

## **Allelic diversity of *OsEPF2* governing stomatal regulation in rice**

### **ABSTRACT**

Rice production has been significantly hindered by frequent drought events in recent times, directly impacting food security and agricultural sustainability. In the current study, a set of 100 diverse rice accessions from 3K RG panel were evaluated for *Epidermal patterning factor 2* (*OsEPF2*) loci which governs the stomatal density on the matured leaves by preventing excessive stomatal development. This plays an important role in drought tolerance in plants. Hence, this gene was selected for analyzing haplotype variation which revealed 27 SNPs and 59 INDELS. Seven significant non-synonymous SNPs, grouped the one hundred accessions into three haplotype groups with the allelic combination CGATGCG for H1, TTGGAAA for H2 and CGGGGCG for H3. This shows the allelic diversity of the population that can be potentially employed in breeding of rice varieties with improved drought tolerance and water use efficiency by assembling superior haplotype.

*Keywords: OsEPF2, allelic diversity, stomata regulation, drought, haplotype breeding.*

### **1.INTRODUCTION**

Rice is one of the core crops in the world food framework, consumed by more than half of the population of the world. In the current scenario of diminishing agricultural acreage and increasing population, there is an urgent need to increase grain production. Over the past decade, global rice production has been severely constrained by frequent drought incidences due to climate change in the rainfed rice agroecosystem (Pandey and Bhandari, 2009). The complexity of this situation is exacerbated in the rainfed and marginal areas which encounter irregular rainfall, high temperatures, intense solar radiation and poor soil quality. When combined with other stresses, drought induces an intricate response in plant's physiology and metabolism (Nahar *et al.*, 2016). Drought stress accompanied by heat stress when it occurs during the reproductive stages of rice, causes the maximum reduction in the yield of rice (Jagdish *et al.*, 2011). These situations also induce the closure of stomatal apertures which further inhibits the gaseous exchange in plants. These tiny openings called stomata play an important role in the interaction between gases, water, and soil-plant-atmosphere systems as well as acts as a barrier against certain pathogens (Buckley, 2005; Jolliffe *et al.*, 2023). The stomatal density can be genetically modified to mitigate either high-temperature stress or drought stress. Enhancement in the stomatal density of the rice plants leads to enhanced stomatal conductance and photosynthetic rate to deal with evaporative cooling during high-temperature stress (Rathnasamy *et al.*, 2022). Conversely, reducing the stomatal density may reduce the stomatal conductance and water loss, thus leading to improved Water Use Efficiency (WUE) and drought tolerance (Caine *et al.*, 2019; Pitaloka *et al.*, 2022). The stomatal density and stomatal patterning are controlled by a regulatory network of genes in *Arabidopsis* among which three genes, namely, Epidermal Patterning Factor (*EPF1*), *EPF2* and *EPFL9/STOMAGEN* among which *EPF1* and *EPF2*

are negative regulators of stomatal development and patterning whereas *EPFL9* is a positive regulator. *EPF2* primarily inhibits the asymmetric cell division in the stomatal lineage and *EPF1* prevents the clustering of stomata at the later stages of the lineage. *EPFL9* regulates the lineage by blocking the interaction of *EPF1* and *EPF2* with the ERECTA family receptors and also affects rice panicle architecture (Guo et al., 2023). Manipulation of any one of the three genes may result in drastic modification of the stomatal density (Mohammed et al., 2019; Hunt et al., 2010). The homolog of *EPF2* in rice influences the density of stomata on mature leaves. It is sensitive to light and its expression gets inhibited under shade (Li et al., 2023). When absent, an excessive number of cells can be observed in stomatal lineage and when overexpressed, there are hardly any stomata (Hunt and Gray, 2009).

Over the years, a number of approaches have been undertaken to impart drought tolerance traits in rice. A genetic variation within the rice gene pool was revealed by screening and characterizing rice germplasm under drought conditions (Swamy and Kumar, 2013). The current study was undertaken with the aim to explore the allelic variations at *EPF2* locus using 100 genotypes from 3K RG panel and any significant variation in the gas exchange parameters was evaluated.

## 2. MATERIALS AND METHODS

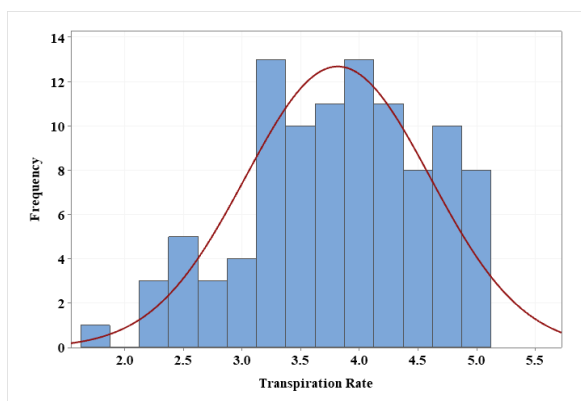
A set of 100 different genotypes from 3K RG panel were sown in Kharif 2021 at Paddy Breeding Station, Tamil Nadu Agricultural University, Coimbatore, India. The standard agronomic practices were followed to raise the plants and a fertilizer dose of 150:50:50 N, P and K kg/ha respectively was applied. Certain gas exchange parameters like photosynthetic rate, transpiration rate, and stomatal conductance were recorded using ADC Bio Scientific- LCI-SD Ultra Compact Photosynthesis system (Promkhambut et al., 2010; Hasan et al., 2017; Munjonjiet al., 2017) from five plants randomly selected in each genotype. Any variation among the target traits was analyzed through descriptive statistics and frequency distribution using Minitab 19 Statistical Software (Allen, 2019).

The investigation of allelic divergence in the *EPF2* gene (LOC\_Os04g38470) within the hundred genotypes involved the retrieval of their sequences from the Rice-SNP seek database in PLINK format, which included only non-synonymous SNPs. The retrieved dataset is then converted into haploview format using gPLINK software (Weeks, 2010). The significant SNPs were determined with a cutoff score of 0.001 (Barrett et al., 2005) and further analyses were carried out by the construction of haplotype groups and Linkage Disequilibrium blocks using Haploview software (version 4.1) (Barrett et al., 2005). The Minitab 19 Statistical Software was used to determine any statistical significance (Allen, 2019).

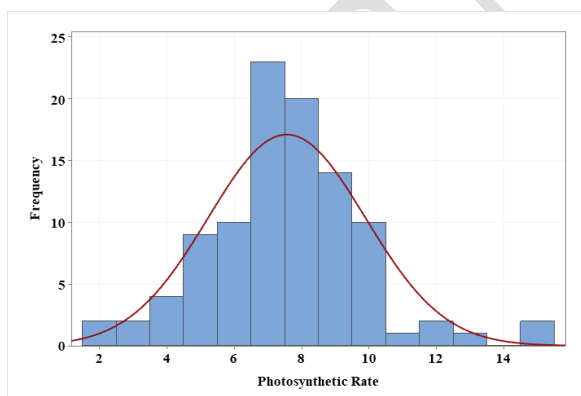
## 3. RESULTS AND DISCUSSION

The gas exchange factors play a major role in the overall growth and development of rice which ultimately affects the productivity of rice. Any variation in the gene, *EPF2* alters the stomatal density on the leaf thus producing an overall effect on the yield of rice by influencing photosynthetic rate and water use efficiency.

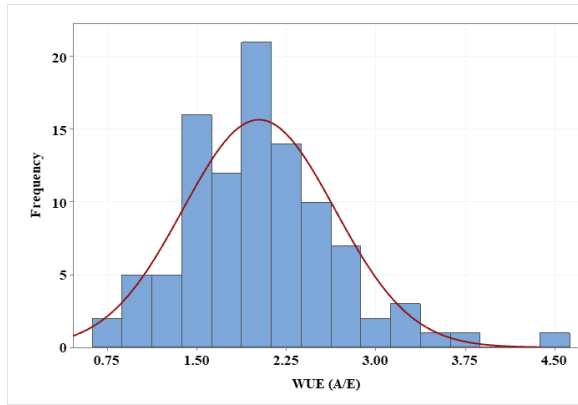
A subgroup constituting 100 accessions from the 3K-RG panel was used for the assessment of transpiration rate, photosynthetic rate, water use efficiency and stomatal conductance. Using Minitab 19 statistical software, histograms were created for all the above-mentioned traits. It was observed that the frequency distribution of these traits among the chosen accessions followed a normal distribution pattern, providing a descriptive visualization of the data distribution for transpiration rate (Fig 1), photosynthetic rate (Fig 2), WUE (Fig 3) and stomatal conductance (Fig 4). From the table 1, it is observed that the mean for transpiration rate, photosynthetic rate, WUE and stomatal conductance 3.82, 7.57, 2.02 and 0.25 respectively while the coefficient of variation is high for the photosynthetic rate (30.84) and WUE (31.48) in the population under study followed by the transpiration rate (20.60). This explains the significant variation in the population for transpiration rate, photosynthetic rate and WUE.



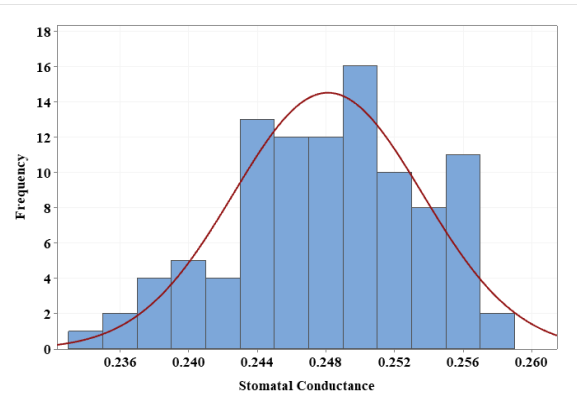
**Fig 1. Frequency distribution for transpiration rate**



**Fig 2. Frequency distribution for photosynthetic rate**



**Fig 3. Frequency distribution for WUE Conductance**



**Fig 4. Frequency distribution for stomatal**

**Table 1. Descriptive statistics for transpiration rate, photosynthetic rate, WUE and stomatal conductance**

Statistics	Transpirationrate	Photosynthetic rate	WUE	Stomatal conductance
Mean	3.82	7.57	2.02	0.25
SE Mean	0.08	0.23	0.06	0.001
SD	0.79	2.33	0.63	0.005
Variance	0.62	5.44	0.41	0.00003
CV	20.60	30.84	31.48	2.22
Minimum	1.76	1.75	0.71	0.23
Maximum	5.12	15.33	4.42	0.26

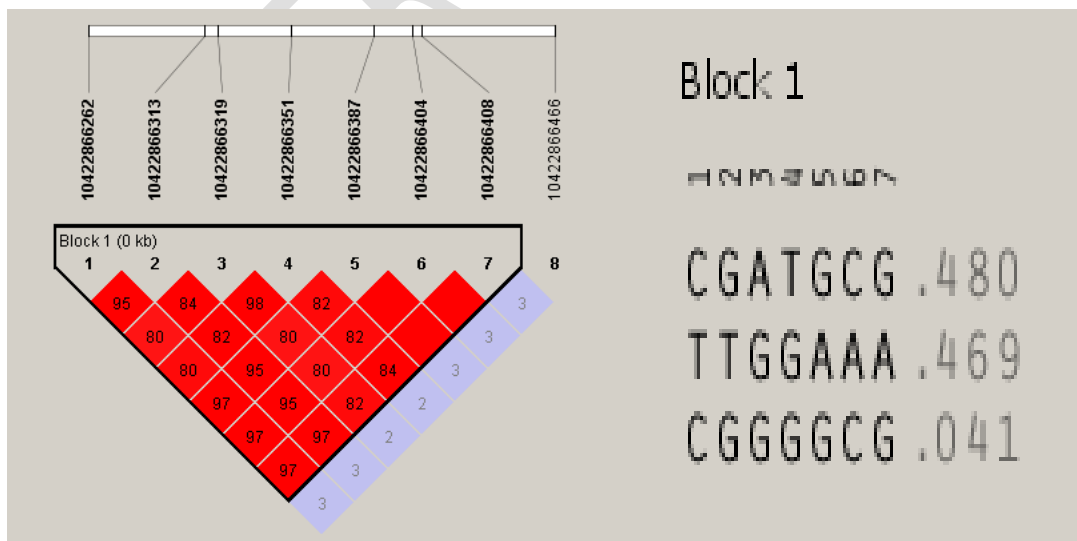
Allelic diversity analysis of *OsEPF2* located in chromosome 4 revealed that the gene contains 27 SNPs and 59 INDELS for the 100 genotypes studied. For the one hundred accessions only the non-synonymous SNPs for the target locus *EPF2* were retrieved from the Rice Seek database. Eight single nucleotide polymorphisms (SNPs) were identified, out of which seven SNPs located at positions chr04-422866262, chr04-422866313, chr04-422866319, chr04-422866351, chr04-422866387, chr04-422866404 and chr04-422866408 played a significant role in determining the haplotype groups leading to the corresponding amino acid changes Phe to Ser, Val to Gly, Gly to Asp, Ala to Ser, Thr to Ala, Arg to Ser and Thr to Ala respectively (Table 2). In a similar manner, Zhao *et al.* (2019) reported twenty-three SNPs, including both synonymous and non-synonymous SNPs, in six known genes that contribute significantly to phenotypic variations in traits related to improved photosynthetic efficiency in both *japonica* and *indica*. Haplotype analysis using Haploview software divided the one hundred accessions into three haplotype groups H1, H2 and H3. This explains the haplotype diversity among the selected accessions. The respective allelic combinations for groups were CGATGCG for H1, TTGGAAA for H2 and CGGGGCG for H3 (Fig 5a). The haplotype groups formed are showing strong

Linkage disequilibrium that was determined using Haploview software (Fig 5b). Out of the 100 accessions under study, forty were found to exhibit H1 allelic combination, forty-five exhibited H2 and four exhibited H3 allelic combination. Chen *et al.* (2020) identified several haplotype groups in 451 different rice accessions including both *japonica* and *indica*, for eight traits concerned with stomatal density and size involving 64 candidate genes. Similarly, Du *et al.* (2022) performed haplotype analysis to study candidate genes, functional SNPs and superior haplotypes associated with flag leaf shape in rice and its adaptation in different rice varieties.

**Table 2: SNP Effect of allelic variation found for the gene *OsePF2***

POSITION	Alt	Effect	Putative impact	HGV S.c	HGV S.p
chr04-22866262	C	Missense variant	Moderate	56T>C	Phe19Ser
chr04-22866313	G	Missense variant	Moderate	107T>G	Val36Gly
chr04-22866319	A	Missense variant	Moderate	113G>A	Gly38Asp
chr04-22866351	T	Missense variant	Moderate	145G>T	Ala49Ser
chr04-22866387	G	Missense variant	Moderate	181A>G	Thr61Ala
chr04-22866404	C	Missense variant	Moderate	198A>C	Arg66Ser
chr04-22866408	G	Missense variant	Moderate	202A>G	Thr68Ala

With the increasing impact of climate fluctuations on global agriculture, there is an urgent need to breed tolerant rice varieties particularly drought tolerant varieties as par with our study. Varieties that are less dependent on water, maintain better nutritional profiles and exhibit higher yields under drought conditions need to be identified urgently to meet out the food requirement under the drastically changing climatic scenario (Khan *et al.*, 2021). This explains the importance of using this gene in future crop improvement breeding applications.



(a) (b)

**Fig 5. LD block explaining Linkage Disequilibrium (a) and haplotype groups based on allelic variation (b)**

#### **4. CONCLUSION**

*In-silico* analysis carried out to understand the allelic diversity of *OsEPF2* detected eight non-synonymous SNPs for the subset of one hundred diverse rice accessions from the Rice 3K-panel. Three different haplotype combinations were revealed when the eight non-synonymous SNPs within the gene *OsEPF2* were analyzed. By using the haplotype-based breeding strategy for rice crop improvement, the allelic variants discovered in this work for the stomata regulating gene *OsEPF2* can be used to create cultivars that are resistant to drought.

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