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# Genetic Inheritance of Rust Resistance in Pearl Millet [*Pennisetum glaucum* (L.) R. Br.]: A Study of $F_1$ $F_2$, and Backcross Generations across Two Environments.

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**Abstract:**

Rust disease caused by *Puccinia substriata* var. *indica* is a major foliar disease affecting pearl millet [*Pennisetum glaucum* (L.) R. Br.], leading to significant yield losses. The present investigation was undertaken to study the inheritance pattern of rust resistance using six generations (P1, P2, F1, F2, B1, B2) derived from three different crosses involving resistant and susceptible inbred lines. In Cross-I (PM-967 × PM-1035), the segregation of F2 and backcross populations conformed to a 3:1 and 1:1 ratio respectively, indicating monogenic dominant inheritance of rust resistance. Similar results were observed in Cross-II (PM-1035 × PM-1103), where F2 and B2 populations followed expected Mendelian ratios, confirming single gene control of resistance with dominance. The F1s of both crosses exhibited complete resistance, reinforcing the dominance of resistance alleles. Cross-III, involving two resistant lines (PM-1013 × PM-1035), further supported the presence of complementary gene action or allelic interactions, though detailed results are pending confirmation.

The study concludes that rust resistance in the evaluated crosses is primarily governed by a single dominant gene, which can be effectively utilized in breeding programs for the development of rust-resistant pearl millet hybrids and varieties. These findings offer significant implications for marker-assisted selection and the deployment of durable rust resistance in pearl millet improvement programs.

**Keywords:** Pearl millet, rust resistance, *Puccinia substriata*, inheritance, monogenic, dominant gene, F2 population, Chi-square analysis

## Introduction

Pearl millet [*Pennisetum glaucum* (L.) R. Br.] is an annual tillering diploid (2n=2x=14) crop, belongs to family *Poaceae*, subfamily *Panicoidae*, commonly known as bajra, cat-tail millet, or bulrush millet in different continents and it is believed to be originated in Africa from where it was imported to India (Krishnaswamy, 1962). Pearl millet is a highly cross-pollinated crop because of protogynous flowering condition and wind borne pollination mechanism, which satisfy one of the essential biological demand for hybrid development.

India is the largest single producer of the crop, both in terms of area and production. The West and Central Africa region has large area under millets, of which more than 90 per cent is pearl millet. In India pearl millet is mainly cultivated in the states of Rajasthan, Uttar Pradesh, Maharashtra, Haryana, Gujarat, Karnataka, Madhya Pradesh, Tamil Nadu and Andhra Pradesh with 7.54 million hectare total area, production of 10.36 million tons with national average productivity of 1374 kg/ha. In Maharashtra, pearl millet is grown on an area of 6.72 lakh hectares and annual production of 5.11 lakh tons with state average productivity is of 761 kg/ha (Anonymous, 2021).

It is nutritionally superior and staple food for millions of people living in unbearable environmental conditions which are characterized by erratic rainfall. In fact, it is the only appropriate and efficient crop for arid and semi-arid circumstances because of its capacity to utilization soil moisture and higher level of heat tolerance than sorghum and maize (Harinarayana *et al.*, 1999). Most of the farmers priorate this crop as low cost, low risk option not only by choice but also by necessity (Harinarayana, 1987).

World Health Organisation has recognised that micronutrient malnutrition resulting from dietary deficiency of one or more micronutrients as a serious human health problem worldwide. It is estimated that over 3 billion people in the world suffer from micronutrient malnutrition and that about 2 billion peoples of these have an iron deficiency (Govindaraj *et al.*, 2011). In Asia about 35 per cent of children between 0-5 years of age suffer from iron and zinc deficiency. It also affects larger segment of population mostly women, infants and children from poor families in the country (Singh *et al.*, 2009). Ascendancy of anaemia in pregnant women is highest (87.5 per cent) in India. Pearl millet is a highly nutritious cereal with high levels of metabolizable energy, protein and more balanced amino acid profile (Andrews and Kumar, 1992). The levels of iron and zinc content in pearl millet cultivars are far higher than those reported in improved wheat and maize varieties (Rai *et al.*, 2008). A large number of genetic variations has been detected for iron and zinc and other minerals in pearl millet (Jambunathan and Subramanian, 1988). It is considered that increasing iron and zinc proportion in these crops could increase the dietary intake of iron and zinc.

Diseases that are considered economically important which includes downy mildew, blast, rust, ergot and smut. Among these several diseases, rust has become a disease of considerable importance in recent years because it severely affects both forage and fodder value and thereby limiting the exploitation of heterosis. Pearl millet leaf rust is known to occur in all the areas where the crop is grown. Due to intensive seed production in the summer season and crop overlapping in some Indian regions, the disease has spread widely. For hybrid seed production, the crop is grown in the post-rainy season during January-April that coincides with cool nights (15-20°C) and warm days (25-34°C). During this period the abundant dew formation occurring on the foliage in the mornings helps urediniospores to germinate and cause infection.

Pearl millet rust disease caused by *Puccinia substriata* var. *indica* Ramachar & Cumm. Occasional out breaks may lead to severe losses in grain yield and forage quality. In the rainy season in India, rust generally occurs after anthesis resulting in little or no reduction in grain yield, though there may be substantial reduction in fodder quality. However, rust is a major grain yield reducer in the post rainy season crop (Andrews *et al*., 1985). Breeding for rust resistance is the only cheapest, eco-friendly and surest measure to overcome this disease. Resistance to rust, in most cases, has been reported to be controlled by single dominant genes (Andrews *et al*. 1985). The field and greenhouse rust screening techniques have been developed and resistance sources have been identified (Singh *et al*. 1997). The information on inheritance of resistance will have a direct bearing on the breeding efficiency for genetic management of this disease. Therefore, improvement of a variety/hybrid for rust resistance is of great importance to increase production and productivity. Hence, there is a need to study the inheritance of rust resistance in pearl millet.

## Materials and Methods

The present investigation inheritance of rust resistance in pearl millet segregation and inheritance of rust resistance in pearl millet was conducted during *Kharif*-2024, at Rallis India Limited Research and Development facility in Hyderabad, India, The lines utilized in the study were essential and specific to certain traits, making them highly suitable for use in for improving overall crop yield .The details of the materials used, the experimental approach and statistical methods followed for conduct of experiment are described below.

### Experimental Material

The experimental material for the present study comprised of nine were received from ICRISAT, The details of these inbreds are given below (Table 1).

**Table 1 Salient features of the pearl millet inbred lines used in the study.**

| Sr. No. | Name of inbred | Feature of inbred lines |
|---|---|---|
| 1 | PM-1103 | Synchronous tillering, early, yielder, susceptible to foliar diseases |
| 2 | PM-967 | Mid-tall, profuse tillering fodder, susceptible to foliar diseases |
| 3 | PM-1013 | high in Fe, Resistant to foliar diseases |
| 4 | PM-1035 | Tillers , long and compact ear heads, Resistant foliar to diseases, |

**Table 2 Details of six generations with respect to three crosses for study of inheritance of rust resistance.**

| Generation | Cross-I | Cross-II | Cross-III |
|---|---|---|---|
| $P_1$ | PM-967 (S) | PM-1035 (R) | PM-1013 (R) |
| $P_2$ | PM-1035 (R) | PM-1103 (S) | PM-1035 (R) |
| $F_1$ | PM-967 x PM-1035 (S x R) | PM-1035 x PM-1103 (R x S) | PM-1013 x PM-1035 (R x R) |
| $F_2$ | $F_1$ selfed | $F_1$ selfed | $F_1$ selfed |
| $B_1$ | $F_1$ × PM-967 | $F_1$ × PM-1035 | $F_1$ × PM-1013 |
| $B_2$ | $F_1$ × PM-1035 | $F_1$ × PM-1103 | $F_1$ × PM-1035 |

**Methodology**

**Hybridization:**

Sowing of crossing block was carried out at Rallis India limited, Research and Development facility at Hyderabad. For inheritance of rust resistance studies we have attempted three crosses were selected *viz.*, PM-967 x PM-1035, PM-1035 x PM-1103 and PM-1013 x PM-1035 (Table 2) with four parents, two with resistance to rust (PM-1035 and PM-1013) and another two susceptible to rust disease (PM-967 and PM-1103). Similarly, for generation mean analysis and inheritance of rust resistance, part of $F_1$ seeds harvested from above crosses were sown during *Kharif*-2024 and selfing of $F_1$'s and backcrosses were done to get $F_2$, $B_1$ and $B_2$ generations, respectively.

**Inheritance of rust resistance**

The generations *viz.*, $P_1$, $P_2$, $F_1$, $F_2$, $B_1$ and $B_2$ of following crosses were grown without replication at at Rallis India limited, Research and Development facility at Hyderabad to study inheritance of rust resistance in pearl millet. All treatments were sown with 3 m row length and spaced at 50 cm apart with 15 cm distance between plants for field screening. Each parent, $F_1$, $B_1$ and $B_2$ are represented in four rows while, $F_2$ were in twenty rows of each. For green house screening phytotron green house facility at Rallis India limited was used.

| Sr. No. | Name of cross | Cross type |
|---|---|---|
| 1. | PM-967 x PM-1035 | Susceptible x Resistant |
| 2. | PM-1035 x PM-1103 | Resistant x Susceptible |
| 3. | PM-1013 x PM-1035 | Resistant x Resistant |

**Screening techniques:**

All the parents, three $F_1$s, three $F_2$s, three $B_1$s and three $B_2$s were screened for *Puccinia substriata* var *indica* in the natural field and greenhouse conditions as per Singh *et al.*, (1997).

## Field and Greenhouse Screening for Rust Resistance

### Field Screening:

The test lines were grown in the central four rows of each plot, with a highly rust-susceptible line used as a spreader or infector planted in every first and fifth row. To initiate infection, the spreader rows were inoculated by dispensing 2 ml of a urediniospore suspension into the whorls of plants 20–25 days after seedling emergence. Additionally, the inoculated seedlings, including both test lines and susceptible checks, were sprayed with urediniospore suspension twice—once at 25 days and again at 40 days after emergence. To promote rust development, the crop was irrigated to maintain high humidity for 2–3 days following inoculation. Rust severity was recorded either on individual plants (in segregating material) or on the entire line 25–40 days post-inoculation, typically at the grain-filling stage, using a modified Cobb scale. Furthermore, rust severity was assessed separately on the lower leaves and the top four leaves to evaluate the progression of the disease.

### Greenhouse Screening:

Screening under controlled conditions was conducted using plastic root trainers, each containing 25 cavities filled with a sterilized mixture of soil, sand, coco peat, and well-decomposed farmyard manure (FYM) in a 2:1:1 ratio by volume. Seeds of test lines from various generations, along with a susceptible check, were sown in the trainers and adequately irrigated. The seedlings were grown for 10–12 days before inoculation. At 15 days old, the seedlings were spray-inoculated with an aqueous suspension of *Puccinia substriata* var. *indica* urediniospores. Post-inoculation, the seedlings were maintained under misting conditions to ensure relative humidity exceeded 90% for 10 days. Rust infection types were recorded 12 days after inoculation.

**Table 3 Rust severity rating scale (0-5) recorded as per Singh *et al.*, (1997)**

| Grade | Rust severity rating scale | Type |
|---|---|---|
| 0 | 0 | Immune/highly resistance (HR) |
| 1 | 0.1-20 | Resistant (R) |
| 2 | 20.1-40 | Moderately resistant (MR) |
| 3 | 40.1-60 | Moderately susceptible (MS) |
| 4 | 60.1-80 | Susceptible (S) |
| 5 | 80.1-100 | Highly susceptible (HS) |

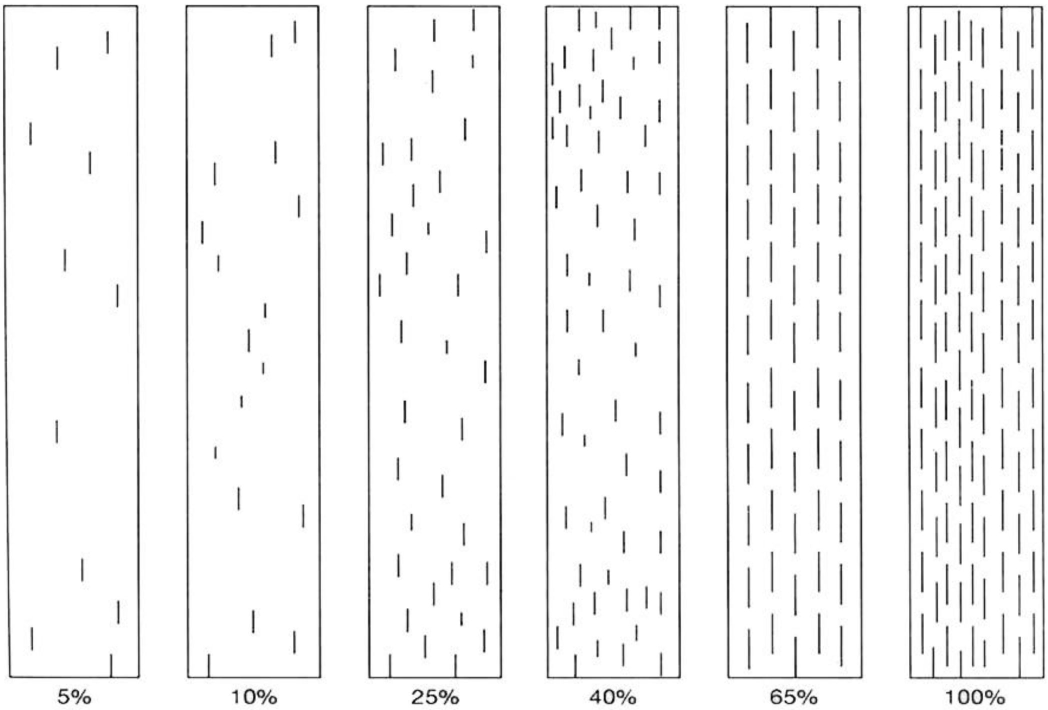


**Fig 1. Rust severity rating scale (Singh *et al.* 1997)**

| 5% | 10% | 25% | 40% | 65% | 100% |
|---|---|---|---|---|---|

**Fig 2. Rust severity rating scale at Filed observations**

**Statistical analysis**

The mean values of randomly selected observational plants for different characters were used for statistical analysis. The following statistical parameters were calculated.

**Chi- square test**

The collected Rust disease segregation data used to conduct statistical analysis using the Chi-square ( $\chi^2$) test, which was applied to the observed segregation data to test the goodness of fit on probable genetic ratios .The goodness of fit test was tested by Chi- square test (Fisher, 1930).

$$\chi^2 = \frac{\sum (O - E)^2}{E}$$

Where,

O = observed frequency.

E = expected frequency.

The significance of Chi- square value was tested against table value with (n-1) degrees of freedom, where ‘n’ is the total number of segregating classes (Stansfield, 1986).

## Results and Discussion

In the present investigation, two rust susceptible (PM-967 and PM-1103) and two rust resistant inbreds (PM-1035 and PM-1013) were selected for study. Three types of crosses *viz*., susceptible x resistant (Cross-I: PM-967 x PM-1035), resistant x susceptible (Cross-II: PM-1035 x PM-1103) and resistant x resistant (Cross-III: PM-1013 x PM-1035) were attempted. Total of three $F_1$ their $F_2$, $B_1$ and $B_2$ generations were developed subsequently. The experiment was conducted along with the $F_1$, $F_2$, $B_1$ and $B_2$ generations and inbreds were scored for their reaction to rust under greenhouse condition and field conditions.

**Cross-I: PM-967 x PM-1035 (S x R)**

The results revealed that in Cross-I: PM-967 x PM-1035 all plants of the susceptible inbred PM-967 (Greenhouse condition 41 plants, Field condition 40 plants) showed susceptibility to rust (score $\geq$3) and spore count at field condition of $P_1$ is 6 x $10^7$ /ml, while for resistant inbred PM-1035 all plants (Greenhouse condition 40 plants, Field condition 40 plants) were resistant (score of $\leq$ 2). Similarly, all plants of $F_1$ of Cross-I were resistant (score of $\leq$ 2) under greenhouse (45 plants) and field (40 plants) conditions.

In, $F_2$ generation of Cross-I, 216 plants were screened at greenhouse condition, of which 159 plants showed resistance (score of $\leq$ 2) and 57 plants showed susceptibility (score $\geq$ 3). Out of 379 $F_2$ plants at field screening, 288 were resistant and 91 were susceptible for rust and spore count was 9.6 x $10^7$/ml. The segregation of $F_2$ at both conditions *viz*., greenhouse and field condition showed good fit to the monogenic ratio of 3:1 with chi-square values of 0.22 and 0.20, respectively.

$B_1$ population of Cross-I showed segregation with respect to rust resistance and susceptibility, while in $B_2$ all plants of both conditions were found resistant. $B_1$ generation of Cross-I, at greenhouse condition out of 74 plants, 41 plants were resistant and 33 were susceptible with chi-square value 0.86. In field condition, 58 plants of $B_1$ were screened, out of that 26 plants showed resistance and 32 showed susceptibility for rust and whereas spore count was 8.8 x $10^7$/ml with chi-square value 0.62. The segregation showed goodness of fit of 1:1 ratio for both the conditions for $B_1$ population of Cross-I which confirms the monogenic ratio of 3:1 for rust inheritance.

**Cross-II: PM-1035 x PM-1103 (R x S)**

In Cross-II (R x S: PM-1035 x PM-1103) all plants of inbred PM-1035 showed resistance (score of ≤ 2) to rust at greenhouse condition (47 plants) and field condition (40), while all plants of inbred PM-1103 were susceptible (score ≥ 3) to rust at greenhouse condition (43 plants) and field condition (38) with the spore count of 8.8 x $10^7$/ml. Similarly, all plants of $F_1$ of Cross-II were resistant (score of ≤ 2) under greenhouse (49 plants) and field condition (40 plants).

In greenhouse condition, for Cross-II, out of 243 plants of $F_2$, 179 were resistant, while 64 were susceptible with chi-square value of 0.23. Out of 392, plants of $F_2$ 305 were resistant and 87 were susceptible with chi-square value 1.64 at field condition and spore count was 6.8 x $10^7$/ml. The segregation in $F_2$ at both the conditions for Cross-II fit to the monogenic ratio 3:1 which confirmed by growing the $B_1$ and $B_2$ populations. All the plants of $B_1$ populations of Cross-II at both the conditions were found to be resistant, while $B_2$ populations of both conditions showed segregation with respect to rust resistance and susceptibility. Out of total 89 plants of $B_2$, 42 were resistant while 47 were susceptible at greenhouse condition. In field condition 29 resistant and 27 susceptible plants were observed out of total 56 plants of $B_2$ and spore count was 1.1 x $10^8$ /ml. The segregation of $B_2$ at both conditions *viz*., greenhouse condition and field condition showed good fit to the ratio of 1:1 with chi-square values of 0.28, and 0.07, respectively.

**Cross-III: PM-1013 x PM-1035 (R x R)**

In Cross-III, PM-1013 x PM-1035 (R x R) all resistant plants were observed for $P_1$, $P_2$, $F_1$, their $F_2$, $B_1$ and $B_2$ in both conditions (Greenhouse $F_2$: 203 plants and Field $F_2$:368 plants). There was no significance difference between the $F_1$ of Cross-I (PM-967 x PM-1035), Cross-II (PM-1035 x PM-1103) and Cross-III (PM-1013 x PM-1035) under greenhouse and field condition for rust resistance, indicated that the resistance is governed by dominant gene in the inheritance of rust resistance in pearl millet. Andrews *et al*., (1985), Sokhi *et al*., (1987), Ramamoorthi *et al*., (1995) and Panna *et al*., (1996) also reported similar results for rust resistance in pearl millet was govern by dominant gene.

The segregation pattern of $F_2$ generation in Cross-I and Cross-II of greenhouse and field condition had good fit to the monogenic ratio of 3: 1 (Table 4). Similar results were also reported by Hanna *et al*., (1985), Panna *et al*., (1996), Ramamoorti and Jehangir (1996) and Sharma *et al*., (2009) on $F_2$ plants of pearl millet. In the $B_1$ population of Cross-I and $B_2$ of Cross-II segregation pattern of greenhouse and field condition was 1 resistant and 1 susceptible; while in $B_2$ population of Cross-I and $B_1$ of Cross-II of green house and field condition, all the plants were resistant. These results confirm single gene control of resistance. Andrews *et al*., (1985) and Ramamoorthi and Jehangir (1996) also observed similar ratio in their studies.

**Table 4 Segregation for rust disease resistance plants in F1, F2 and Back generations and test of goodness of fit based Rust disease data across two environments.**

| Cross | Envt. | Generation | No. of observed plant | | | No. of expected plant | | Expected ratio (3:1) | | $\chi^2$ | P |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | R | S | Total | R | S | R | S | | |
| Cross-I (S x R) PM-967 x PM-1035 | Green house | $P_1$ | 0 | 41 | 41 | - | - | - | - | - | - |
| | | $P_2$ | 40 | 0 | 40 | - | - | - | - | - | - |
| | | $F_1$ | 45 | 0 | 45 | - | - | - | - | - | - |
| | | $F_2$ | 159 | 57 | 216 | 162 | 54 | 3 | 1 | 0.22 | 0.63 |
| | | $B_1$ | 41 | 33 | 74 | 37 | 37 | 1 | 1 | 0.86 | 0.35 |
| | | $B_2$ | 71 | 0 | 71 | - | - | - | - | - | - |
| | Field | $P_1$ | 0 | 40 | 40 | - | - | - | - | - | - |
| | | $P_2$ | 40 | 0 | 40 | - | - | - | - | - | - |
| | | $F_1$ | 40 | 0 | 40 | - | - | - | - | - | - |
| | | $F_2$ | 288 | 91 | 379 | 284.25 | 94.75 | 3 | 1 | 0.20 | 0.65 |
| | | $B_1$ | 26 | 32 | 58 | 29 | 29 | 1 | 1 | 0.62 | 0.41 |
| | | $B_2$ | 55 | 0 | 55 | - | - | - | - | - | - |
| Cross-II (R x S) PM-1035 x PM-1103 | Green house | $P_1$ | 47 | 0 | 47 | - | - | - | - | - | - |
| | | $P_2$ | 0 | 43 | 43 | - | - | - | - | - | - |
| | | $F_1$ | 49 | 0 | 49 | - | - | - | - | - | - |
| | | $F_2$ | 179 | 64 | 243 | 182.25 | 60.75 | 3 | 1 | 0.23 | 0.62 |
| | | $B_1$ | 81 | 0 | 81 | - | - | - | - | - | - |
| | | $B_2$ | 42 | 47 | 89 | 6.5 | 6.5 | 1 | 1 | 0.28 | 0.60 |
| | Field | $P_1$ | 40 | 0 | 40 | - | - | - | - | - | - |
| | | $P_2$ | 0 | 38 | 38 | - | - | - | - | - | - |
| | | $F_1$ | 40 | 0 | 40 | - | - | - | - | - | - |
| | | $F_2$ | 305 | 87 | 392 | 294 | 98 | 3 | 1 | 1.64 | 0.23 |
| | | $B_1$ | 51 | 0 | 51 | - | - | - | - | - | - |
| | | $B_2$ | 29 | 27 | 56 | 28 | 28 | 1 | 1 | 0.07 | 0.81 |
| Cross-III (R x R) PM-1013 x PM-1035 | Green house | $P_1$ | 51 | 0 | 51 | - | - | - | - | - | - |
| | | $P_2$ | 54 | 0 | 54 | - | - | - | - | - | - |
| | | $F_1$ | 59 | 0 | 59 | - | - | - | - | - | - |
| | | $F_2$ | 203 | 0 | 203 | - | - | - | - | - | - |
| | | $B_1$ | 76 | 0 | 76 | - | - | - | - | - | - |
| | | $B_2$ | 81 | 0 | 81 | - | - | - | - | - | - |
| | Field | $P_1$ | 40 | 0 | 40 | - | - | - | - | - | - |
| | | $P_2$ | 40 | 0 | 40 | - | - | - | - | - | - |
| | | $F_1$ | 40 | 0 | 40 | - | - | - | - | - | - |
| | | $F_2$ | 368 | 0 | 368 | - | - | - | - | - | - |
| | | $B_1$ | 51 | 0 | 51 | - | - | - | - | - | - |
| | | $B_2$ | 53 | 0 | 53 | - | - | - | - | - | - |

## Conclusions

The present investigation inheritance of rust resistance in pearl millet and their three crosses were selected *viz*., PM-967 x PM-1035, PM-1035 x PM-1103 and PM-1013 x PM-1035 involving susceptible and resistant parents to study inheritance of rust resistance. Gene action and segregation study were estimated using standard procedures. All the plants from each generation of three crosses were scored for rust disease intensity at different stages by using the 0-5 scale.

The inheritance pattern of rust resistance in pearl millet was investigated using three crosses: Cross I (PM-967 × PM-1035), Cross II (PM-1035 × PM-1103), and Cross III (PM-1013 × PM-1035). All $F_1$ hybrids from these crosses exhibited a resistant reaction to rust, with no significant differences observed among them. This uniform resistance suggests the involvement of the same gene conferring resistance across all three crosses. The expression of resistance in the $F_1$ generation further indicates that rust resistance is governed by a Single dominant gene. In the $F_2$ populations of Cross I and Cross II, the segregation pattern for rust resistance followed a 3:1 ratio (resistant: susceptible), which is consistent with Mendelian inheritance of a single dominant gene. This was further confirmed by the analysis of backcross progenies, which also supported the involvement of a single dominant gene. In Cross III (PM-1013 × PM-1035), where both parents were resistant (R × R), all plants in the $F_1$, $F_2$, and backcross generations exhibited resistance, indicating that the gene responsible for resistance is homozygous and identical in both parental lines. These results collectively confirm that rust resistance in the studied genotypes of pearl millet is controlled by a single dominant gene, likely shared among the resistant parents.

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