Indian J. Genet. Plant Breed., (2024); 84(1): 124-126
https://doi.org/10.31742/ISGPB.84.1.13

ISSN: 0975-6906

www.isgpb.org

SHORT RESEARCH ARTICLE

Leaf rolling and senescence scores as selection criteria to identify drought-tolerant genotypes in basmati rice (*Oryza sativa* L.)

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Abstract

Thirty-eight genotypes of basmati rice were evaluated to identify drought-tolerant genotypes by using leaf rolling and leaf senescence scores under drought conditions, and further validation was done with PCR studies using drought tolerance genes. The genotypes Taravadi, Pusa Sugandh-5, Hariyana Basmati-1, Hariyana basmati-2, Palim basmati, Vallabh Nagina-1, Ranbir basmati, Sanwal basmati, Vallabh basmati-21, Vallabh basmati-22, Vallabh basmati-24, Kasturi, Malviya and Type-3 were found as suitable drought tolerant genotypes. The selection criteria based on leaf rolling and senescence could be useful for breeding drought-tolerant genotypes of basmati rice.

Keywords: Basmati rice, drought, leaf rolling, molecular marker, QTL, senescence.

One of the primary factors reducing the yield of rice is drought. Crop phenology, plant development, and the timing, length, and degree of drought stress all significantly impact on drought sensitivity. In rice, the quantitative trait loci for these traits have been identified (Salunkhe et al. 2011). Two droughts tolerant QTLs were selected for the present study *i.e.,* QTL-qSN-4b and QTL-rn7a (Table 1).

Thirty-eight genotypes were evaluated in a Randomized Block Design in three replications, which were induced with drought stress before the onset of the reproductive stage, during *kharif* 2022 at Crop Research Centre and Technology Park of SVPAUT, Meerut with a spacing of 30x20 cm^2. Leaf rolling and senescence scores (Table 2) were recorded as per the standard evaluation system given by IRRI (2014). Leaves were collected from 90-95 days old plants of 38 basmati genotypes, IR 64 (drought susceptible genotype) and N22 (drought tolerant genotype) from the experimental plot into sterilized plastic poly-bags and kept in the thermo-insulated icebox. Then, these leaf samples were stored at -80°C in a deep freezer to isolate genomic DNA. Isolation of DNA was done based on the modified protocol of cetyl-trimethyl ammonium bromide (CTAB) by Doyle and Doyle (1987). Amplification reactions were performed in BIO-RAD My Cycler™. The bands were observed through Alpha Infotech gel documentation on a 2% agarose gel.

Selection of draught tolerant genotypes

Moisture stress imposition was started before panicle initiation of the crop and given up to 15 days during the reproductive stage by withholding the water in the field. Four soil samples were collected from the stressed field for every five days after imposing the drought condition and soil moisture of the bulked sample was measured by using the gravimetric method and observed a drastic change in the soil moisture content such as 39.76, 30.27, 13.20 and 10.44%, respectively before the initial imposition, after five days, 10 days and at the final stage of drought treatment, *i.e.,* after 15 days during *kharif* 2022 (Fig. 1). Especially, after 15 days with cut off irrigation, severe leaf rolling and leaf drying was observed among most of the genotypes. The reproductive stage started at the first week of August during *kharif*. Two

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How to cite this article: Veerala P., Chand P., Das T.R., Gangwar L.K. and Kumar R. 2024. Leaf rolling and senescence scores as selection criteria to identify drought-tolerant genotypes in basmati rice. Indian J. Genet. Plant Breed., **84**(1): 124-126.

Source of support: Nil

Conflict of interest: None.

Received: May 2023 **Revised:** Nov. 2023 **Accepted:** Dec. 2023

Table 1. Position, Flanking markers, LOD and PVE values for the selected QTLs in rice

S. No.	QTL/gene	Ch. No.	Start	End	LOD	Marker Interval-1	Marker Interval-2	Trait name	PVE (%)	Reference
1.	rn7a	7	11391449	13444846	14.18	C39	RM214	Root number	11.70	Li et al. 2005
2.	qSN-4b	4	31356068	33083265	13.20	RM255	RM349	Spikelet number per panicle	12.85	Zou et al. 2005

Table 2. Leaf Rolling and Senescence scores of 38 basmati rice genotypes

Leaf rolling score	Phenotype of leaves	Name of genotypes	Leaf senescence score
0	Healthy leaves	Pusa Basmati-6, Punjab basmati -5, Pusa Sugandh-5, Vallabh basmati-21, Malviya and Taravadi.	1
		Pusa Basmati-1609	5
1	Leaves start to fold (shallow V shape)	Pusa Basmati-1637, Improved Pusa Basmati-1,Basmati-370,Basmati-386,Pant basmati-2,Punjab basmati-2,Punjab basmati-4, Hariyana Basmati-1, Hariyana basmati-2, Sanwal basmati, Vallabh basmati-22, Vallabh basmati-24 and Type-3.	1
		Pusa Basmati-1121, Pusa Basmati-1718, Pusa Basmati-1728, Pant basmati-1, Pant sugandh dhan-15, and Pusa Sugandh-3	5
3	Folded leaves (Deep V shape)	Pusa Basmati 1612, Ranbir basmati, Vallabh basmati-23, Vallabh Nagina-1, CSR-30 and Kasturi.	1
		Basmati-564, Punjab basmati-3, Pusa Basmati-1509, Pusa Basmati-1692, Pusa Basmati-1 and Palim Basmati	5

leaf samples were collected from all the 40 rice genotypes, one from before drought imposition and other from drought treated one after panicle exertion for recording leaf rolling and leaf senescence scores (Table 2) and comparative leaf samples are depicted in Supplementary Fig. 1. The symptoms of leaf senescence were categorized as, 1: slow and late natural green colored leaves; 5: intermediate, yellow upper leaves and 9: early and fast, all yellow leaves and dead. The lines Pusa Basmati 1612, Ranbir basmati, Vallabh basmati-23, Vallabh Nagina-1, CSR-30 and Kasturi may possess different mechanisms such as crop water usage and water application efficiency, which could be employed in turn for drought experiments, there are also drought escape mechanisms, drought avoidance mechanisms, and flexibility in leaf rolling and unrolling. Duan et al. (2018) suggested that traits like leaf rolling and leaf drying were suitable traits for simple selection improvement in drought tolerance. However, no genotype in the present study fell in the category of 5 (fully cupped leaves, U shape), 7 (touching leaf margin, O shape and 9 (tightly rolled leaves).

Amplification of the isolated samples of DNA from leaf samples of 38 basmati rice genotypes along with IR-64 and N-22 (15 days reproductive stress treatment) was performed with reliable primers. Four drought tolerance genes (*vg0712623096, vg0431750843, vg0432626757* and *vg0433031562*) specific molecular markers were used to detect bands among these 40 rice genotypes. All four drought-tolerant genes or markers produced distinct,

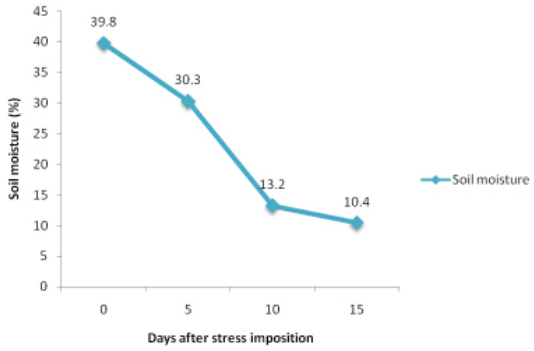

Fig. 1. The percentage of Soil moisture recorded at 60 cm deep in a moisture stressed rice field during *kharif* 2022

reproducible bands and showed positive linkage with drought-tolerant genes in 40 rice genotypes used in the present investigation, with each of the reactions at least thrice by a slight change in the protocol. In addition, the band pattern shown by N22 was also considered for screening the genotypes as it is a well-known drought-tolerant genotype. The amplification products of the four molecular markers were used to detect the drought tolerance gene in basmati rice (Table 3). Similar findings on the assessment of phenotypic and molecular data for screening rice genotypes for drought resistance are reported earlier (Matin et al. 2022).

It is necessary to create screening techniques that are straightforward, repeatable, and predictive of performance in the target environment to find sources of drought

resistance. Traditional methods of assessing drought resistance features such as stay-green, leaf senescence, and leaf rolling have relied on subjective, error-prone, poorly quantified, and time-consuming manual assessments. Generally, rice leaves undergo leaf rolling in response to the severity of moisture stress. Leaf rolling and senescence were plant responses, especially plants in the family Poaceae, which correlate with reducing leaf expansion, lower stomatal conductance, and transpiration rate. Therefore, leaf rolling and senescence scores can assist scientists in developing plants that withstand to water stress. These traits might be used to evaluate basmati rice varieties in drought environments for further validation. The four primers belong to two QTLs, primer *vg0712623096* (LOC_Os07g22450) from rn7a located on chromosome 7 encoding NAC domain-containing protein and the primers *vg0431750843* (LOC_Os04g53310) encoding chloroplast precursor soluble starch synthase 3, *vg0432626757* (LOC_Os04g54850) encoding pectin acetylesterase domain-containing protein and *vg0433031562* (LOC_Os04g55520) encoding AP2 domain-containing protein from qSN-4b located on chromosome 4. Considering the overall study of visual scoring and PCR studies, for drought tolerance screening of 38 basmati rice genotypes, the genotypes Taravadi, Pusa Sugandh-5, Hariyana Basmati-1, Hariyana basmati-2, Palim basmati, Sanwal basmati, Ranbir basmati, Vallabh basmati-21, Vallabh basmati-22, Vallabh basmati-24, Vallabh Nagina-1, Kasturi, Malviya and Type-3 were considered as desirable drought tolerant genotypes as they have characteristic drought tolerant genomic regions in comparison with other genotypes and 2-3 irrigations can be saved in comparison with others, which could be utilized for the betterment of marker-assisted selection (MAS) and also very useful in developing the drought resistant\ tolerant variety or RILs and NILs which can be used in future as drought tolerant lines/genotypes.

Supplementary material

The Supplementary Fig. S1 is provided, www.isgpb.org

Authors' contribution

Conceptualization of research (PC, LKG), Designing of the experiments (PC, PV), Contribution of experimental materials (PV, PC, LKG), Execution of field/lab data collection (PV, RK, PC), Analysis of data and interpretation (PC, PV, TRD, LKG), Preparation of the manuscript (PV, TRD, PC)

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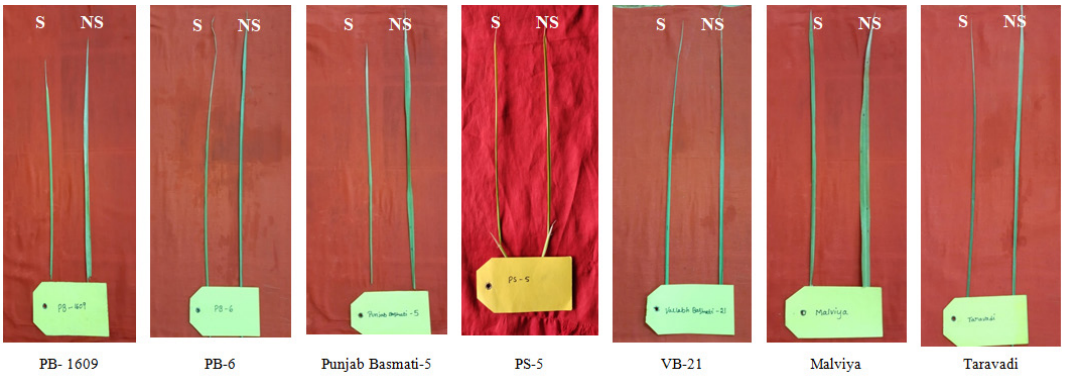
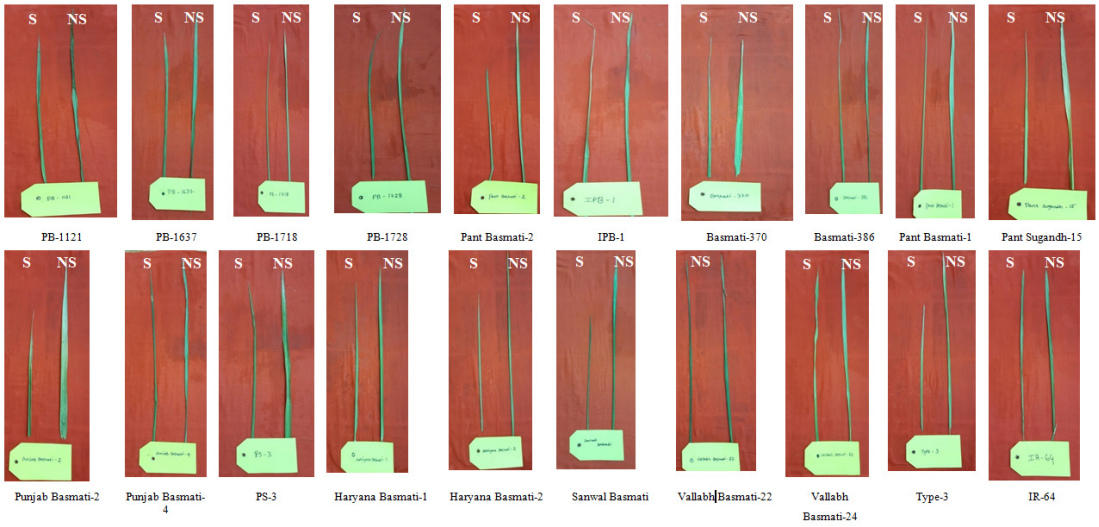


i) Genotypes leaf rolling score-0



ii) Genotypes leaf rolling score-1

iii)Genotypes leaf rolling score-3

(S: leaf harvested from plant under stress NS: leaf harvested from plant before stress imposition)

Supplementary Fig. 1. Comparison of leaf rolling of basmati rice under stress and non stress conditions