Micro-morphological diversity of rice (*Oryza sativa* L.*)* as seen under foldscope

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ARTICLE INFO	ABSTRACT
	Rice being the global grain, its genetic diversity is essential to support farmers' adaptation to climate change for sustainable production. Genetic variability analysis is essential to identify the diverse genotypes and to use them in hybridization programs. Although several advanced molecular techniques are now being used to characterize plants, morphological characterization is always preferred owing to their ease of detection. However, not all morphological traits can be observed through naked eyes. Observing micro-morphological variations requires the help of specialized optical instruments. "Foldscope" is a simple and portable optical instrument, which offers a great opportunity to exploit micro-morphological variations in crop plants. Hence, the current study was aimed at the micro-morphological characterization of rice crop using a foldscope. A total of 24 elite rice genotypes including checks were evaluated using Randomized Complete Block Design during *Kharif* 2018 at Agricultural Research Station Gangavati, to explore their genetic diversity. Five often neglected micro-morphological traits but associated with the traits of economic importance were recorded using 'foldscope' to assess the variability existing among the selected genotypes. The analysis of variance revealed substantial variations across all genotypes for all the characteristics investigated. The traits *viz.*, length of leaf serrations, length of hairs on the lemma, and root hair length exhibited higher GCV, PCV, heritability, and GAM most likely because of additive gene effects. So, selection for these traits may be effective. The study also showed that foldscope can be effectively used in agriculture to study micro-morphological diversity between crop genotypes.

Introduction

The rapidly changing climate and the expanding population with the associated threat of food shortage are the major issues affecting the progress of the country. Rice is a predominant food crop in the world (Diwan and Shenoy, 2001). It is the primary source of dietary carbohydrates for billions of people throughout the world (Babu *et al.,* 2012). Rice is the staple meal of around half of the world's rising population, and its demand will continue to climb as the global population grows. By 2030, it is anticipated that we would need to produce 40% more rice to feed the world's 5.0 billion rice

consumers (Khush, 2005; Tiwari *et al.,* 2020). Being one of the important centers of rice diversity, India harbors vast diversity both at inter- and intra-species levels. Indian rice varieties harbor a huge amount of genetic diversity. Crop morphological variability which forms the basis for their ability to adapt to their changing environment cannot be neglected while studying the diversity among individuals and breeding for better crop plants. However, in recent decades, the trait-based improvement programmes have driven breeders to rely on a small number of parents, resulting in phenotypic similarity and a loss of gene diversity (Singh *et al.,* 2016). In this context, morphological diversity assessment of rice is of prime importance in rice breeding. The germplasms with unique traits can serve as good parents for hybridization programs. But in the quest of characterizing the rice genotypes based on major morphological characters affecting rice yield and quality, some microscopic morphological characters like lemma hairs, root hair length, leaf serrations, *etc* are often neglected. Their role can be known only when they are characterized morphologically first, followed by dissecting their molecular basis.

The root of the plant is indeed a vital organ for nutrition absorption. In nutrient-deficient soils, rice root trait variation is linked to increased nutrient efficiency and crop productivity. In reaction to nutritional deficiency, some rice genotypes lengthen and densify their root hairs, especially when P and K levels are low. Plants with long root hairs can often acquire more P and K and are better suited to nutrient-deficient soils than those with short root hairs. Thus, root hairs play a crucial role in nutrient absorption (Klinsawang *et al.,* 2018).

The major plant physiological processes like Light harvesting, gas exchange, starch buildup, and phytohormone synthesis are all dependent on plant leaves. The morphology of leaf shape, which is predominantly dictated by the size of leaf serrations, has a considerable impact on these processes. Variations in leaf serration features also add to the plant's biodiversity. As a result, the evolution of leaf serration is a fascinating process that has piqued the interest of many experts (Kong *et al.,* 2019). Trichomes, commonly known as hairs or pubescence, are unicellular or multicellular epidermal structures found on the surface of plant organs including leaves, stems, flowers, hulls (glumes), and roots. Trichomes on the leaf surface are vital for plant development and productivity in rice as they contribute to photosynthesis as well as transpiration and respiration processes (Hu *et al.,* 2013). The rice grain is made up of an edible portion called the rice caryopsis that is covered by a coating called the hull (husk). The husk protects the rice caryopsis with its bigger lemma and smaller palea (Roy and Shill, 2020). The hairs on the lemma and palea are known to impact milling and to harbour pathogens. Burkholderia glumae, a bacterial pathogen that causes panicle blight in rice, is one such case (BPBR). Pathogens first appear on the surface of the glumes and then invade the glume hairs and cells on the palea and lemma edges (Li *et al.,* 2017). Foldscope is a low-cost paper microscope that can be used to study various microscopic morphological characters such as leaf trichomes, root hairs, leaf serrations, and lemma hairs. Foldscope being portable and easy to handle, can be taken to fields and microscopic morphological characters can be assessed easily. It has wide range of applications in various fields like food testing and nutrition, sanitary care, environment control, and agricultural applications (Jadhav *et al.,* 2020). There are few reports where foldscope has been used as a powerful tool in the field of agriculture. Maheshwari *et al.* (2018) used Foldscope as an intelligent disease detection tool for Tomato early blight. Sharma and Nischal (2019) have used a foldscope to assess the seed quality in wheat, maize, moong dal, and black dal. Diwan and Chikkanaragund (2019) studied rice root hair length, length, and density of rice lemma, and trichome . Trichome length and density in pigeon pea (*Cajanus cajan* (l.) Millsp) observed by Satish *et al.* (2020).

In the present study, an effort was made to characterize the 24 elite rice genotypes for their neglected yet useful microscopic morphological traits using Foldscope.

Material and Methods

The present investigation was carried out at the Plant Molecular Biology Laboratory, Department of Genetics and Plant Breeding, College of Agriculture, UAS Raichur, Karnataka. The field investigations were carried out during *Kharif* 2018 at the Agricultural Research Station, Gangavathi, which represents the Tungabhadra command area's

irrigated transplanted rice belt. The research station is located in Karnataka's Northern Dry Zone between 150_15'40" North latitude and 760_31'40" East longitude, at an elevation of 419m above MSL (Mean Sea Level). The experimental material for the current study comprised of 24 rice genotypes which include both early and medium maturing advanced breeding lines. The experiment was conducted in three replications using Randomized Complete Block Design (RCBD). All the genotypes were randomized to minimize errors due to soil heterogeneity. The samples were taken from the experimental plot for recording observations for lemma and leaf traits; however, for recording root hair length, the seeds were taken from the plot and allowed for germination in germination chambers.

Inserting a sample placed on a microscope slide, turning on the LED, and seeing the sample while panning and focusing with the thumbs is all that is required to operate the foldscope. The sample can be examined by holding the foldscope with both hands and positioning the eye near enough to the microlens that the eyebrows contact the paper. Panning is accomplished by positioning the thumbs at opposite ends of the top stage and moving them in tandem, translating both the optics and lighting stages while maintaining the stages aligned. The identical thumb placement is used for focusing, only the thumbs are pulled apart (or pushed together). This produces tension (or compression) along the optics stage, resulting in (or +) deflection of the micro-lens owing to flexure of the sample-mounting stage's supporting structure. Unlike typical microscopes, the foldscope fixes the sample in place as the optics and lighting stages move in unison (Cybulski *et al.,* 2014).

The kit also includes magnets that can be stuck onto the foldscope to attach it to a smartphone which allows taking pictures of the magnification. In the present study, rice root, leaf and grain morphological characters were studied using foldscope. A photograph of the foldscope instrument is shown in Figure S1.

Root hair length
Randomly selected healthy seeds of each genotype were kept for germination in a germination chamber under optimal conditions. After germination and root growth, roots of about 1 mm length were cut and fixed on the glass slide with clear sticky tape. The slide was also fixed with the measuring grid provided with the foldscope tool kit, for easy measurement of root hair length. The prepared slides were observed under the foldscope using LED. Photographs were taken under 3 different microscopic fields for each genotype with the unchanged magnification of the camera.

Length of leaf serrations
The matured leaves of 45-50 days old were collected from randomly selected plants of all the selected genotypes in each replication. A thin section of thread-like leaf margin was dissected out and mounted on the glass slide and fixed with adhesive tape. The serration pattern of leaves was observed by panning and focusing, turning on the LED. Photographs were taken in 3 different microscopic fields of each sample without changing the magnification of the mobile camera.

The density of leaf trichomes
Rice leaf trichome density was calculated using the method published by Maiti *et al* (1980). 45–50 day old matured leaf samples randomly picked from the rice plant were chopped into one square centimetre size and cooked in 20 ml of water in tiny glass vials for 15 minutes in a hot water bath maintained at 85°C. After removing the leaves, the water was drained out and heated for 20 minutes at 80 °C with 20 cc of 96 percent ethanol added. The alcohol was then drained out, and the boiling procedure with ethanol was repeated to thoroughly eliminate the chlorophyll from the leaves. The alcohol was poured out again and 90 % lactic acid was added and cooked at 85°C for 30–45 minutes, until the leaf segments were transparent. The vials were then cooled, and leaf fragments were extracted and mounted on clean slides using adhesive tape to analyse the trichome density with the foldscope. For each sample, the number of trichomes per millimetre area was counted under constant magnification, and trichome density was calculated.

Length and density of hairs on the lemma
Rice seeds of 12–15% moisture content were taken randomly from all the selected 24 genotypes. The seed coat was dissected out by separating the lemma and palea. A small piece of lemma was fixed on the glass slide using adhesive tape. The upper surface of the lemma was observed under the foldscope for length and density of hairs per unit area. Photographs were taken under three different

microscopic fields for each sample without changing the magnification of the camera and the observations were recorded in replications to minimize errors.

Analysis

The photographs of the samples focused under foldscope and captured with the help of a mobile camera with unchanged magnification were fed into the ImageJ imaging software. ImageJ is a Java image processing and analysis software inspired by the National Institute of Health (NIH) and the Laboratory for Optical and Computational Instrumentation (LOCI). The software analyzes the image formats and computes area and pixel value statistics for user-specified choices. It can calculate distances and angles, as well as generate density histograms and line profile charts. ImageJ user-written plugins (Fiji and MBF ImageJ) allow users to tackle practically any problem with the image processing or analysis (Rueden *et al.,* 2017). Length of leaf serrations, lemma hairs, and root hairs were measured using ImageJ software and density of leaf trichomes, lemma hairs per millimeter square area in each microscopic field

were counted manually. Windostat version 8.5 software was used to analyse the data.

Results and Discussion

ANOVA for the rice leaf, root, and grain morphological traits studied using foldscope

Analysis of variance (ANOVA) for various morphological characters studied using foldscope revealed that the genotypes under investigation differed significantly for all the traits studied both at 5% and 1% level of significance. This revealed the existence of significant morphological variability among the genotypes included in the study, and the selection for these morphological traits could be effective. Table 1 shows the ANOVA for various morphological traits studied using foldscope; while genetic variability parameters like Vg, Vp, GCV, PCV, heritability, and GAM (Genetic Advance as percent of Mean) for morphological characters studied using foldscope are given in Table 2. Table S1 shows the mean performance of genotypes for various characters studied.

Table 1: ANOVA for rice root, leaf and grain morphological traits studied using foldscope.

Source of variation	DF	RHL	LLS	LTD	LHL	DHL
Treatment	23	8882.29**	5668.91**	0.33**	3549.58**	3.59**
Replication	1	226.70	17.02	0.04	7.75	0.26
Error	23	119.97	184.66	0.11	76.95	0.72
CV		4.72	4.60	12.44	4.70	13.72

(Note: ** significant at 1% level of significance)

Table 2: Genetic variability parameters for rice root, leaf and grain morphological traits studied using foldscope

Sl. No.	Characters	Mean	Range		Variance		CV %		H (%)	GA	GAM
			Min.	Max.	Vg	Vp	GCV	PCV			
1	RHL	232.27	122.87	384.26	4381.16	4501.13	28.50	28.89	97.34	134.52	57.92
2	LLS	295.68	194.12	400.04	2742.13	2926.78	17.71	18.30	93.69	104.41	35.31
3	LTD	3.07	2.39	3.98	0.09	0.24	9.77	15.82	38.12	0.38	12.38
4	LHL	186.52	119.51	272.86	1736.32	1813.27	22.34	22.83	95.76	84.00	45.03
5	DHL	6.17	3.82	9.55	1.44	2.16	19.43	23.79	66.70	2.02	32.73

Abbreviations: DF- Degrees of freedom; **CV-** Coefficient of variation; **Vg-** Genotypic Variance; **Vp-**Phenotypic variance; **GCV & PCV-** Genotypic and Phenotypic coefficients of variation; **H-**heritability in broad sense; **GA-** Genetic advance; **GAM-** Genetic advance as percent of mean. **RHL-**Root hair length in micrometers; **LLS-**length of leaf serrations in micrometers; **LTD-**Leaf trichome density (per mm^2); **LHL-**Length of hairs on lemma in micrometers; **DHL-**Density of hairs on lemma (per mm^2).

Estimation of mean and other genetic parameters for rice leaf, root and grain morphological characters studied using foldscope.

Root hair length

The cylindrical extensions of root epidermal cells are known as root hairs. They vastly increase root diameter as well as root surface area, thus assisting in nutrient acquisition, microbe interactions and plant anchorage (Grierson and Schiefelbein, 2002).

The genotypes showed a mean value of 232.27 micrometers for the trait with the maximum and minimum values of 384.26μm (IET-25497) and 122.87μm (BPT-5204), respectively. The GCV and PCV values were almost equal (28.50 and 28.89) with a minor difference indicating a negligible influence of the environment on the trait. Nestler *et al*. (2016) has earlier reported a maximum root hair length of 395 μm and an average length of 122 μm through *in situ* imaging of rice root hairs in intact soil. The researchers also reported that root hair length remains constant in phosphorus fertilized and unfertilized soil, indicating the negligible influence of the environment on the trait.

The trait has shown higher heritability coupled with high genetic advance (97.34 and 57.92 respectively). It may be due to additive gene action governing the trait. Thus, selection for this trait will be effective for improving nutrient and water uptake efficiency of crop plants. Figure 1 shows the foldscopic view of rice root hairs.
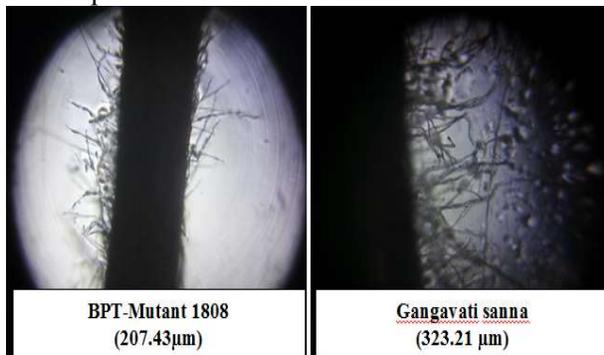

Figure 1: Foldscopic view of rice root hairs.

Length of leaf serrations

The genotypes studied had a wide range of variability for the trait, ranging from 194.12 to 400.04 μm with a mean value of 295.68 μm. The lowest length of leaf serration was recorded in BPT-Mutant 1802 (194.12 μm) and the highest was

recorded in SMW-09-32 (400.04 μm). The trait recorded moderate GCV and PCV of 17.71 and 18.30 respectively. Heritability (93.69) and GAM (35.31) were found higher, providing a good scope for selection. Figure 2 shows the photograph of rice leaf serrations captured using foldscope.
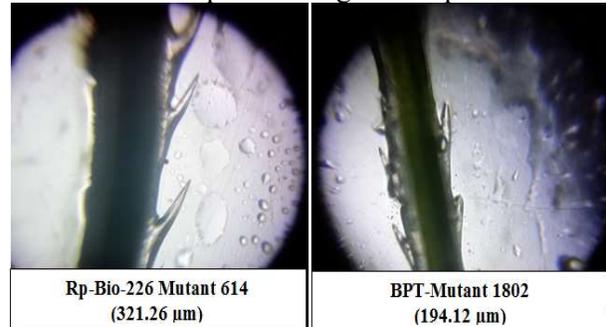

Figure 2: Foldscopic view of rice leaf serrations

Leaf trichome density.

Trichomes on the leaf surface are vital for plant development in rice because they contribute to physiological activities such as photosynthesis, transpiration, respiration, and resistance to biotic and abiotic stress, influencing productivity. (Li *et al.,* 2010).

Hamaoka *et al.* (2017) identified BLANKET LEAF hairy-leaf gene on chromosome 6 of wild *Oryza nivara* which lengthens the macro-hairs. They reported that the hairy-leaf character increases leaf surface temperature and Water Use Efficiency by limiting leaf transpiration. As a result, the characteristic may be advantageous in breeding of rice cultivars that are amenable to water-saving farming practices. In the current study, the density of leaf trichomes ranged from 2.39 (Gangavati sanna) to 3.98 per mm^2 (Rp-Bio-226 Mutant 614) and the mean trichome density was found to be 3.07 mm^2. The results are in agreement with Hu *et al*. (2013) who had reported significant differences in pubescent density of different rice genotypes through microscopic studies. Scanning electron microscopic studies of leaf trichomes by Amsagowri (2017) showed that trichome density of rice accessions differ significantly between accessions ranging from 0.41 to 8.56 per mm^2, and higher trichome density of rice leaves is associated with the lesser ovipositional preference of yellow stem borer thus offering resistance to the pest. Foldscopic study of Satish *et al*. (2020) also

showed the presence of significant variation among the pigeonpea genotypes for pod trichome length and density.

The trait showed low GCV (9.77) coupled with moderate values of PCV (15.82), heritability (38.12) and GAM (12.38). So, the selection based on phenotype may not be effective for the trait as it might be under the control of non-additive gene actions as well. Figure 3 shows the foldscopic view of leaf trichomes in rice genotypes.
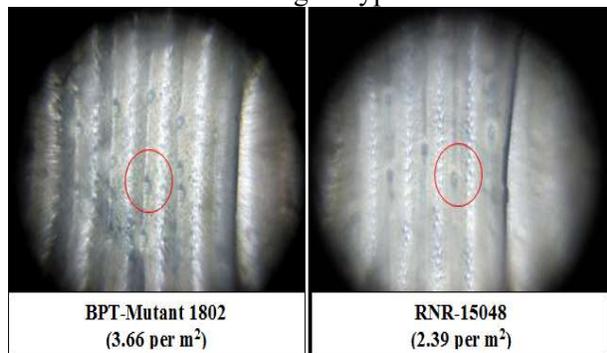

Figure 3: Foldscopic view of rice leaf trichomes

Length of hairs on the lemma
Rice hulls with hairs or pubescence are problematic for transporting. Besides being prickly, they also minimize the weight per unit volume (Hu *et al.,* 2013).The range of lemma hair length varied from 119.51 to 272.86 μm with a mean value of 186.52. The genotype BPT-Mutant 1808 recorded the minimum length of lemma hairs (119.51 μm) whereas IET-25520 showed maximum lemma hair length (272.86 μm). Higher values of GCV (22.34), PCV (22.83), heritability (95.76), and GAM (45.03) were recorded indicating that the trait is governed by additive genes and phenotypic selection is effective. So, the selection of genotypes with short lemma hairs might be rewarding. Li *et al.* (2010) also obtained similar results through scanning electron microscopic studies of rice accessions with the length ranging from 15 to 150 micrometers.

The density of hairs on the lemma
Glabrous leaves and hull are important agronomic traits in rice that facilitates easy harvest and follow-up processes. Glabrous rice varieties are preferred over pubescent ones as they are noted for high yield, resistant to lodging, and superior grain quality (Li *et al.,* 2010). The genotypes with a lesser density of hairs on lemma are preferred for cultivation over hairy types, as glabrous cultivars

produce less dust than pubescent ones during processing. (Jodon, 1965). In the current study, the density of lemma hairs ranged from 3.82 to 9.55 per mm^2 with a mean of 6.17 hairs per mm^2 area. The genotype IET-22066 exhibited the lowest lemma hair density (3.82) whereas BPT-Mutant 1808 recorded a higher density of lemma hairs per mm^2 area (9.55). The genotypic coefficient of variation was found moderate (19.43) whereas the PCV, heritability, and GAM was high with the values 23.79, 66.70, and 32.73 respectively; indicating the additive gene actions controlling the trait. Hamaoka *et al*. (2017) had earlier reported non-significant variation of hull pubescence among different rice genotypes through scanning electron microscopic observations. Figure 4 shows the photograph of lemma hairs captured using foldscope.
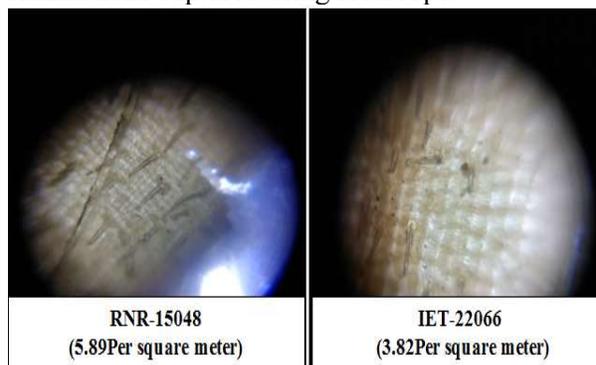

Figure 4: Foldscopic view of rice lemma hairs

The top five genotypes selected based on their performance of various morphological characters studied using foldscope are presented in Table S2, where genotypes are listed in decreasing order of their trait performance for the traits root hair length and leaf trichome density and length of leaf serrations whereas in increasing order for length and density of lemma hairs.

Conclusion
The results of the present investigation helped to identify superior rice genotypes for each micro-morphological trait studied here. These genotypes can be used to improve rice plants for specific morphological traits based on the breeding objective set. The above studied foldscopic traits can also be used as criteria of selection which directly or indirectly affect the acceptance of the varieties by the farmers as well as consumers. The genetics of these traits can be studied in the future

to dissect their linkage behavior and they can be used as morphological markers while breeding for elite cultivars. The results also evidently showed the application of foldscope in agriculture for crop morphological diversity assessment. Being simple, portable, light, and easy to handle, the instrument can be used to assess various agronomically important morphological traits that cannot be neglected while breeding for better crop types.

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Conflict of interest

The authors declare that they have no conflict of interest.

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