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Genetic diversity and association mapping for salinity tolerance in Bangladeshi rice landraces

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ABSTRACT

Breeding for salinity tolerance using Bangladeshi rice landraces and understand genetic diversity has been limited by the complex and polygenic nature of salt tolerance in rice genotypes. A genetic diversity and association mapping analysis was conducted using 96 germplasm accessions with variable response to salt stress at the seedling stage. These included 86 landraces and 10 *indica* varieties and lines including Nona Bokra, from southern Bangladesh. A total of 220 alleles were detected at 58 Simple Sequence Repeat (SSR) marker loci randomly distributed on all 12 rice chromosomes and 8 Sequence Tagged Site (STS) markers developed for genes *SKC1*, *DST*, and *SalT*. The average gene diversity was 0.5075 and polymorphism information content value was 0.4426, respectively. Cluster analysis revealed that 68 and 21 accessions were clustered into 2 distinct groups, possibly corresponding to *indica* and *japonica* groups, respectively and the remaining 7 landraces were classified as an admixed group. In addition to Wn11463, the STS marker for *SKC1*, RM22418 on Chr. 8 was significantly associated with salinity tolerance, at the location of a QTL detected in previous studies. Our findings of favorable alleles associated with salinity tolerance in Bangladeshi rice landraces, as well as the development of STS markers for salt tolerance genes, will be helpful in future efforts to breed salinity tolerance in rice.

1. Introduction

Soil salinity is one of the most important environmental factors restricting rice production. Rice is classified as a salt sensitive crop, especially in the early growth stages [1]. There are about 380 million hectares of saline soils globally, which are widely distributed in arid and semi-arid areas as well as seasonally dry coastal areas [2]. In Bangladesh 2.8 million hectares of rice land in coastal areas are currently affected by salinity [3]. Salt tolerant varieties are considered to be the most economical and effective way to increase crop production on saline lands [3].

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Table 1 – STS markers for three salt-tolerant genes.

Gene	Marker	Forward primer sequence (5′–3′)	Reverse primer sequence (5′–3′)
SKC1	Wn11463	TCCTCCTTCTCTCGCAAC	GATCCACTCGTCACAGG
	Wn11466	GCTTCCCAATAATTTCGACCT	CCCACCAATACTAAAGATCCTG
SalT	Wn13900	GTACGGGTTCACATCCTC	ACCCTCTATTAATTCACTACCA
	Wn13902	CACCAGCGTCATACTCT	CAAAACTGAGTAGGAATACCGTGA
	Wn13903	CTGTATCAACTGCATTCGTGT	GCTTGGTCAAACTCCGT
DST	Th32637	TCGTATAGTAGGCTTTCATGGC	TTTCACAGGTGCGAGAGCTT
	Th32638	AGAGAAGCCAAGAAATCGAC	TCCAAGCTCCACCTACTCC
	Th32639	CTATTTGGCTTCGCAAGGACA	CGCCCACTTTAATCATATTCCCT

Many studies show that salinity tolerance is a complex trait controlled by quantitative trait loci (QTL) [4]. For example, 11 QTL for seedling survival were identified on chromosomes 1, 4, 6, 7, and 9 using a Nona Bokra × Koshihikari $F_{2:3}$ population. One major QTL for shoot K^+ concentration on chromosome 1 (*qSKC-1*) explained 40.1% of the total phenotypic variance [5]. *SKC1* was subsequently map-based cloned; it encodes a Na^+ transporter of the HKT type and is involved in Na^+ and K^+ homeostasis [6]. Another QTL, *SalTol*, was fine-mapped to the same region and also acts mainly to control shoot Na^+/K^+ homeostasis, suggesting that *SKC1* may be the causal gene underlying the *SalTol* QTL [7]. A salt-induced gene *SalT*, identified previously, was found to co-localize with *SalTol* and was 2.4 Mb away from *SKC1* [8].

Landraces are currently being exploited as preferred potential donors of abiotic stress tolerance traits because of their local adaptation [4]. For instance, favorable alleles at the *SKC1* and *SalTol* loci were derived from *indica* landraces Nona Bokra [6] and Pokkali [7], respectively. With close genetic similarity to current cultivars, the tolerance traits could readily be introduced into commercial breeding lines [4].

The southern part of Bangladesh is well known for high salinity and popular landraces from the region are well adapted and regarded as possessing some resistance to salt stress, particularly at the seedling stage [9]. The situation is further increased by selection of rice landraces, which has happened in the case of association analysis using collected germplasm from this region. An evaluation of genetic diversity and identification of markers in Bangladesh rice landraces could provide useful information for genetic improvement of salt tolerance.

SSR have been the predominant molecular markers used in kinship and population studies because they are multiallelic, reproducible, PCR-based, and generally selectively neutral [10]. They can be applied for genetic diversity and association analysis of important agronomic and quality traits in rice [11–15].

In the present study, 96 rice accessions from southern Bangladesh were subjected to a genetic diversity and association mapping study using SSR and STS markers. The main objective of the present study was to: 1) characterize the genetic diversity and population structure of Bangladesh rice landraces; 2) develop novel STS markers for salt-tolerance genes and confirm their effect; and 3) identify loci significantly associated with salinity tolerance in rice.

2. Materials and methods

2.1. Rice materials

A total of 96 rice accessions were collected by Bangladesh Institute of Nuclear Agriculture (BINA) and used in this study. They included 86 landraces from southern Bangladesh, 9 *indica* varieties and lines, and salt tolerant Nona Bokra, the donor of *SKC1*, was used as the tolerant control (Table S1).

2.2. Screening for salinity tolerance

Hydroponic system based on the IRRI protocol [16] was used in the glasshouse at BINA to evaluate the salt tolerance responses of rice genotypes at the seedling stage. Three replications of 20 plants were tested under salt stress of 12 dS m^{-1}. The modified standard evaluation score (SES) of IRRI [17] was used to assess visual symptoms of salt toxicity 21 days after sowing. Binadhan-8 was used as a second tolerant control and Binadhan-7 was the susceptible control.

2.3. Marker genotyping

DNA was extracted from 6–8 individuals in each accession following the method of Zheng et al. [18]. To facilitate

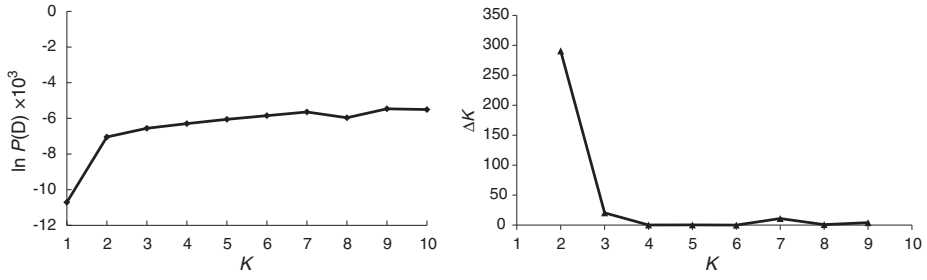

Fig. 1 – Average ln *P*(D) with *K* = 1–10 and Δ*K* with *K* = 2–9.