Phenotypic Characterization of Diverse Rice Fertility Restorers

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Authors’ contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Characterization of restorer lines of rice based on seedling and plant morphology contributes in assessment of varietal identity and further its utilization in development of hybrids. With this objective the work was carried over with 90 restorer lines with three replications during Kharif season 2018. The experiment conducted under Rice Improvement Project at Seed Breeding Farm, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur (M.P.). Out of 41 morphological traits as per DUS guidelines, variability was reported for all traits except leaf sheath anthocyanin colouration, presence of leaf auricle, presence of leaf collar, presence of leaf ligules, white colour of leaf ligules, absence of stem anthocyanin colouration of internodes, male sterility and presence of secondary branching in panicle. The genotypes Mahamaya, ANP-526 and ANP-553 revealed distinctness for most of the traits.

Keywords: Characterization; DUS guidelines; rice improvement project; rice restorer lines.
1. INTRODUCTION

Rice is the semi-aquatic, monocot normally grown as an annual plant that belongs to family Poaceae, sub-family Bambusoideae and Genus Oryzae. Among 24 known species of rice, 22 are wild and only two are cultivated species i.e., O. sativa (Asiatic rice) and O. glaberima (African rice). The species sativa is subdivided based on ecotype as indica (India), japonica (Japan) and javanica (Indonesia) [1]. Rice is a staple food for almost half of the world population and it claimed more than one-fifth of its share in calorie consumption. Globally, rice fulfils about 60% of dietary, 20% of calorie and 14% of protein requirement, which highlighted its significance in the human diet [2,3]. According to world population review 2019, India holding the second rank occupied a global share of 17.7% in terms of population, with the increasing growth rate of 1.02% yearly [4]. This called breeder's attention to increase production and productivity of rice, to meet the growing demands of the population. Thus, the exploitation of diversity is needed for the development of hybrids, which shows higher productivity per day along with good quality traits. Restorer lines male fertile lines, used as a pollen parent in commercial seed production plots serve this purpose through commercial exploitation of heterosis. Three line systems is being practiced in rice hybrid breeding programs to obtain a breakthrough in yield potential.

With the view of protecting genotypes under PPV & FR (2001), they are examined for compliance with DUS criteria needed for registration. Characterization revealed common knowledge about the uniqueness of a variety from existing variety, following standards of DUS guidelines. Restorer line helps us to revive the male sterility of a line and high genetic variability for the specific traits characteristic of restorer parents are the prerequisites for selection and development of superior hybrid parents. However, for sustained and continuous improvement, contemporary variability ought to be created by introgressing new sources of desired genes from landraces into elite scientific discipline background. The agro-morphological characterization should eventually lead to a system of recording and storing useful data that can be readily retrieved and made available to others and help in planning breeding programs [3]. It helps in tracing traits exhibiting correlation and linkage among themselves and yield. Hence, provided elementary information regarding plant breeding program would help in the choice of parents for hybrid production in the future. Keeping aforesaid in view, the present investigation enumerated 90 fertility restorer lines of rice to explicate the existing variability through characterization.

2. MATERIALS AND METHODS

The current research was conducted with 90 fertility restorer lines of rice with the aim to assess phenotypic variation based on morphological characterization, which could be act as a marker for identification of elite genotype. The experiment was carried out under Rice Improvement Project, at Seed Breeding Farm, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur. The seeds were sown on a raised seedbed in order to ensure the uniform germination and establishment, and subsequently 30-day-old seedlings were transplanted into the experimental field. Each genotype was grown in a single row of 4 m length with a row to row spacing of 20 cm and plant to plant spacing of 15 cm. Recommended agronomic package of practices was followed for the proper field management during the experiment. The exclusive one among all would be further utilized in hybridization programme. All the observations were recorded as per DUS guidelines. Based on botany and assessment method several morphological, quantitative and quality traits were studied.

3. RESULT AND DISCUSSION

The ninety restorer lines were keenly observed because the visual assessment of variability in a population reported is necessary for a plant breeder to begin a judicious Breeding programme. The present investigation depicted no variability for eight morphological traits viz., leaf sheath anthocyanin colouration, auricle presence, leaf collar presence, leaf ligules presence, white colour of leaf ligules, absence of internodes anthocynin colouration, secondary branching presence and male sterility, as shown in Table 1 and Fig. 1. The corresponding findings were also noticed before for traits like presence of ligules, auricle and collar [5,6] and for trait panicle presence of secondary branching [7]. Therefore, the frequent occurrence of the specific trait put them in a reluctant category and could be avoided for the next selections of restorers.
Basal leaf sheath colour

Green

Purple (JR 1009)

Anthocyanin colouration of collar

Green

Purple (Mahamaya)

Anthocyanin colouration of auricles

Purple (Mahamaya)

Green
Spikelet colour of stigma

- White
- Purple (ANP 526)

Panicle exertion

- Well Exerted
- Mostly Exerted (NPT 40-018*)

Panicle curvature of main axis

- Semi-straight
- Deflexed
- Drooping (ANP 553)
Secondary branching

Flag Leaf Attitude (early)

Sterile lemma colour (ANP 526)
Purple colour tip of lemma (ANP 526)
Anthocyanin keel colour (ANP 526)

Fig 1. Morphological markers for varietal identification
As per Tables 1 and 3, trait are classified into varied class and depicting the phenotypic diversity among the restorers. And the frequently occurring traits clearly signified through maximum frequency per cent. Thus, genes governing such none or least variable traits are considered to be consensus with high adaptability for the Madhya Pradesh geographical location. The least variability comes with 13 trait reporting frequency under range of 90–100% and 05 traits under 80-90% range. Dark intensity of green colour has sufficient frequent occurrence and this was in consonance with the findings of earlier workers [6,8,9]. Maximum frequency of leaf pubescence of blade surface reported were weak (75%). Split ligules (80%) trait exhibited commonness in comparison with acute (10%). Straw colour lemma palea colour observed in 82 genotypes, as shown in Fig. 2. The rest restores have unique index value for this particular trait, signifies its use as marker phenotypic trait in breeding programme such that ANP 526 with purple spot/ furrow on straw.

**Fig 2. Frequency distribution of morphological traits showing variability**
| Character | Class | Frequency | Percentage |
|-----------|-------|-----------|------------|
| **LEAF**  |       |           |            |
| 1. Leaf: Intensity of green colour | Light | 09 | 10.00 |
|            | Medium | 77 | 85.56 |
|            | Dark   | 04 | 4.44  |
| 2. Leaf: Anthocyanin colouration | Absent | 89 | 98.89 |
|            | Present | 01 | 1.11  |
| 4. Leaf: Distribution of anthocyanin colouration | On tip only | 00 | 00.00 |
|            | On margin only | 00 | 00.00 |
|            | In blotches only | 01 | 1.11  |
|            | Uniform | 00 | 00.00 |
| 5. Leaf Sheath: Anthocyanin colouration | Absent | 90 | 100.00 |
|            | Present | 00 | 00.00 |
| 6. Flag leaf: Attitude of blade | Erect | 52 | 57.70 |
|            | Semi-erect | 37 | 41.10 |
|            | Horizontal | 00 | 00.00 |
|            | Deflexed | 01 | 1.11  |
| **LEMMA** |       |           |            |
| 8. Lemma and Palea colour | Straw | 82 | 91.10 |
|            | Gold and gold furrow on straw background | 02 | 2.22 |
|            | Brown spots on straw | 00 | 00.00 |
|            | Brown furrow on straw | 00 | 00.00 |
|            | Brown | 04 | 4.44  |
|            | Reddish to light purple | 00 | 00.00 |
|            | Purple spot/ furrow on straw | 02 | 2.22 |
|            | Purple | 00 | 00.00 |
|            | Black | 00 | 00.00 |
| **PANICLE** |       |           |            |
| 9. Panicle: Curvature of main axis | Straight | 00 | 00.00 |
|            | Semi-straight | 18 | 20.00 |
|            | Deflexed | 70 | 77.70 |
|            | Dropping | 02 | 2.22  |
| 10. Panicle: Awns | Absent | 56 | 62.20 |
|            | Present | 34 | 37.70 |
| 11. Panicle: Presence of secondary branching | Absent | 00 | 00.00 |
|            | Present | 90 | 100.00 |
| 12. Panicle: secondary branching | Weak | 04 | 4.44  |
|            | Strong | 76 | 84.40 |
|            | Clustered | 10 | 11.11 |
| 13. Panicle: Attitude of branches | Erect | 00 | 00.00 |
|            | Erect to semi-erect | 14 | 15.55 |
|            | Semi-erect | 29 | 32.20 |
|            | Semi-erect to spreading | 47 | 52.22 |
|            | Spreading | 00 | 00.00 |
| 14. Panicle: exertion | Partly exerted | 00 | 00.00 |
|            | Mostly exerted | 04 | 4.44  |
|            | Well exerted | 86 | 95.50 |
### Visual Assessment by observation of individual plant or part of plants

| LEAF | Description | Basal Leaf : Sheath colour | Leaf: Pubescence of blade surface | Leaf: Auricles | Leaf: Anthocyanin colorations of auricles | Leaf: Collar | Leaf: Anthocyanin colouration of collar | Leaf: Ligule | Leaf: Shape of ligule | Leaf: Colour of ligule | Stem: Attitude | Stem: Anthocyanin colouration of nodes | Stem: Intensity of anthocyanin coloration of nodes | Stem: Anthocyanin colouration of internodes | Lemma: Density of pubescence of lemma | Lemma: Anthocyanin colouration of keel | Lemma: Anthocyanin colouration of area below apex |
|------|-------------|-----------------------------|---------------------------------|----------------|------------------------------------------|--------------|----------------------------------------|-------------|---------------------|----------------------|----------------|-------------------------------------|------------------------------------------|---------------------------------------------|-----------------------------------------------|------------------------------------------------|------------------------------------------------|
| 1    | Basal Leaf : Sheath colour | Green | 89 | 98.80 | | | | | | | | | | | | | | |
|      | Light Purple | 01 | 1.11 | | | | | | | | | | | | | | | |
|      | Purple Lines | 00 | 0.00 | | | | | | | | | | | | | | | |
|      | Uniform Purple | 00 | 0.00 | | | | | | | | | | | | | | | |
| 15   | Leaf: Pubescence of blade surface | Absent | 00 | 0.00 | Weak | 75 | 83.33 | Medium | 15 | 16.67 | Strong | 00 | 0.00 | Very Strong | 00 | 0.00 | |
| 16   | Leaf: Auricles | Absent | 00 | 0.00 | Present | 90 | 100.00 | | | | | | | | | | |
| 17   | Leaf: Anthocyanin colorations of auricles | Colourless | 89 | 98.80 | Light Purple | 00 | 0.00 | Purple | 01 | 1.11 | | | | | | | | |
| 18   | Leaf: Collar | Absent | 00 | 0.00 | Present | 90 | 100.00 | | | | | | | | | | |
| 19   | Leaf: Anthocyanin colouration of collar | Absent | 89 | 98.80 | Present | 01 | 1.11 | | | | | | | | | | |
| 20   | Leaf: Ligule | Absent | 00 | 0.00 | Present | 90 | 100.00 | | | | | | | | | | |
| 21   | Leaf: Shape of ligule | Truncate | 00 | 0.00 | Acute | 10 | 11.11 | Split | 80 | 88.80 | | | | | | | |
| 22   | Leaf: Colour of ligule | White | 90 | 100.00 | Light Purple | 00 | 0.00 | Purple | 00 | 0.00 | | | | | | | |
| 23   | Culm: Attitude | Erect | 18 | 20.00 | Semi-erect | 61 | 67.70 | Open | 09 | 10.00 | Spreading | 02 | 2.22 | | | | |
| 24   | Stem: Anthocyanin colouration of nodes | Absent | 89 | 98.80 | Present | 01 | 1.11 | | | | | | | | | | |
| 25   | Stem: Intensity of anthocyanin coloration of nodes | Weak | 00 | 0.00 | Medium | 01 | 1.11 | Strong | 00 | 0.00 | | | | | | | |
| 26   | Stem: Anthocyanin colouration of internodes | Absent | 90 | 100.00 | Present | 00 | 0.00 | | | | | | | | | | |
| 27   | Spikelet: Density of pubescence of lemma | Absent | 00 | 0.00 | Weak | 51 | 56.60 | Medium | 39 | 43.30 | Strong | 00 | 0.00 | Very strong | 00 | 0.00 | |
| 28   | Lemma: Anthocyanin colouration of keel | Absent / very weak | 88 | 97.70 | Weak | 01 | 1.11 | Medium | 00 | 0.00 | Strong | 01 | 1.11 | Very strong | 00 | 0.00 | |
| 29   | Lemma: Anthocyanin colouration of area below apex | Absent | 88 | 97.70 | Weak | 00 | 0.00 | Medium | 01 | 1.11 | Strong | 00 | 0.00 | | | | |
Most of fertility restorer lines fall into erect (52), followed by semi-erect (11) and deflexed (02) flag leaf attitude categories. Based on the spikelet density of pubescence of lemma entire experimental material was characterized as weak (51) and medium (39). Eighty six genotypes exhibited white stigma colour, while only four unique accessions had shown purple colour. Majority (78) genotype reported early followed by medium (11) and late (01) leaf senescence. Variation observed among ninety genotypes for traits related to panicle such as for curvature of main axis: semi-straight (18), deflexed (70) and dropping (02); for awns: absent (56) and present (34), among these thirty four genotypes, in majority awns were found only in upper half (21) followed by only in tip (13), while yellow white colour of awn (30) were observed in majority of restorers followed by brown (02) and purple (02); for panicle exertion eighty six genotypes were well exerted and only four were mostly exerted; for secondary branching: maximum genotype exhibited strong (76) followed by clustered (10) and weak (04). In panicle attitude of branches, variability is present with semi-erect to spreading (47) followed by semi-erect (29) and erect to

| Visual Assessment by observation of individual plant or part of plants | Very strong | 01 | 1.11 |
|---|---|---|---|
| 30 Lemma: Anthocyanin colouration of apex | Absent | 87 | 96.60 |
| | Weak | 00 | 00.00 |
| | Medium | 02 | 2.22 |
| | Strong | 00 | 00.00 |
| | Very strong | 01 | 1.11 |
| 31 Spikelet: Colour of tip of lemma | White | 00 | 00.00 |
| | Yellow | 85 | 94.44 |
| | Brown | 5 | 5.55 |
| | Red | 00 | 00.00 |
| | Purple | 00 | 00.00 |
| | Black | 00 | 00.00 |
| 32 Sterile lemma colour | Straw | 89 | 98.80 |
| | Gold | 00 | 00.00 |
| | Red | 00 | 00.00 |
| | Purple | 01 | 1.11 |

**PANICLE**

| Panicle: Distribution of awn | Tip only | 13 | 14.40 |
|---|---|---|---|
| | Upper half only | 21 | 23.30 |
| | Whole length | 01 | 1.11 |
| 34 Spikelet: Colour of stigma | White | 86 | 95.50 |
| | Light green | 00 | 00.00 |
| | Yellow | 00 | 00.00 |
| | Light purple | 00 | 00.00 |
| | Purple | 04 | 4.44 |
| 35 Panicle: Colour of awns | Yellow white | 30 | 33.30 |
| | Yellow brown | 00 | 00.00 |
| | Brown | 02 | 2.22 |
| | Reddish brown | 00 | 00.00 |
| | Light red | 00 | 00.00 |
| | Red | 00 | 00.00 |
| | Light purple | 00 | 00.00 |
| | Purple | 02 | 2.22 |
| | Black | 00 | 00.00 |
| 36 Panicle: Length of longest awn | Very short | 00 | 00.00 |
| | Short | 02 | 2.22 |
| | Medium | 32 | 35.50 |
| | Long | 00 | 00.00 |
| | Very long | 00 | 00.00 |
| 37 Male Sterility | Present | 90 | 100 |
| | Absent | 00 | 00 |
The frequency distribution of some of the important traits is presented in Fig. 2 in form of pie chart. According to above results of characterization, the traits leaf pubescence of blade, leaf anthocyanin colouration of collar, culm attitude, spikelet density of pubescence of lemma, lemma anthocyanin colouration of keel, lemma anthocyanin colouration of area below apex, lemma anthocyanin colouration of apex and spikelet colour of stigma were reported with some distinctness among genotypes [6]. While, some previous workers revealed similar findings for traits leaf anthocyanin colouration of auricle and panicle colour of awns [8,9,10], for panicle presence of awns [10,11], for lemma anthocyanin colouration of apex and spikelet colour of stigma [12]. However contradictory results were found in case of ligule shape, absences of leaf auricle and leaf collar in the landraces of rice of East India [13].

Characterization of 90 fertility restorer lines for 34 Agro-morphological traits had highlighted some genotypes with distinctness in morphology which is shown in Table 2. Among ninety genotype enquired, ANP 526 addressed specificity for traits viz, Leaf anthocyanin colouration, Leaf Distribution of anthocyanin colouration (In blotches only), Lemma: Anthocyanin colouration of Keel (strong), Lemma: Anthocyanin colouration of apex (very strong) and Spikelet colour of stigma(Purple); Mahamaya for purple colour of stigma, anthocyanin colouration of auricle and collar; JR 1009 for light purple basal leaf sheath colour; ANP 553 for deflexed flag leaf attitude of blade; NPT 37 and JR 4322-2 for short bold and Laxmi 144 for short slender decorticated grain shape. The four genotypes expressed purple colour of stigma were ANP 526, Mahamaya, NPT 14-12 and JR 1301.

Table 2. Agro-morphological characterization with unique assessments found under index value

| S.No. | Characteristics                                      | Index value                       | Unique genotype                              |
|-------|------------------------------------------------------|-----------------------------------|---------------------------------------------|
| 1     | Leaf Intensity of green colour (GC)                   | Dark                              | JR-1004, RP 5911-52-13-3-2-2-1, ANP-553, PS-2, NPT 89*IR-36, PS-2, NPT 40-018* PUSA BASMATI |
| 2     | Leaf Distribution of anthocyanin colouration (DAC)    | In blotches only                  | ANP-526                                      |
| 3     | Flag leaf attitude (FLA)                             | Deflexed                          | ANP-553                                      |
| 4     | Basal leaf: Sheath colour                            | Light purple                      | JR-1009                                      |
| 5     | Leaf Anthocyanin colouration of auricles             | Present                           | Mahamaya                                     |
| 6     | Stem Anthocyanin colouration of collar               | Present                           | JR-1021’                                    |
| 7     | Stem Intensity of anthocyanin colouration of nodes (IAC) | Medium                           | JR-1021’                                    |
| 8     | Lemma Palea Colour (LPC)                             | Purple spot / furrow on straw     | ANP 526                                      |
| 9     | Lemma Palea Anthocyanin colouration of keel          | Strong                            | ANP 526                                      |
| 10    | Lemma Palea Anthocyanin colouration of area below apex | Medium Very strong                | ANP 526, RPHR 619                           |
| 11    | Lemma Palea Anthocyanin colouration of apex          | Medium Very strong                | Mahamaya, ANP526, RPHR 619                  |
| 12    | Sterile lemma Colour                                 | Purple                            | ANP 526                                      |
| 13    | Colour of stigma                                     | Purple                            | Mahamaya, ANP 526, RPHR 619, NPT 14-12       |
Table 3. Classification of variability on the basis of frequency range

| S.NO. | Frequency range | Variability   | Traits | Remark                      |
|-------|-----------------|---------------|--------|-----------------------------|
| 1.    | 100             | No Variability| 08     | Highly Frequent trait       |
| 2.    | 80-99           | Least Variability| 18    | Relatively highly frequent  |
| 3.    | 60-79           | Medium Variability| 03    | Medium Frequent             |
| 4.    | <59             | High Variability | 08    | Least Frequent              |

4. CONCLUSION

From ancient times, the visual observations scoring of genotypes were used as an aid to determine genetic diversity in gene pool. The existence of distinctness, uniformity and stability is confirmed through characterization of traits under studies which were analysed as per DUS guidelines. Therefore, out of 34 morphological traits seven traits- leaf sheath anthocyanin colouration, presence of leaf auricle, presence of leaf collar, presence of leaf ligule, white colour of leaf ligule, absence of stem anthocyanin colouration of internodes and presence of secondary branching in panicle were found to be monomorphic; while fourteen traits like- basal leaf sheath colour (green, light purple), leaf anthocyanin colouration, Auricle colour (colourless, purple), anthocyanin colouration of collar, stem anthocyanin colouration of node, leaf pubescence of blade surface (weak, medium), ligule shape (acute, split), spikelet density of pubescence of lemma (weak, medium), colour of sterile lemma, stigma colour, leaf senescence, awns, awns distribution, panicle exertion were dimorphic. Sufficient amount of variability was recorded for the remaining traits. Among all traits under study, Lemma palea colour was the only tetramorphic trait showing maximum variability.

The genotype ANP 526 and Mahamaya were reported as unique genotypes in terms of many qualitative traits Thus, these restorer lines can be further utilized as morphological markers in plant breeding programme.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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