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SHORT RESEARCH ARTICLE

Analysis of inheritance in a novel seedling stage zebra leaf mutant, Pusa Zebra 18 in rice (*Oryza sativa* L.)

Amaresh, S. Gopala Krishnan*, K. K. Vinod, C. Viswanathan[1], S.V.A.C.R Mithra[2], Ranjith Kumar Ellur, P.K. Bhowmick, Haritha Bollinedi, M. Nagarajan[3], Gaurav Dhawan, Pankaj Kumar and A. K. Singh

Abstract

A novel zebra leaf mutant, Pusa Zebra 18 derived spontaneously from F_2 intercross population of Pusa 44 near-isogenic lines (NILs) exhibits distinguishable yellowish cross bands across the green leaf blade, specifically expressed in the seedling stage from 6 DAT, which diffuses progressively, leading to the recovery of the normal green leaf by 30 DAT. We elucidated the genetics and nature of inheritance for zebra trait in F_2 and BC_1F_2 populations by crossing Pusa 6B with Pusa Zebra 18. The leaves of the F_1 were a normal green color, indicating that the seedling stage zebra leaf trait in Pusa Zebra 18 is recessive. Chi-square goodness of fit analysis of these segregating populations showed that the segregation ratio was fitting to the Mendelian segregation ratio of 3:1. The seedling stage zebra leaf mutation in Pusa Zebra 18 is monogenic recessive in nature. Further, mapping, fine mapping, cloning, and functional characterization need to be carried out, which will help gain insight into the mechanism involved in chlorophyll biosynthesis and regulation studies in rice.

Keywords: Leaf color, Zebra mutant, Pusa Zebra 18, inheritance, segregation

Two pigments, chlorophyll and carotenoid, which are critical for photosynthesis, are primarily responsible for the color of the leaves (Li et al. 2010; Li et al. 2012). Any modification to these pigments, whether in quantity or quality, causes variations in the typical green color of the leaf (He et al. 2012; Feng et al. 2019). Most of these pigment deficiencies in leaves are commonly associated with mutations, often referred as leaf color mutants (Zhao et al. 2014). More than 208 leaf-color rice mutants have been reported in rice (Deng et al. 2014; Sheng et al. 2014), of which zebra mutants are named for the characteristic cross bands on their leaf blades that alternate between green and yellow or white bands in rice (Kusumi et al. 2000). The genetic improvement of rice can be aided by the discovery of genes that cause chlorophyll deficiency (Chai et al. 2011; Liu et al. 2013; Feng et al. 2019) and use of rice leaf-color mutants as a morphological marker in hybrid rice breeding (Wang et al. 2009).

Pusa Zebra 18 is a novel zebra leaf mutant, which exhibits a distinct zebra leaf patterning and phenological stage of expression from earlier reported zebra mutants with yellowish white bands across green leaf blades from the seedling stage from 6 days after transplanting (DAT) to 30 DAT (Amaresh et al. 2023). However, there is a need to elucidate the nature of the inheritance of the zebra leaf mutation in Pusa Zebra 18 to explore its potential value in rice breeding. Hence, the present study was conducted to determine the genetics of zebra leaf trait specifically expressed at the seedling stage in Pusa Zebra 18.

In order to characterize the inheritance of the seedling

Division of Genetics, ICAR-Indian Agricultural Research Institute, New Delhi 110 012, India.

[1]Division of Plant Physiology, ICAR-Indian Agricultural Research Institute, New Delhi 110 012, India.

[2]Division of Molecular Biology and Biotechnology, ICAR-National Institute of Plant Biotechnology, New Delhi 110 012, India.

[3]IARI-Rice Breeding and Genetics Research Centre, Aduthurai 621 101, Tamil Nadu, India.

***Corresponding Author:** S. Gopala Krishnan, Division of Genetics, ICAR-Indian Agricultural Research Institute, New Delhi 110 012, India, E-Mail: gopal_icar@yahoo.co.in

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stage zebra leaf mutation, the plant materials included the seedling stage zebra leaf mutant, Pusa Zebra 18 derived spontaneously from F_2 intercross population of Pusa 44 NILs possessing the quantitative trait loci (QTLs) for reproductive stage drought tolerance, other genotype and Pusa 6B possessing dark green leaves throughout the seedling stage is a maintainer line of the superfine grain aromatic rice hybrid, Pusa RH 10.

Mapping populations were developed by crossing seedling stage zebra leaf mutant, Pusa Zebra 18 (PZ 18) with Pusa 6B, during the off-season 2019-2020 at Rice Breeding and Genetics Research Centre (RBGRC), ICAR-IARI, Aduthurai, Tamil Nadu. The F_1 seeds from the cross PZ18/ Pusa 6B were raised at the experimental farm of the Division of Genetics, ICAR-Indian Agricultural Research Institute (ICAR-IARI), New Delhi, during *kharif* 2020. The F_1 plants were selfed to generate F_2 populations. During *kharif* 2021, the zebra plants from the segregating F_2 population were identified, backcrossed to develop BC_1F_1 and selfed to generate BC_1F_2 population at RBGRC, ICAR-IARI, and Aduthurai. All the BC_1F_2 were raised at ICAR-IARI, New Delhi, during *kharif* 2022, and one of the BC_1F_2 populations segregating for seedling stage leaf color was selected and phenotyped for leaf color trait at the seedling stage. Each of the individual plants from these F_2 and BC_1F_2 populations was phenotyped visually for leaf color trait. Zebra leaves scored as Z and normal green leaves scored as N. Statistical analysis with expected Mendelian segregation ratio using the chi-square (χ^2) test, the observed phenotypic data of F_2 populations were analyzed for the goodness of fit.

Mode of inheritance of zebra leaf color trait at seedling stage in Pusa Zebra 18

The F_1s from the crosses were found to produce normal green-colored leaves from the seedling stage to maturity, indicating that the zebra leaf color is recessive. It was observed that on the basis of leaf colour out of 834 plants, 630 plants were producing completely normal green leaves (N) like Pusa 6B, while 204 plants were expressing zebra leaf color phenotype (Z) like Pusa Zebra18 at the seedling stage. In another F_2 population out of 1001 plants, 756 plants were producing completely normal green leaves (N) while 245 plants were expressing zebra leaf color phenotype (Z) at the seedling stage. The chi-square test for goodness of fit showed that the observation recorded for the leaf color phenotype at the seedling stage had a good fit to the mendelian segregation ratio of 3:1 ($p = 0.72$ and $p = 0.70$), indicating that the zebra leaf color trait in the cross, Pusa Zebra 18/Pusa 6B is governed by a single recessive gene (Table 1).

Segregation for zebra leaf trait in the backcross populations derived from Pusa Zebra 18/ Pusa 6B// Pusa 6B

It was observed that on the basis of leaf color, out of 469 BC_1F_2 plants from one of the segregating BC_1F_2 populations, 342 plants were producing completely normal green leaves (N), while 127 plants were expressing zebra leaf color phenotype (Z) at the seedling stage. The chi-square test for goodness of fit showed that the observation recorded for the leaf color phenotype at the seedling stage had a good fit to the Mendelian segregation ratio of 3:1 ($p = 0.30$). In another BC_1F_2 population out of 462 plants from the segregating BC_1F_2 population, 333 plants were producing completely normal green leaves (N), while 129 plants were expressing zebra leaf color phenotype (Z) at the seedling stage. The chi-square test for goodness of fit showed that the observation recorded for the leaf color phenotype at the seedling stage had a good fit to the Mendelian segregation ratio of 3:1 ($p = 0.15$) (Table 2).

Based on segregation analysis, the inheritance of the zebra leaf trait in the mutant Pusa Zebra 18 in F_2 and the segregating BC_1F_2 populations was found to be monogenic recessive in nature. All the 17 zebra leaf trait mutants, including *z-1 to z-6* (Kinoshita and Takahashi 1991), *zn* (Kinoshita and Takamure 1984), z-7 to *z-13* (Sanchez and

Table 1. Segregation for seedling stage zebra leaf trait in the F_2 populations from the cross, Pusa Zebra 18/Pusa 6B

Cross (PZ 18/ Pusa 6B)	Total no. of F_2 plants	No. of F_2 plants		Expected ratio	p-value
		Normal green leaves	Zebra patterned leaves		
Population 1	834	630	204	3:1	0.72
Population 2	1001	756	245	3:1	0.70

Table 2. Segregation for seedling stage zebra leaf trait in the segregating BC1F2 populations from the cross, Pusa Zebra 18/ Pusa 6B// Pusa 6B

Segregating Backcrosses (Pusa Zebra 18/ Pusa 6B// Pusa 6B)	Total no. of BC_1F_2 plants	No. of BC_1F_2 plants		Expected ratio	p-value
		Normal green leaves	Zebra patterned leaves		
Population 1	469	342	127	3:1	0.30
Population 2	462	333	129	3:1	0.15

Khush, 1992), *Zl -2* (Zhao et al. 2014), *z-15* (Wang et al. 2009), *z-16* (Liu et al. 2018) have been found to be monogenic recessive. The inheritance of the zebra leaf trait in the mutant, Pusa Zebra 18, also exhibited a typical Mendelian segregation ratio of 3:1, indicating that the mutation governing the zebra leaf trait by a single recessive gene. However, the phenological stage (seedling stage from 6 DAT to 30 DAT) of zebra leaf color expression differs from all the earlier reported zebra leaf mutants, making it a novel seedling stage zebra mutation (Amaresh et al. 2023).

Authors' contribution

Identification of mutant and Conceptualization of research (GKS); Designing of the experiments (GKS, A, KKV, VC, SVACRM, RKE, HB, AKS); Contribution of experimental materials (GKS); Execution of field/lab experiments and data collection (GKS, A, MN, GD, PK, PKB); Analysis of data and interpretation (GKS, A, KKV); Preparation of manuscript (A, GKS).

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