Short Communication

Expression, isolation and identification of submergence induced Genes from FR13A through RT-PCR

Abstract

The present study was undertaken to identify submergence-induced genes, *OsARP* (Antiporter regulating protein) from the submergence tolerant rice (*Oryza sativa L.*) cultivar, *Indica* (FR13A). We analyzed submergence-tolerant genetic material FR13A to improve flooding tolerance in rice and, specifically, complete submergence tolerance. Healthy leaf samples collected from plant material, FR13A through complete submergence of 0, 1, 3, 5 & 7days, the gene was expressed in the shoot. We isolated mRNA through Trizol kit and mRNA converted to cDNA through Reverse Transcriptase (RT). The *OsARP* gene was first expressed into *E. coli* and antibody was produced by using purified recombinant protein. The expression of *OsARP* protein was detected under submergence stresses. We examined the expression and subcellular localization of *OsARP* in a submergence tolerant rice cultivar FR13A. Gradient PCR of cDNA with these three genes (*OsARP ,OsGGT, OsMGD*) were amplified from 3, 7days submerged by annealing temperature (^{0}C) of 62, 64 and 66. This PCR containing cDNA of *OsARP* gene was present clear amplification by Tm of 64^{0}C than other *OsGGT, OsMGD* genes. A time-course study showed that *OsARP*-gene expression increased in FR13A during submergence of 7 days. This indicates that *OsARP* is a membrane bound protein of rice, which is expressed under submergence stresses.

Keywords: cDNA, submergenc, *OsARP*, mRNA, *indica,* environments

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Introduction

Rice (*Oryza sativa L.*) is the only cereal that can be cultivated in the frequently flooded river deltas of South-East and South Asia. Flash-flooding adversely affect at least 16% of the rice lands of the world (~22mha) and 1 billion USD annual loss in South & South-east Asia. Submergence can result in yield losses of up to 100% depending on different climatic & agronomic factors.[1] More than 2.0 million-hectare areas are affected by different grades of flash floods and reduces 5% average yield in Bangladesh.[2] In this circumstance, improvement of germplasm is likely the best option to withstand submergence and stabilize productivity in these environments. High frequency regeneration of plants from *in vitro* cultured tissues and cells are a pre-requisite for successful application of tissue culture and genetic engineering technologies for crop improvement.[3] The present study was undertaken to identify submergence-induced genes from the submergence-tolerant rice cultivar, *indica* (FR13A) for crop improvement through genetic engineering technologies. Rice has adapted to flood-prone environments, i.e. where excessive flooding limits growth and yields, by either submergence tolerance or elongation ability.[4] The functional roles of this OsChaC (OsARP) protein lie in the tolerance of submergence by regulating membrane bound transporter/antiporter in higher plants. In addition, it was confirmed that three isoforms like this *OsARP* gene are present also in *Arabidopsis* genome. Therefore, expression analysis of *ChaC/OsARP* gene will be conducted in different rice cultivars and subsequently transgenic crops will be developed through *Agrobacterium* mediated transformation.

Materials and methods

Healthy leaf samples collected from plant material, FR13A through complete submergence of 0, 1, 3, 5 & 7 days, the gene was expressed in the leaves through stress. RNA was extracted from the leaves of FR13A using the Trizol Reagent at Biotechnology Lab., Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh. Primer mix incubated for 5min at 65ºC and then placed on ice for 1min. cDNA synthesis mix was added to primer mix. The mixture was spinned down at 3000 rpm for 1minute by centrifuge, then gently mixed and incubated for 50 min at 50ºC. The reaction was terminated for 5min at 85ºC and placed on ice for 1 min. The mixture was spinned down at 3000 rpm for 1 minute by centrifuge and then 1µl RNase free H_2O was added. The tube was incubated for 20min at 37ºC. The cDNA samples were stored at-20°C. Finally, mRNA converted to cDNA through Reverse Transcriptase (RT) of 3 & 7days submerged samples. cDNA samples were evaluated both quantitatively and qualitatively using spectrophotometer and agarose gel electrophoresis, respectively.

Results and discussion

We analyzed submergence-tolerant genetic material FR13A to improve flooding tolerance in rice and, specifically, tolerance of complete submergence. The rice (*O. sativa L. subsp. indica*) cation transporter gene *OsCTP* (accession No. AB112061) was reported earlier; it encodes a protein of 137 amino acids having an atomic mass of 15kDa[5] under salt, drought and submergence stresses. Healthy leaf samples collected from plant material, FR13A through complete submergence of 0,1,2,3,4,5,6 & 7 days, the gene was expressed in the shoot. We isolated mRNA through Trizol kit and cDNA was then synthesized from this mRNA through Reverse Transcriptase (RT). We examined the expression and sub cellular localization of *OsARP* in a submergence tolerant rice cultivar FR13A. The target genes, *OsARP, OsGGT, OsMGD* we examined the expression using cDNA through RT-PCR during submergence of 3, & 7 days. Gradient PCR of cDNA

with these three genes were cloned from 3, & 7days submerged by annealing temperature (°C) of 58, 60, 64, 66, 68, and 70. This PCR containing cDNA of *OsARP* gene was present clear amplification by Tm of 64°C than another two *OsGGT, OsMGD* genes. A time-course study showed that *OsARP*-gene expression increased in FR13A during submergence of 7 days. This indicates that *OsARP* is a membrane bound protein of rice, which is expressed under submergence stresses. The study of abiotic stress response has advanced considerably in recent years. Analyzing of a single stress in plants can be very different from the conditions encountered by plants in the field where several stresses may be occurred simultaneously.[7] This can alter plant metabolism in a novel manner that may be different from that caused by each of the different stresses applied individually, and may require a new type of response that would not have been induced by each of the individual stresses.[8] Therefore, some genes that can be expressed by specific stress might be expressed in other stress as well. The expression of putative vacuolar antiporter regulator *OsARP* was wide spread in all the plant parts under salt stress. The salt stress increased the transcripts levels of OsNHX1 in roots and shoots indicating that ionic stress played major role for the expression of this gene in different plant parts.[8] Therefore, expression analysis of *OsARP* gene will be conducted in different rice cultivars and subsequently transgenic crops will be developed through *Agrobacterium* mediated transformation.

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None.

Conflict of interest

The author declares no conflict of interest.

Refrences

1. Neeraja CN Maghirang-Rodriguez R, Pamplona A, Heuer S, et al. A marker-assisted backcross approach for developing submergence-tolerant rice cultivars. *Theor Appl Genet*. 2007;115(6):767–776.
2. Iftekharuddaula KM, Newaz MA, Salam MA, et al. Rapid and high-precision marker assisted backcrossing to introgress theSUB1 QTL into BR11, the rainfed lowland rice mega variety of Bangladesh. *Euphytica.* 2011;178(1):83–97.
3. Ray BP, Hassan L, Nasiruddin KM. *In vitro* Regeneration of Brinjal (*Solanum melongena L*). *Bangladesh J Agril Res*. 2011;36(3):397–406.
4. Mohanty HK, Chaudhary RC. *Breeding for submergence tolerance in rice in India*. In: Progress in rainfed lowland rice, Philippines: International Rice Research Institute; 1986. p. 191–200.
5. Qi Y, Yamauchi Y, Ling J, et al. The submergence-induced gene OsCTP in rice (*Oryza sativa* L.) is similar to Escherichia coli cation transport protein Cha C. *Plant Sci*. 2005;168(1):15–22.
6. Mittler R, Merquiol E, Hallak-Herr E, et al. Living under a "dormant" canopy: a molecular acclimation mechanism of the desert plant Retama raetam. *Plant J*. 2001;25(4):407–416.
7. Rizhsky L, Liang H, Mittler R. The combined effect of drought stress and heat shock on gene expression in tobacco. *Plant Physiol*. 2002;130(3):1143–1151.
8. Fukuda A, Nakamura A, Tagiri A, et al. Function, intracellular localization and the importance in salt tolerance of a vacuolar Na+/H+ antiporter from rice. *Plant Cell Physiol*. 2004;45(2):146–159.