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## Research Article

### Analyzing rice (*Oryza sativa* L.) panicle structure of 122 RILs using P-TRAP software for spikelet related traits

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#### Abstract

Panicle architecture is shown to have a direct impact on rice grain yield, making it an important characteristic for rice varietal improvement. In this study, we focus on the Panicle Trait Phenotyping Tool (P-TRAP), freely accessible, platform-independent software for analyzing rice panicle architecture, as one of the ways for generating comprehensive and reproducible panicle structure data and identifying superior breeding lines. In this analysis, the rice panicle structures of 122 RILs along with their parents were analyzed using P-TRAP software. P-TRAP assesses nine spikelet characteristics and its impact on grain yield. All traits had positive and significant associations and have a direct influence on grain yield. The findings indicate that most characteristics' coefficients of variation had lower values, with the exception of the characteristics spikelet number, spikelet area and grain yield. In comparison to other traits, the standard error for the traits spikelet number and grain yield had a larger value. To better understand their genetic basis, these quantitative traits can be exploited in genome-wide association analyses. Hence, analyzing these traits is crucial. The P-TRAP tool is preferred because it reduces human effort and provides data in a shorter time span when compared to the traditional method.

**Keywords:** Rice, spikelet, p-trap, grain yield

#### INTRODUCTION

Rice is a staple food consumed globally by a majority of the world's population. The structure of the rice panicle is critical for breeding because it has a direct impact on the number of grains per panicle and as a result, the overall rice grain yield is also impacted. Panicle branching is a complicated process driven by genetic, environmental and hormonal influences (Xing and Zhang, 2010). Rice species differ in terms of morphological characteristics (such as panicle complexity), ecological, environmental, abiotic and biotic stress tolerance (Vaughan *et al.*, 2003). For breeding programmes to be designed and to improve production, variability is a prerequisite (Lakshmi *et al.*, 2022). The best criterion for boosting productivity is grain

yield (Prathiksha *et al.*, 2022). Panicle morphological traits must be defined and measured in order to maximize the diversity of rice panicle resources. Plant phenotyping comprises screening of large collections of accessions for novel intriguing features and accessing existing phenotypic data to determine the genes involved in their diversity so that these genes can be used in plant breeding. The standard approach for gathering information includes tedious manual measurements of specified traits such as panicle length, the number of branches, the number of grains, branch order and grain size. The manual phenotypic analysis takes time depending on the complexity of the panicle, and it is not possible to analyze

and quantify all features (such as branch and spikelet location in the panicle) to get an accurate view of panicle architecture. Furthermore, manual phenotyping is usually damages the plant, making it impossible to evaluate all the features with the same panicle. Considering the significance of gene discovery and crop development, it is critical to automate such time consuming and laborious operations (AL-Tam *et al.*, 2013).

The P-TRAP is an open source software that is used to evaluate the panicle architecture and grain properties at high throughput. It is written in the Java programming language. It offers a user-friendly interface that works on a variety of platforms. This is used to examine the structure of the panicle, count the grains and examine the seed form. These tools can be used individually or collectively on the same image. Comma Separated Values (CSV) or Extensible Mark-up Language (XML) formats are used to extract the results. P-TRAP software, in comparison to manual data collection methods, offers more reliable results in a shorter time frame and collects more data (AL-Tam *et al.*, 2013).

## MATERIALS AND METHODS

The current research was conducted at the Research Farm, Department of Genetics and Plant Breeding, Indira Gandhi Agricultural University, Raipur (Chhattisgarh) during *kharif* 2019 using Randomized Block Design (RBD) with two replications. The experimental material comprising of 122 RILs with their parents (Danteshwari x Dagad deshi) were grown in two rows with row to row and plant to plant spacing of 20 × 20 cm maintained under two environmental conditions (irrigated - IR and rainfed - RF). In IR conditions, seeds were sown in nurseries and seedlings were transplanted as a single plant after twenty-one days and in RF conditions, seeds were sown directly. The rice panicles from the irrigated condition were obtained for further analysis. The JPG images of the 122 RIL populations along with parents were taken and

analysis was performed on these images. The captured images were analyzed for nine grain related traits which are spikelets number (mm), spikelet length (mm), spikelet width (mm), spikelet area (mm), spikelet perimeter (mm), spikelet circularity (mm), spikelet compactness (mm), spikelet ellipticity (mm), aspect ratio (mm) and grain yield (g). The panicle structure analysis was performed for the above nine traits using P-TRAP software (version: P-TRAP 201306052037), developed by Universidade do Algrave, Faro-Portugal and IRD, Montpellier-France. The results were extracted in CSV format. The images of the panicles were captured using Canon EOS 1300D camera. The image size was 5184 x 3456px at 72 dpi. These images were uploaded on to the P-TRAP tool one after the other and its structure was evaluated. The trait grain yield per plot was recorded from the field under irrigated conditions. The association for grain yield and nine grain related traits were analyzed using Microsoft Excel 2010 software and the density distribution histogram was generated using XLSTAT 2021 software.

## RESULTS AND DISCUSSION

The objective of this study was to evaluate how the grain traits of a rice panicle influence the overall grain yield. The mean, range, standard deviation, standard error and coefficient of variation are calculated for the grain yield and nine grain traits which are represented in **Table 1**. The correlation between grain yield and nine grain traits is shown in **Table 2**. The density distribution histogram along with the normal distribution of 122 RILs including their parents for the nine traits are shown in **Graph 1**. The detection and quantification of the panicle and grain of the parents Danteshwari and Dagad deshi are shown in **Fig. 1 and Fig. 2**.

The rice panicle images were analyzed using a panicle trait phenotyping tool among 122 lines and their parents for nine grain traits.

**Table 1. Mean, range and standard deviation for grain yield and nine grain traits**

Characters	Mean	Range		Standard Deviation	Standard Error	Coefficient of Variation
		Min	Max			
Spikelets number	186.88	73.00	422.00	66.99	6.02	35.85
Spikelet length	1.87	1.35	2.71	0.36	0.03	19.50
Spikelet width	1.09	0.95	1.32	0.16	0.01	14.79
Spikelet area	24.93	15.44	41.45	5.94	0.53	23.84
Spikelet perimeter	5.19	3.97	7.21	0.92	0.08	17.69
Spikelet circularity	0.77	0.69	0.84	0.11	0.01	13.73
Spikelet compactness	0.84	0.73	0.93	0.12	0.01	13.89
Spikelet ellipticity	0.94	0.92	0.98	0.12	0.01	12.93
Aspect ratio	1.62	1.28	2.17	0.28	0.03	17.31
Grain yield	145.48	68.00	273.00	37.69	3.38	25.91

**Table 2. Analysis of Correlation coefficient for grain yield and nine grain traits**

Traits	SN	SL	SW	SA	SP	Sci	Sco	SE	AR	GY
SN	<b>1.00</b>									
SL	0.08	<b>1.00</b>								
SW	0.15	0.81**	<b>1.00</b>							
SA	-0.02	0.97**	0.80**	<b>1.00</b>						
SP	0.12	0.99**	0.87**	0.96**	<b>1.00</b>					
Sci	0.36**	0.39**	0.78**	0.29**	0.48**	<b>1.00</b>				
Sco	0.37**	0.36**	0.76**	0.26**	0.45**	1.00**	<b>1.00</b>			
SE	0.37**	0.60**	0.85**	0.48**	0.67**	0.96**	0.95**	<b>1.00</b>		
AR	0.26**	0.93**	0.70**	0.81**	0.92**	0.47**	0.44**	0.68**	<b>1.00</b>	
GY	0.20*	0.38**	0.47**	0.33**	0.42**	0.45**	0.44**	0.49**	0.40**	<b>1.00</b>

\*Significant at 5%, \*\* Significant at 1% probability level

SN-Spikelets number, SL-Spikelet length, SW- Spikelet width, SA- Spikelet area, SP- Spikelet perimeter, Sci-Spikelet circularity, Sco-Spikelet compactness, SE-Spikelet ellipticity, AR- Aspect ratio, GY-Grain yield per plot

The number of spikelets per panicle plays a crucial role in influencing the grain yield of the crop. The average performance ranged from 73.00 to 422 with a mean value 186.88, standard deviation 66.99, standard error 6.02 and a high coefficient of variation 35.85. Spikelet's number showed positive and highly significant association with the traits spikelet circularity, spikelet compactness, spikelet ellipticity and aspect ratio with the exception of the trait grain yield, which showed positive and significant association. The traits spikelet circularity, spikelet compactness, spikelet ellipticity showed a moderately weak association. So, an increase in the number of spikelets per panicle is important in enhancing the grain yield in rice.

The range of the mean performance was from 1.35 to 2.71 with a mean value 1.87, standard deviation 0.36, standard error 0.03 and moderate coefficient of variation 19.50. Spikelet's length showed positive and highly significant association with the traits spikelet width, spikelet area, spikelet perimeter, spikelet circularity, spikelet compactness, spikelet ellipticity, aspect ratio and grain yield. The traits spikelet width, spikelet area, spikelet perimeter and aspect ratio showed a very strong association but in the case of spikelet ellipticity, spikelet circularity, spikelet compactness and grain yield were moderately weak associations. As per the analysis carried-out, it is found that the grain length of the RIL population varied from short to medium. The grain length between 1 to 2.2, 2.3 to 3.3 and 3.4 and above are categorized as short, medium and long respectively (Saichuk, 2005).

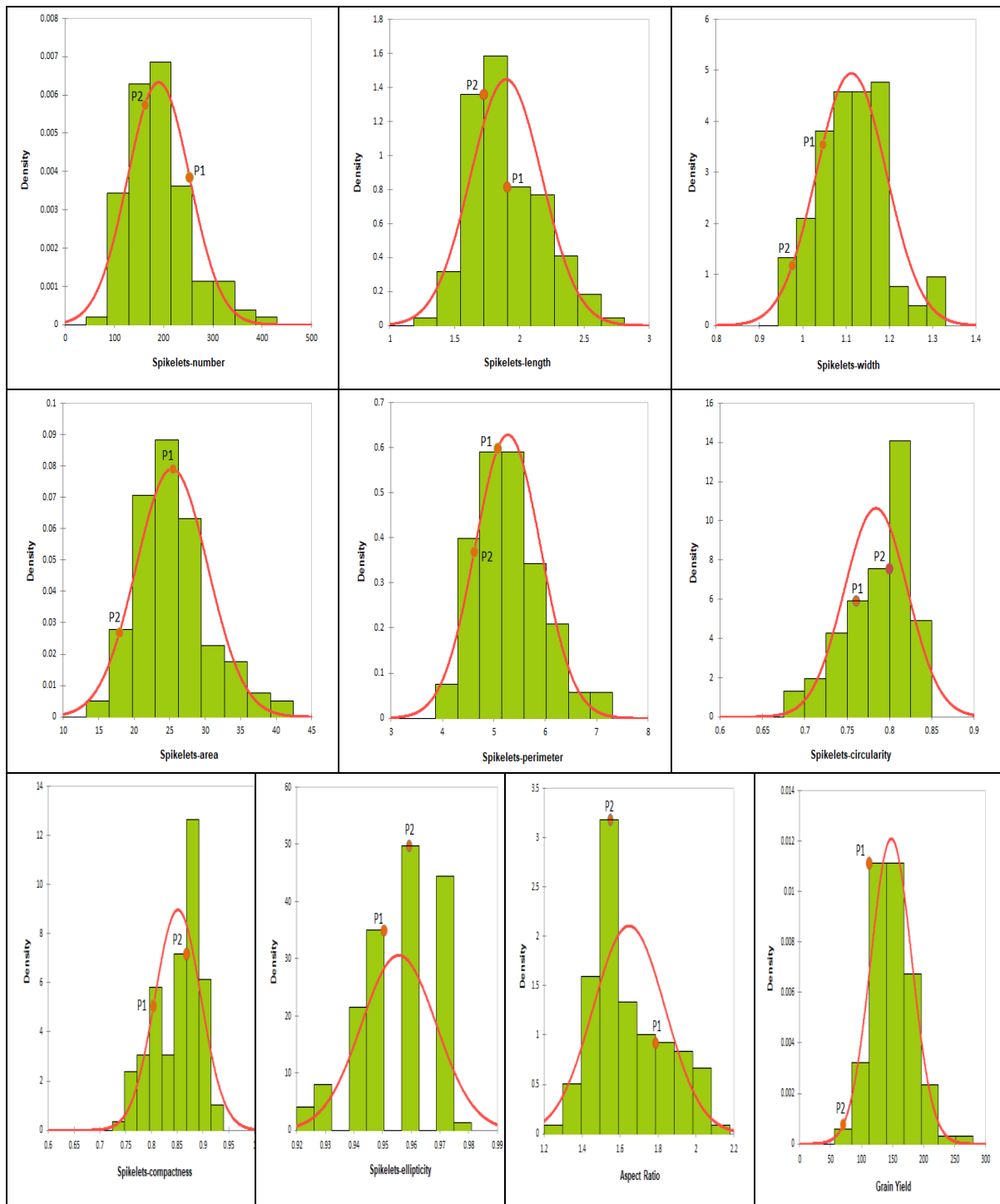
The average spikelet width ranged from 0.95 to 1.32 with a mean value of 1.09, standard deviation 0.16, standard error 0.01 and moderate coefficient of variation of 14.79. The traits spikelet area, spikelet perimeter, spikelet circularity, spikelet compactness, spikelet ellipticity and

aspect ratio, all demonstrated a positive, highly significant, and very strong association, whereas grain yield depicted a positive, highly significant and moderately weak association with the trait spikelet width. On calculating the grain-length to width ratio, it was found that the grain shape falls under the "bold" category i.e. 1.42 to 2.05 according to IRRI Scale (Slender->3.0, Medium-2.1 to 3.0, Bold-1.1 to 2.0, Round-< 1.0).

The range of the spikelet's area was from 15.44 to 41.45 with a mean value 24.93, standard deviation 5.94, standard error 0.53 and a high coefficient of variation of 23.84. The traits spikelet perimeter, spikelet circularity, spikelet compactness, spikelet ellipticity, aspect ratio and grain yield all indicated a positive and highly significant relationship with spikelet area. Spikelet perimeter and aspect ratio had a very strong association, while spikelet ellipticity and grain yield had a moderately weak association, whereas spikelet circularity and spikelet compactness had a very weak association.

The mean perimeter performance for the spikelet ranged from 3.97 to 7.21 with a mean of 5.19, standard deviation 0.92, standard error 0.08 and moderate coefficient of variation 17.69. The traits spikelet circularity, spikelet compactness, spikelet ellipticity, aspect ratio and grain yield all revealed a positive and very significant relationship with spikelet perimeter. Spikelet ellipticity and aspect ratio had a very strong association, but spikelet circularity, compactness and grain yield had a relatively weak association.

The range was from 0.69 to 0.84 with a mean value 0.77, standard deviation 0.11, standard error 0.01 and moderate coefficient of variation 13.73. The traits spikelet compactness, spikelet ellipticity, aspect ratio and grain yield all demonstrated a positive and highly significant



Graph 1. Histogram based on density for nine grain traits of 122 lines with their parents (P1-Danteshwari and P2-Dagad deshi)



(A)Detection of Panicle structureEnlarged view



(B)Detection of GrainEnlarged view



Fig. 1. Detection and quantification of rice panicle - Danteshwari



Fig. 2. Detection and quantification of rice panicle – Dagad deshi

relationship with spikelet circularity. Spikelet compactness revealed a very strong and perfect correlation ( $r=1.00$ ); spikelet ellipticity showed a very strong association and on the other hand, aspect ratio and grain yield had a moderately weak association.

The association between the grain's area and its perimeter is known as spikelet compactness (Burger and Burge, 2008; Rosin, 2015). The grain weight of a panicle is proportional to its compactness. The mean performance for the spikelet's compactness ranged from 0.73 to 0.93 with a mean value 0.84, standard deviation 0.12, standard error 0.01 and moderate coefficient of variation 13.89. The traits spikelet ellipticity, aspect ratio and grain yield revealed a positive and highly significant association with spikelet compactness. Spikelet ellipticity had a very strong association whereas, the traits aspect ratio and grain yield were found to have a moderately weak association.

The average ellipticity of the spikelets varied from 0.92 to 0.98 with a mean value 0.94, standard deviation 0.12, standard error 0.01 and moderate coefficient of variation 12.93. It has a strong, positive, and significant association with the trait aspect ratio.

The aspect ratio is the relationship between the grain's major (length) and minor (width) axes (Al-Tam, 2013). The average aspect ratio performance varied from 1.28 to 2.17 with a mean value 1.62, standard deviation 0.28, standard error 0.03 and moderate coefficient of variation 17.31.

Grain yield is a key trait among all yield contributing traits. The average grain yield performance ranged from 68.00 to 273 with a mean value of 145.48, a standard deviation of 37.69, a standard error of 3.38, and a high coefficient of variation of 25.91. Grain yield had a positive, highly significant relationship with all traits except spikelet-number, which had a significant positive and very weak association.

Considering grain yield as the most important aspect of agriculture, various grain-related traits that are related to yield should be considered for grain yield enhancement. Because of environmental influences, there is a lot of variation in the traits spikelets number and grain yield. All of the other nine traits were positively related and significant with grain yield, implying that if the other traits are improved, yield would increase.

The results revealed that the coefficient of variation for a majority of traits have lower values except for the traits spikelets number, spikelet area and grain yield. The standard error is calculated for all the traits which infers that the traits spikelet width, spikelet circularity, spikelet compactness, spikelet ellipticity, spikelet length, spikelet perimeter, aspect ratio and spikelet area have lower values. But the trait spikelets number (6.02) and

grain yield (3.38) showed a slightly higher value. Hence, it can be concluded that all the grain traits except spikelets number and grain yield have very less variations since, the used material in this study is from the same RIL population.

In the present experiment, the result revealed that the association between a majority of the traits are positive except for the trait spikelets number which is not associated or independent with spikelet length, spikelet width, spikelet area and spikelet perimeter. The above discussed traits influence grain yield directly. The analysis of these traits consumes more time when performing manually but while using P-TRAP it is simplified and it takes less time to analyse these traits by using a rice panicle image in the software.

The improvement of panicle structure through genetics is associated to yield and grain quality traits (Crowell *et al.*, 2014). It has a direct impact on the quantity of grains per panicle and, as a result, the overall rice production. P-TRAP is distinctive in that it can simultaneously measure panicle structure parameters and quantify grain related traits such as spikelet number per panicle and several spikelet dimensional traits such as length, width, circularity, ellipticity, perimeter, area, compactness, and aspect ratio, which is not possible with other software of similar function (Pasion *et al.*, 2019). Unlike P-TRAP, which can detect the shape and size of spikelets threshed out from panicles irrespective of whether or not they are linked to rachis branches, Smart Grain can only determine the shape and size of spikelets threshed out from panicles (Tanabata *et al.*, 2012). PASTAR (Panicle Structure Analyzer for Rice) (Ikeda *et al.*, 2010) is comparable to P-TRAP in that it can count spikelets and analyse the length and number of rachis branches, but it is not publicly available and does not measure spikelet dimensional traits.

In panicle trait analysis, PANorama (Crowell *et al.*, 2014) is faster and more accurate than P-TRAP version 20130213220. PANorama, on the other hand, only evaluates rachis and rachis branch qualities, not spikelet-related traits; therefore P-TRAP is more valuable in that regard. To quickly estimate the number of spikelets per panicle, an image analysis method and a 5-point calibration model was used (Zhao *et al.*, 2015). Although this method eliminates the necessity for high-resolution photos, it only allows for the measurement of one panicle characteristic. Multi-angle imaging was developed as another way for focusing on only one trait, the number of panicles per plant during heading (Duan *et al.*, 2015). For rice panicle post-anthesis development analysis, a simple and noninvasive technique based on X-ray computed tomography was developed (Jhala and Thaker, 2015). The ultimate goal of this method is to provide an accurate prediction of the best crop harvest time and to make breeding for increased grain yield easier.

Panicle-SEG is a more contemporary approach for phenotyping rice panicles (Xiong *et al.*, 2017). This software, on the other hand, concentrates on panicle segmentation and generates information about the heading phase and panicle development based on maturity tests and changes in panicle area and color in the field. Panicle-SEG is unable to make precise measurements of rachis branches and spikelets. Despite the availability of new approaches, P-TRAP was employed in the panicle architecture study to account for both panicle structural variation and spikelet features. P-TRAP was also employed in recent genome-wide association studies to generate rice panicle phenotypic data (Rebolledo *et al.*, 2016).

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