

DOCTORAL THESIS

Molecular studies of some developmental and reproductive traits of Rayada rice

Bin Rahman, A N M Rubaiyath

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Abstract

Crop domestication and subsequent breeding or directional selection have narrowed the genetic diversity of elite varieties whereas land races, ecotypes, wild relatives growing on native preferences still keep genetic diversities of stress tolerances. Rayada is such an exceptional ecotype, variant of typical deepwater rice, completely endemic to certain areas of Madhumati river tracts of Bangladesh and still shares some features of wild rices. Multiple physiological features of Rayadas are distinctly different from typical deepwater rice. In this PhD project, we have studied the specialty of Rayada rice and identified that Rayada has special tolerances to prolonged flood, submergence and cold along with longer root system and prompt recovery capacity after water stress. All these features make it as an elite resource of stress tolerance and might become a new focus of rice germplasm research.

Among all deepwater rices, Rayada is the only exception, having virtually no seed dormancy, but both physiological and molecular bases of this trait are completely unknown. We examined the non-dormant nature of Rayadas as a natural variant of deepwater rice. After comparing features of freshly harvested seeds of Rayada with those of typical deepwater rice variety, we identified several concerted features; for instance, less ABA content in freshly harvested seeds; faster ABA catabolism and enhanced ROS accumulation after imbibition. Moreover, after analyzing stepwise gene expressions of 32 bZIPs in seed germination, mild and severe water stresses among three extreme ecotypes including Rayadas together with homology search with reported genes, we identified OsbZIP84 as a candidate gene for the regulation of ABA catabolism in Rayada rice. ABA content and expression analysis of OsbZIP84 and ABA8oxs in four growth and developmental stages along with phenotyping of mutant revealed the function of OSbZIP84 in the dormancy regulation of Rayada rice.

Submergence tolerance during seed germination is one of the rare traits of rice, even among cereals. Except few physiological indications of tolerance, most other molecular signaling network is not known. We identified several positive and negative regulators of shoot development under submergence inducing the capacity of shoot development of Rayada rice under oxidative stress. We

successfully developed a condition supplemented with riboflavin and H₂O₂ where intolerant genotypes successfully developed shoot under submergence. However, induced shoot development was completely inhibited by glucose, ABA and mitochondrial complex IV inhibitor signifying ABA and glucose as negative regulators, whereas ROS, riboflavin and mitochondrial complex IV as positive regulators. Gene expression analysis of α -amylases revealed H₂O₂ supplementation mimicked aerobic gene expression pattern. Plausible mechanisms of riboflavin and H₂O₂ function in submergence tolerance were also discussed.

Finally, we isolated a novel mutant of Rayada variety with Kaladigha background and having four interesting phenotypes of practical implications. Mutant plant shows purple pigmentation throughout the plants organs along with dense and elongated trichomes on the adaxial leaf surface. In addition, the same mutant also shows high frequency of stigma exsertion. But ultimately, we observed that the mutant plant is completely sterile. The possible reason of the sterility was found being related to the stigma receptivity. Severe reduction of ROS accumulation in stigmas of mutant plant was observed after fluorescent H₂DCF-DA staining. However, pollen grains are completely viable with normal shape and size. Interestingly, the fertility was partially restored after humidifying the panicles. Mutant progeny showed dense black coloration in seeds with significant reduction of grain weight. Moreover, it showed segregating ratio of 3:1 for purple pigmentation, suggesting single gene mutation nature. Other phenotypic features confirmed the mutant as a Rayada variety with Kaladigha background, not a seed contamination. After extensive data mining of these four phenotypes, we identified maize Lc gene with three similar phenotypes reported earlier excluding stigma exsertion, hence considered as candidate gene of this mutant. The gene expression of maize Lc homolog of rice, OsbHLH13, was exceptionally up-regulated in the purple mutant. Further studies of genetic characterization may open up the practical implications of this mesmerizing mutant.

In summary, Rayada is a primitive deepwater rice ecotype that can offer many traits and genetic resources that are badly needed in rice breeding for stress tolerance and the time is mature to do the more detailed research with rapid advances in genome research weaponry .

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