerance were subjected to the phenotyping for reproductive stage drought tolerance as reported by Fischer *et al* 2012.

RESULTS AND DISCUSSIONS

Genotyping: On genotyping 200 plants in the 4th recurrent selection cycle with the reported markers for salinity tolerance - RM3412 linked to *Salto1* QTL located on chromosome 1 (Thomson *et al* 2010) and drought tolerance - RM511 linked to *qDTY12.1* QTL located on chromosome 12 (Arvind kumar *et al* 2012), we had identified 48 plants showing positive banding pattern for salinity tolerance, 17 plants showing positive banding pattern for drought tolerance and 10 plants were identified to carry both drought and salinity tolerance banding patterns. (Fig. 1)

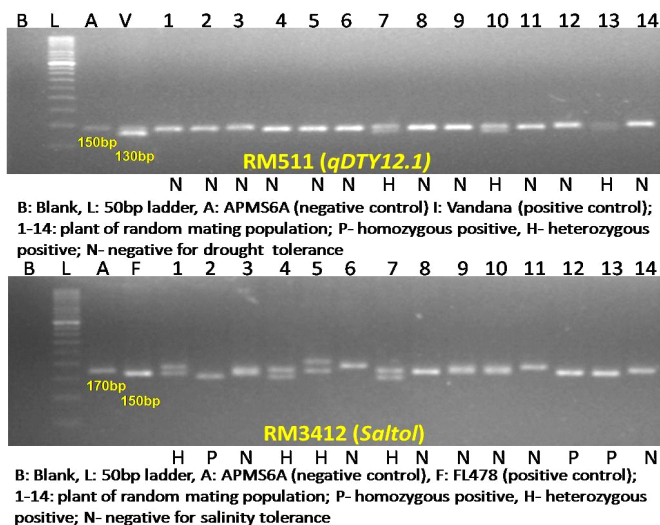


Fig.1. Genotyping for *Salto1* and *qDTY12.1* QTLs.

Five plants of each of these tolerant lines were raised in three row pedigree method during *Kharif* 2015 and evaluated with the help of markers for salinity and drought tolerance. Salt tolerant lines further segregated as 67 plants and 39 plants which were homozygous and heterozygous respectively. Drought tolerant lines showed 22 plants homozygous and 12 plants heterozygous for drought tolerance QTL. Further genotyping of plants from salinity-drought tolerant lines are resulted in obtaining three plants with genes for the QTLs of salinity and drought tolerance.

Phenotyping for Agromorphological Traits: Statistical analysis on R-software of crop duration, tillering ability, plant height and single plant yield of individual plants from IR 58025B maintainer base population, 4th and 5th cycles of recurrent selection showed that, there was a negative correlation between crop duration and tillering ability (Fig. 2). On analyzing plants for tillering ability, large number plants of GMS maintainer population, it was observed that on an average productive tillers of 10-15; in the 4th recurrent selection cycle 10-12 tillers containing plants were dominant, followed by 5-10 tillers and also plants with 5-30 tillers were also seen; In the 5th cycle

was observed to be dominant with 5-20 tillers and also plants with 10-20 tillers were frequent (Fig. 3). When plant height was observed among these three populations, lesser variations among plant height was seen in base population; more variance in plant height was there in the 4th cycle and 5th cycle has showed plants with stable plant height (Fig. 4). On observing for crop duration among these three populations, early, medium and late duration plants were seen in base population and variable crop duration was observed in the 4th recurrent selection cycle; and a stabilized duration range of 95-125 days was seen in the 5th cycle but very less number of late plants were also obtained (Fig. 5). Coming to the single plant yield, yield of single plants was distributed in the range of 5-60grams but 15-20grams per plant was predominant in DRCP105 maintainer population; in the 4th cycle single plant yield was slightly increased than base population and up to 90grams per plant was also seen but up to 25grams per plant was regular. In the same way, the 5th cycle was observed to be with the plants of decreased single plant yield than to the 4th cycle but added advantage was found as plants with tolerance to salinity and drought stresses (Fig. 6).

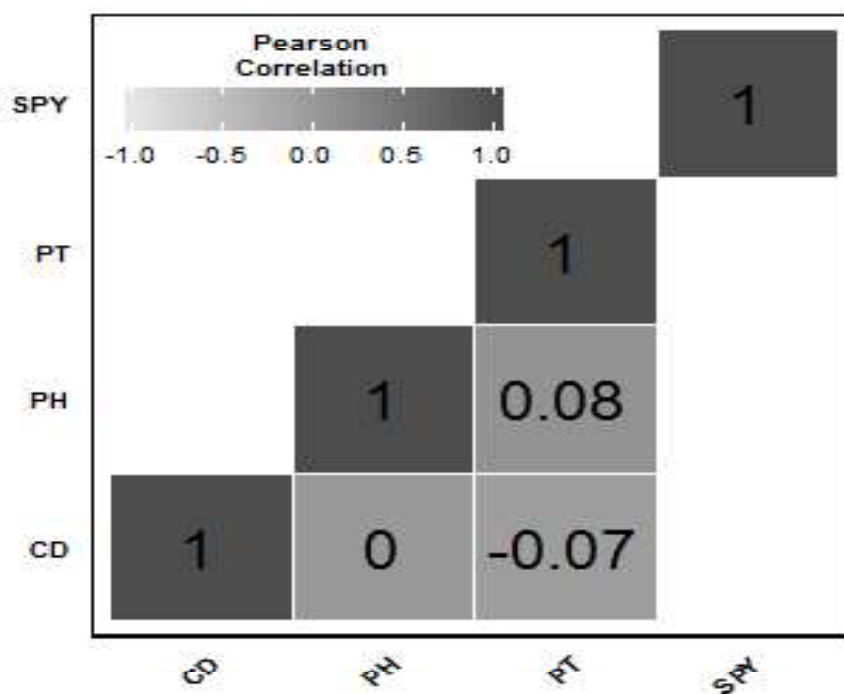


Fig.2. Correlation among DRCP105 maintainer population, the 4th and 5th recurrent selection cycles

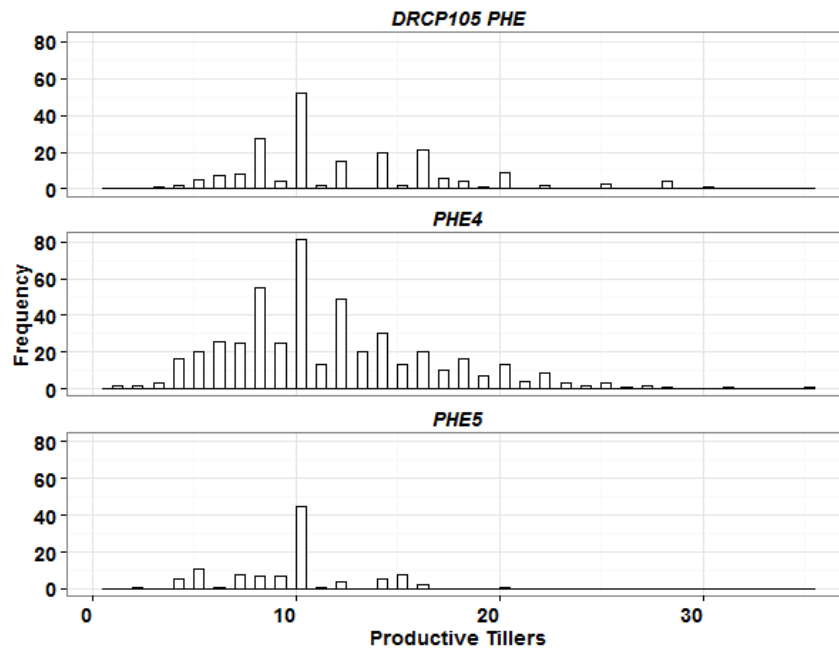


Fig. 3. Comparison of Tillering Ability of DRCP105 Maintainer Population, the 4th and 5th cycles of Recurrent Selection

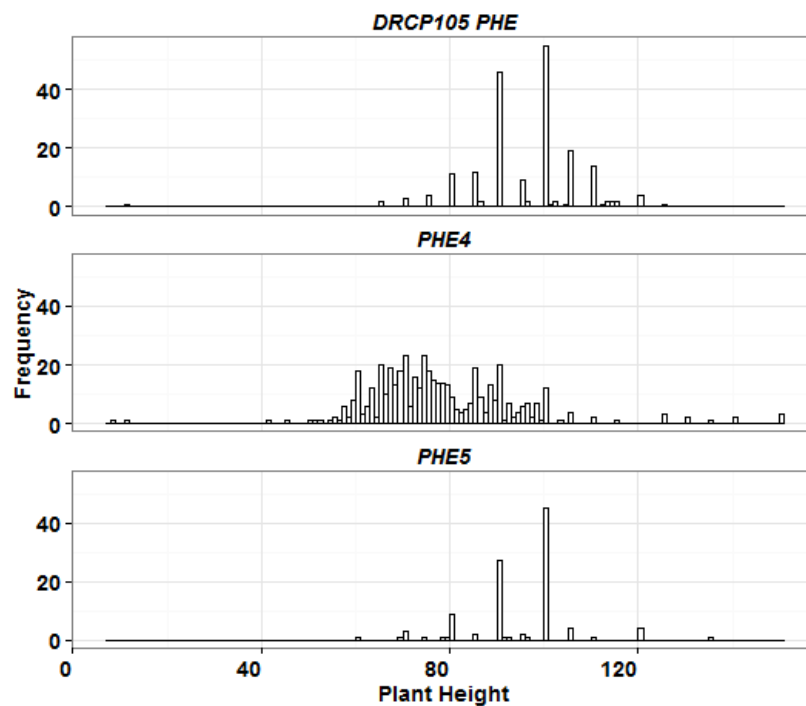


Fig. 4. Comparison of Plant Height of the 4th and 5th cycles of Recurrent Selection with DRCP105 Maintainer Population

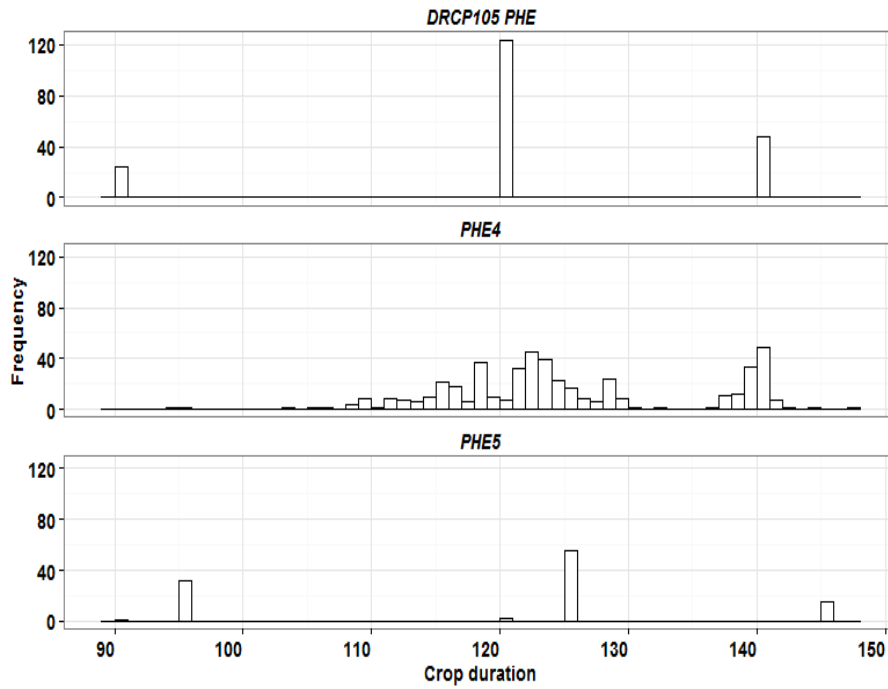


Fig. 5. Comparison of Crop Duration of the 4th and 5th cycles of Recurrent Selection with DRCP105 Maintainer Population

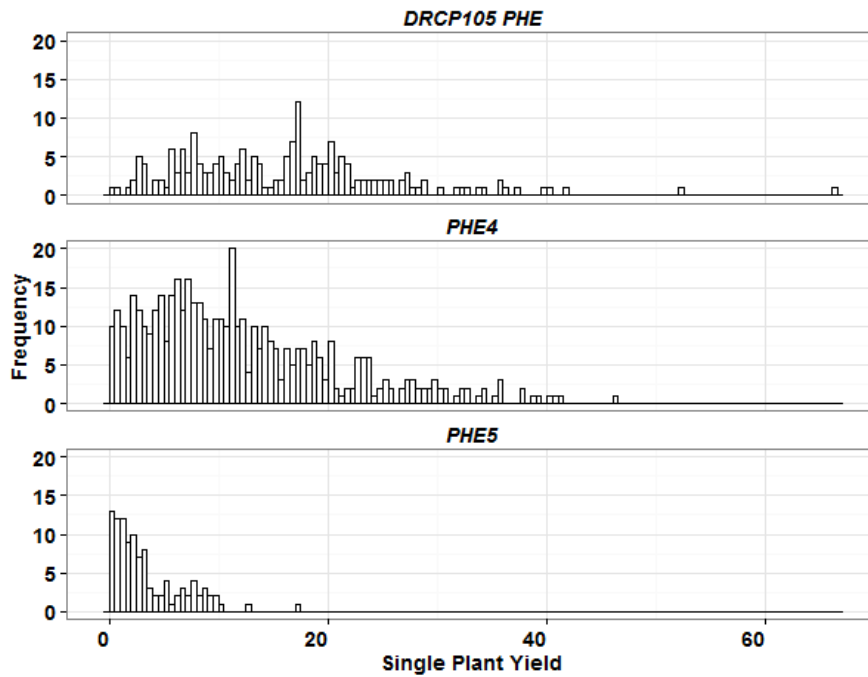


Fig.6. Comparison of Single Plant Yield of the 4th and 5th cycles of Recurrent Selection with DRCP105 Maintainer Population

Drought and salinity are major abiotic constraints for the rice production. Drought affects the progress of rice production in rainfed areas of Asia and Sub-Saharan Africa (Fischer *et al* 2012). Excessive irrigation with poor quality of water and lack of proper drainage facility exposes rice to salinity stress in inland areas (Ismail *et al* 2010). Therefore, the breeders aim in improving rice for salinity and drought stress tolerance. Our current study includes marker assisted recurrent selection using genetic male sterility to improve rice for salinity and drought tolerance. Our aim was to develop suitable maintainer lines which are incorporated with these traits. We could identify prominent maintainer lines that are having tolerance towards salinity and drought individually; and also the plants having traits of both salinity and drought tolerance were seen. These plants were analyzed for phenotypic traits (SES, 2013). Phenotyping for rice seedling stage salinity tolerance was performed and the data generated

is being analyzed for correlation with genotyping. Currently, the rice lines are being phenotyped for reproductive stage drought tolerance by means of late planting in the field. The results of salinity and drought tolerance screening are in progress. Therefore, it could be concluded that these maintainer lines could be potentially used to produce improved IR58025A CMS lines by repeated backcrossing with improved salinity and drought tolerance. Further, these stress tolerance lines will be evaluated for grain quality traits, including aroma.

ACKNOWLEDGEMENT

The first author is highly thankful to Department of Science and Technology, SERB, Govt of India for the fellowship received from the project No. SERB/ No.SR/FT/LS-28/ 2011. The support received from ICAR-IIRR to carry out this research work is highly acknowledged.

REFERENCES

- Abrol, I.P. and Sehgal J. 1994. Degraded lands and their rehabilitation in India, In: Soil Management for Sustainable Agriculture in Dryland Areas, T.D. Biswas, G. Narayanswamy, J.S.P. Yadav, G. Dev, J.C. Katyal and P.S. Sidhu (Eds.), Bulletin No. 16, Indian Society of Soil Science, New Delhi, India, p. 107 - 118.
- Asch, F., Dingkuh, M. and Doerffling, K. 1997. Physiological stresses of irrigated rice caused by soil salinity in the Sahel, In: K.M. Miezán, M.C.S. Wopereis, M. Dingkuhn, J. Deckers, T.F. Randolph (Eds.), *Irrigated Rice in the Sahel: Prospects for Sustainable Development*, West Africa Rice Development Association, Bouake, Cote d'Ivoire, p. 247 - 273.
- Bajaj, S., Targolli, J., Liu, L.F., Ho, T.H.D. and Wu, R. 1999. Transgenic approaches to increase dehydration-stress tolerance in plants. *Mol Breed.*, **5**: 493 - 503. [\[Cross Ref\]](#)
- Bernier, J., Kumar, A., Serraj, R., Spaner, D. and Atlin, G. 2008. Review: breeding upland rice for drought resistance. *J. Sci. Food Agric.*, **88**: 927 - 939. [\[Cross Ref\]](#)
- Chang, T.T., Loresto G.C., OToole, J.C. and Armenta-Soto, J.L. 1982. Strategy and methodology of breeding rice for drought-prone areas. In: *Drought resistance in crops with emphasis on rice*, Los Baflos, Philippines: IRR1, p. 217 - 44.
- Dey, M.M. and Upadhyaya, H.K. 1996. Yield loss due to drought, cold and submergence in Asia, In: *Rice Research in Asia: Progress and Priorities*, R.E. Evenson, R.W. Herdt, M. Hossain (Eds.), CAB International and IRRI, Manila, Philippines, p. 291 - 303.
- Dudley, J.W. and Lambert, R.J. 2004. 100 generations of selection for oil and protein in corn. *Plant Breed. Rev.*, **24**(1): 79 - 110. [\[Cross Ref\]](#)
- Fischer, K.S., Fukai, S., Kumar, A., Leung, H. and Jongdee B. 2012. Field phenotyping strategies and breeding for adaptation of rice to drought. *Front Physiol.* **3**(282): 1 - 21. [\[Cross Ref\]](#)
- Hull, F.H. 1945. Recurrent Selection for Specific Combining Ability in Corn. *J. Am. Soc. Agron.*, **37**: 134-145. [\[Cross Ref\]](#)
- Gregorio, G.B., Senadhira, D. and Mendoza, R.D. 1997. Screening Rice for Salinity Tolerance. IRR1 Discussion Paper Series No.22. The International Rice Research Institute, Manila, The Philippines.
- Fuzimski, H. 1979. Recurrent Selection by Using Genetic Male Sterility for Rice Improvement. *JARQ.*, **13** (3): 153 - 156.
- Hossain, M. 1995. Sustaining food security for fragile environments in Asia: achievements, challenges, and implications for rice research. In: *Fragile Lives in Fragile Ecosystems*, Proceedings of International Rice Research Conference, 13-17 February, IRRI, Los Banos, Laguna, Philippines, p. 2 - 23.
- International Rice Research Institute (IRRI). 1993. 1993-1995. IRRI rice almanac. LosBanos, Philippines. IRRI.
- Ismail, A.M., Thomson, M.J., Vergara G.V., Rahman M.A., Singh, R.K. and Gregorio, G.B. 2010. Designing resilient rice varieties for coastal deltas using modern breeding tools. In: *Tropical Deltas and coastal zones: food production, communities and environment at the land-water interface*, C.T. Hoanh, B.W. Szuster, K.S. Pheng, A.D. Nobel and A.M. Ismail, Wallingford, CAB, p. 154-65. [\[Cross Ref\]](#)
- Ismail, A.M., Heuer, S., Thomson, M.J. and Wissuwa, M. 2007. Genetic and genomic approaches to develop rice germplasm for problem soils. *Plant Mol. Biol.*, **65**: 547-70. [\[Cross Ref\]](#)

- Lang, N.T., Buu, B.C. and Ismail, A. 2008. Molecular mapping and marker assisted selection for salt tolerance in rice (*Oryza sativa* L.). *Omonrice*, **16**: 50-56.
- O'Toole, J.C. and Chang, T.T. 1979. Drought resistance in cereals - rice: a case study. In: Stress physiology in crop plants, H. Mussell, R. Staples, (Eds.), *John Wiley & Sons, Inc*, p. 373-405.
- Rekha, G., Padmavathi, G., Abhilash, V., Kousik, M.B.V.N., Balachandran, S.M., Sundaram, R.M. and Senguttuvel, P. 2018. A protocol for rapid screening of rice lines for seedling stage salinity tolerance. *Electron. J. Plant Breed.*, **9**(3): 993-1001. [\[Cross Ref\]](#)
- Shaheen, A.S., Sindhumole, P., Swapnil, G.W. and Sajini, S. 2017. Molecular characterization of rice (*Oryza sativa* L.) genotypes for drought tolerance using two SSR markers. *Electron. J. Plant Breed.*, **8**(2): 474-479. [\[Cross Ref\]](#)
- Dixit, S., Mallikarjuna Swamy, B.P., Prashant Vikram, Ahmed, H.U., Sta Cruz M.T., Modesto Amante, Dinesh Atri, Hei Leung and Arvind Kumar. 2012. Fine mapping of QTLs for rice grain yield under drought reveals sub-QTLs conferring a response to variable drought severities. *Theor. Appl. Genet.*, **125**: 155-169. [\[Cross Ref\]](#)
- Singh, K., Gopalakrishnan, S., Singh, V.P., Prabhu, K.V., Mohapatra, T., Singh, N.K., Sharma, T.R., Nagarajan, M., Vinod, K.K., Devinder Singh, Singh, U.D., Subhash Chander, Atwal, S.S., Rakesh Seth, Vikas, K. Singh, Ranjith, K. Ellur, Atul Singh, Deepti Anand, Apurva Khanna, Sheel Yadav, Nitika Goel, Ashutosh Singh, Asif, B. Shikari, Anita Singh and Balram Marathi. 2011. Marker assisted selection: a paradigm shift in Basmati breeding. *Indian J. Genet. Plant Breed.*, **71** (2): 120-128.
- Singh, R.K., Redoña, E.D. and Refuerzo, L. 2010. Varietal improvement for abiotic stress tolerance in crop plants: special reference to salinity in rice. In: Abiotic stress adaptation in plants: physiological, molecular and genomic foundation. A. Pareek, S.K. Sopory, H.J. Bohnert, Govindjee, (Eds.), New York, *Springer*, p. 387-415. [\[Cross Ref\]](#)
- Standard Evaluation System for Rice (SES), International Rice Research Institute, Philippines, 5th edition, 2013.
- Thomson, M.J., de Ocampo, M., Egdane, J., Rahman, M.A., Sajise, A.G., Adorada, D.L. and Ismail, A.M. 2010. Characterizing the *Salto1* quantitative trait locus for salinity tolerance in rice. *Rice*, **3**: 148-160. [\[Cross Ref\]](#)
- Tyagi AK, A Mohanty, S Bajaj, A Chaudhury and SC Maheshwari, 1999. Transgenic rice: a valuable monocot system for crop improvement and gene research, *Crit. Rev. Biotechnol.*, **19**: 41-79. [\[Cross Ref\]](#)
- Virmani, S.S., Viraktamath, B.C., Casa, C.L., Toledo, R.S., Lopez, M.T. and Manalo, J.O. 1997. Hybrid rice breeding manual. IRRI, Philippines, 13
- Wang, X.W., Lai, J.R., Fan, L. and Zhang, R.B. 1996. Effects of recurrent selection on populations of various generations in wheat by using the Tai Gu single dominant male-sterile gene. *J. Agric. Sci.*, **126** (4): 397-402. [\[Cross Ref\]](#)
- Werner, B.K. and Wilcox, J.R. 2004. Recurrent selection for yield in Glycine max using genetic male-sterility. *Euphytica.*, **50** (1): 19-26. [\[Cross Ref\]](#)
- Zheng, K., Subudhi, P.K., Domingo, J., Maopanty, G. and Huang, N. 1995. Rapid DNA isolation for marker assisted selection in rice breeding. *Rice Genetics Newsletter*. **12**: 255-258.