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Surveying Allelic diversity of *OsSRO1c* in 3K rice germplasm

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Abstract
Rice production is severely constrained by drought stress, which causes enormous economic losses. The global climate change is evolving into a more significant problem. Enhancing agricultural yield in the drought-prone rainfed areas has become critical in light of the current and projected global food demand. Due to their complexity and multigenic regulation of drought tolerance features, breeding for drought tolerant rice varieties is a challenging task that would significantly impede present research. Drought hinders plant development and decreases crop yield. Stomatal aperture regulates CO_2 uptake and water loss to the atmosphere, and thus plays a significant role in agricultural yield gain and drought tolerance. The current study was attempted to find the allelic diversity of abiotic stress related gene, *OsSRO1c* in a diverse set of 3K rice genome panel. *Insilico* analysis of *OsSRO1c* sequence among the 186 rice genotypes revealed the presence of four nonsynonymous SNP. Three haplotypes (H1, H2 and H3) were identified for *OsSRO1c* based on four non synonymous SNPs.

Keywords: Surveying, *OsSRO1c*, germplasm, synonymous, SNPs

Introduction
Rice production needs to be increased by at least 50% by 2050 to meet the growing requirements of global population. But, any further increase in rice production needs to overcome challenges *viz.*, yield plateau, declining land, labour and water resources and challenges due to climate change. Agricultural production losses frequently occur due to different abiotic stresses as well as from increased frequency of extreme weather conditions (Mittler and Blumwald, 2010) [5]. Plants have evolved to overcome various environmental cues through a range of physiological, biochemical, and molecular responses. Drought being one of the important abiotic stresses causing declines in crop yield can be overcome by employing several strategies to reduce the water loss under drought condition. One such strategy is to reduce the water loss through stomata. Stomata have a greater role to play under drought condition in regulating the water loss through stomatal pores.
Genes in the SRO (Similar to RCD One) family have been examined for their sensitivity to abiotic stressors as well as changes in expression during development (Jaspers *et al*. 2010) [3]. *OsSRO1c*, a rice ortholog of SRO, was discovered and described as a downstream *SNAC1*-regulated gene (You *et al*., 2013) [7]. Under drought stress, it was discovered that guard cells express *OsSRO1c* predominately. The *OsSRO1c* mutant displayed increased drought sensitivity. By controlling H_2O_2 homeostasis, *OsSRO1c* overexpression improved stomatal closure and decreased water loss. Through interactions with several stress-related proteins, the OsSRO1c is engaged in oxidative stress and controls the stress response. Haplotype breeding can benefit from knowledge of *OsSRO1c* allelic variations. This research was carried out to ascertain the allelic variants and haplotype analysis of *OsSRO1c* in 3K RG panel subset.

Materials and Methods
A diverse subset of 186 rice accessions from the 3K panel was used for the study. The 186 genotypes were found to have 36 different origins indicating its diverse geographic distribution. To retrieve data on *OsSRO1c*, allelic variation for these 186 diverse rice lines, the SNP seek database was used. Haplotype analysis was carried out by downloading the allelic variants of the gene *OsSRO1c* in PLINK format and utilizing the SNP seek database to visualize the nucleotide polymorphisms present in the *OsSRO1c* (LOC_Os03g12820). SNP seek database was exploited to perform haplotype analysis for *OsSRO1c* with the reference Nipponbare genome and by adopting default parameters with Calinski criteria for k-group determination. The '3kfiltered' SNP set available in the SNP seek database for the analysis.

The filtered dataset was obtained from the Base SNP set with alternative allele frequency of at least 0.01, proportion of missing calls per SNP as 0.2 (Mansueto *et al.*, 2016) [4] and this was available in the SNP seek database. Allele mining was carried out by selecting only the non-synonymous SNPs. Obtained results were converted into haploview file using PLINK (Purcell *et al*., 2007) [6] and used for haplotype analysis using Haploview (version 4.1) (Barrett *et al*., 2005) [2]

Results and Discussion

Surveying Allelic diversity of *OsSRO1c*

The allelic diversity of these diverse rice lines was carried out using the SNPs available at SNP seek database. Allelic diversity analysis of *OsSRO1c* revealed that the gene contains 25 SNPs and 18 INDELs for the screened 186 genotypes surveyed. Of the 25 SNPs four SNPs located in the *OsSRO1c* was found to be non-synonymous leading to Arg32Ser; Lys126Glu; Pro377Leu and Gln381Arg (Table 2). Out of the four non-synonymous SNPs interestingly we identified two SNPs (chr03-6895363 and chr03-6895645) causing novel variations in the first exon and two SNPs (chr03-6896729 and chr03-6896741) causing variations in the second exon region of the *OsSRO1c* allele. These four non-synonymous SNPs formed three haplotype groups (Fig 1). SIMILAR TO RCD ONE (SRO) a plant specific gene family is involved in growth and abiotic stress reactions. SRO proteins can be divided into two groups and five subgroups according to the presence of the poly (ADP-ribose) polymerase catalytic (PARP) and C-terminal RCD1-SRO-TAF4 domains (Jaspers *et al*. 2010) [3]. Previous studies on *OsSRO1c* clearly states that it is a direct target of the drought stress-related transcription factor SNAC1 (You *et al*. 2013) [7]. *OsSRO1c* promotes stomatal closure and H2O2 accumulation in rice via a novel pathway involving the SNAC1 and DST regulators (You *et al*. 2014) [8]. The *OsSRO1c* is induced under multiple stress conditions including drought, salt, cold, heat, UV, wounding, and ABA treatments. Compared to other members of the gene family, *OsSRO1c* showed greater expression and responsiveness under various multiple stress conditions (Ahlfors *et al*. 2004 and Jaspers *et al*. 2010) [1, 3]. This explains why it is crucial to use this gene in crop improvement breeding applications.

Table 1: Non synonymous SNPs allelic variations in *OsSRO1c* gene for the subset of 186 diverse rice lines

Position	Position	Allele		Type
chr03-6895363	Exon 1	1bp	C/A	SNP
chr03-6895645	Exon 1	1bp	A/G	SNP
chr03-6896729	Exon 2	1bp	C/T	SNP
chr03-6896741	Exon 2	1bp	A/G	SNP

Table 2: SNP Effect of allelic variation found for the gene *OsSRO1c*

POSITION	Alt (ANN)	Effect (ANN)	Putative_impact (ANN)	HGV S.c (ANN)	HGV S.p (ANN)
chr03-6895363	A	missense_variant	MODERATE	c.94C>A	p.Arg32Ser
chr03-6895645	G	missense_variant	MODERATE	c.376A>G	p.Lys126Glu
chr03-6896729	T	missense_variant	MODERATE	c.1130C>T	p.Pro377Leu
chr03-6896741	G	missense_variant	MODERATE	c.1142A>G	p.Gln381Arg

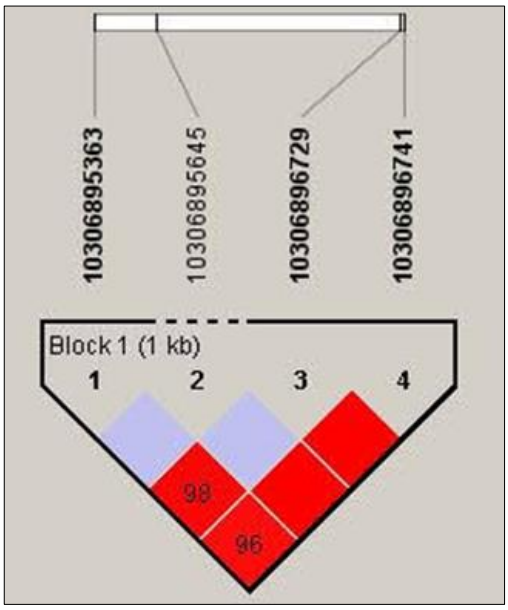

Fig 1: Pairwise Linkage Disequilibrium plot of non-synonymous SNPs in *OsSRO1c* using Haplo View

Conclusion

Insilico analysis carried out to understand the allelic diversity of *OsSRO1c* was found to have four non synonymous SNPs surveyed for the studied subset of 186 diverse rice accessions from the Rice 3K-panel. The haplotype analysis for the four non-synonymous SNP found within the gene *OsSRO1c*, three haplotype combinations were observed. The allelic variations found in this study for the stomata regulating gene *OsSRO1c* can be deployed by employing haplotype-based breeding method of rice crop improvement.

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