# Identification of rust resistance in hybrid parents and advanced breeding lines of pearl millet

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Citation: Sharma R, Thakur RP, Rai KN, Gupta SK, Rao VP, Rao AS and Suresh Kumar. Identification of rust resistance in hybrid parents and advanced breeding lines of pearl millet. Journal of SAT Agricultural Research 7.

## Abstract

Rust caused by *Puccinia substriata* var *indica* is one of the major diseases affecting both forage and grain production in pearl millet, particularly in the postrainy cool season (*rabi*) crop. The disease can be best managed through host plant resistance. In all, 214 advanced breeding lines, including 126 designated B-lines, 23 designated R-lines and 65 potential R-lines were evaluated for rust resistance in the disease nursery during the postrainy season 2008–09 under natural epiphytotic conditions. Eight lines (1 B-line, 7 R-lines) that showed resistance (≤10% rust severity) in the field screen were evaluated in the greenhouse by artificial inoculation of potted seedlings to confirm their resistance. One B-line (ICMB 96222) and three R-lines (ICMR 0699, ICMP 451-P8 and ICMP 451-P6) were resistant while the other four R-lines were susceptible. The four confirmed resistant lines could be useful resistance sources for breeding rust resistant hybrid parental lines and their hybrids.

## Introduction

Pearl millet (*Pennisetum glaucum*) is an important grain, forage and stover crop, grown on more than 26 million ha in arid and semi-arid regions of Asia and Africa, where it is often a basic staple among the poorest people. It is also emerging as an important fodder and feed crop in Brazil. In India, the crop is grown on 9.3 million ha (http://www.icrisat.org/PearlMillet/PearlMillet.htm). Single-cross $F_1$ hybrids based on cytoplasmic-nuclear male-sterility (CMS) system have contributed significantly in increasing pearl millet productivity in India.

Rust, caused by the fungus *Puccinia substriata* var *indica* is one of the major diseases affecting both forage and grain production in pearl millet. Rust has been observed throughout India. In northern India, the disease does not frequently occur until flowering time in September when temperatures are somewhat moderate. In other regions of the country, rust may attack even the seedling stage, causing substantial reduction in yield. The disease is of major concern in peninsular India where pearl millet is planted during the postrainy season (*rabi*) and rust infection and disease development is favored by lower temperatures during this season. However, pearl millet rust has also been reported in central and peninsular India in the summer season (March–May) crop where seed production is carried out. All growth stages of the plant are susceptible to rust attack, and under favorable environment, plants can wither before flowering due to severe rust infection (Ramakrishnan and Sunderam 1956, Rachie and Majmudar 1980). Rust infection of pearl millet forage has been reported to cause up to 51% reduction in digestible dry matter yield (Monson et al. 1986). Resistance to rust has been reported in some pearl millet germplasm accessions and breeding lines (Wilson 1993, Singh et al. 1997). However, lines that were resistant in India became susceptible in USA indicating existence of different physiological races in India and USA (Wilson 1991, Tapsoba and Wilson 1996). Such information on existence of physiological races of the pathogen in India is not available as yet. Although a number of germplasm accessions and some breeding lines have shown good level of rust resistance (Singh et al. 1997), identification of resistance in elite advanced breeding lines is likely to be more useful and effective in resistance breeding than transferring resistance from a germplasm accession, which requires more time and resources. The present investigation, therefore, was undertaken to evaluate advanced hybrid parental lines of pearl millet for resistance to rust. Such resistant lines then could be used effectively to develop rust resistant hybrid parents and their hybrids in a relatively short time.

## Materials and methods

In all, 214 advanced breeding lines, including 126 designated B-lines, 23 designated R-lines and 65 potential R-lines were evaluated for rust resistance in the disease

nursery at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India during postrainy season (November–March) 2008–09 and the resistant lines were further screened in the greenhouse to confirm their resistance.

**Field screening.** Pearl millet hybrid parental lines were screened in the field under natural epiphytotic conditions. The experiment was conducted in a complete randomized block design with two replications, 1 row of 2 m length/entry in each replication. Plants were thinned to 20 plants/row 15 days after planting and other agronomic practices were followed as per local practices. Rust severity (%) was recorded at soft dough stage using the modified Cobb scale of 0–100% severity (Singh et al. 1997). Rust severity on lower leaves and top four leaves was recorded separately to indicate the disease progress.

**Greenhouse screening.** Test lines along with susceptible check (ICMB 89111) were planted in 15-cm diameter pots (10 seeds/pot) filled with sterilized soil-sand-FYM (farmyard manure) mix (2:1:1) and placed in a greenhouse bay maintained at 30±1°C. The pot-grown seedlings (15 days old) were spray-inoculated with an aqueous urediniospores suspension (about $1 \times 10^5$ spores $ml^{-1}$) of *P. substriata* var *indica* and exposed to high humidity (>90% RH) under misting for 10 days. Rust severity (%) was recorded 15 days after inoculation using a modified Cobb scale (Singh et al. 1997). Plants were also observed for hypersensitive response (chlorotic specks without rust pustules) and the reaction was recorded.

## Results and discussion

In general, rust severity in susceptible lines was quite high both on lower leaves (mean severity 52%) and on the top four leaves (mean severity 21%) of plants indicating adequate disease pressure for an effective field screening. In the 126 designated B-lines, rust severity in the lower leaves ranged from 10% (ICMB 96222) to 95% (ICMB 91444) with a mean of 55%, while in the top four leaves it ranged from 3% (ICMB 96222) to 60% (ICMB 91444 and ICMB 91666) with an average of 22%. Based on lower leaves observation, only one line (ICMB 96222) was resistant and the remaining 125 lines were highly susceptible, indicating high level of susceptibility in the designated B-lines to Patancheru isolate of *P. substriata* var *indica* (Table 1). On top four leaves, none of the B-lines was free from rust, while 17 lines were resistant (1–10% severity), 58 moderately resistant (11–20% severity), 30 susceptible (21–30% severity) and remaining 21 lines were highly susceptible (>30% severity) to rust.

In the 23 designated R-lines, rust severity on the lower leaves ranged from 3% (ICMR 06999) to 100% (ICMR 06222) with an average of 54%, while on the top four leaves it ranged from 0 (ICMR 06999 and ICMR 07999) to 100% (ICMR 06222) with an average of 25%. Considering rust severity on the top four leaves, 5 lines were rust free, 4 lines resistant, 4 lines each moderately resistant and susceptible and the remaining 6 were highly susceptible to rust.

In the 65 potential R-lines, rust severity ranged from 0 to 100% on lower leaves, and 0 to 90% on the top four leaves. Only one line (K-08-18395) was rust free in the field screen. Two lines recorded 1–10% severity (resistant) on lower leaves and 51 lines were highly susceptible with >30% severity. Three of the 65 potential R-lines were rust free on top four leaves, 20 resistant, 23 moderately resistant, 9 susceptible and 10 were highly susceptible.

Under field screen, rust severity on top four leaves of a plant is critical as these leaves mainly contribute to photosynthesis and grain filling in the panicle. Plants showing high rust severity on top four leaves often set poor seed and seed remain shriveled and undersized.

**Table 1. Performance of hybrid seed parents of pearl millet for rust resistance during the 2008–09 postrainy season under field conditions at ICRISAT, Patancheru, India.**

| | | No. of lines in rust severity (%) class[1] | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Lower leaves | | | | | Top four leaves | | | | |
| Trial | No. of lines | 0 (HR) | 1–10 (R) | 11–20 (MR) | 21–30 (S) | >30 (HS) | 0 (HR) | 1–10 (R) | 11–20 (MR) | 21–30 (S) | >30 (HS) |
| Designated B-lines | 126 | – | 1 | – | – | 125 | 0 | 17 | 58 | 30 | 21 |
| Designated R-lines | 23 | – | 4 | – | – | 19 | 5 | 4 | 4 | 4 | 6 |
| Potential R-lines | 65 | 1 | 2 | 3 | 8 | 51 | 3 | 20 | 23 | 9 | 10 |
| Total | 214 | 1 | 7 | 3 | 8 | 195 | 8 | 41 | 85 | 43 | 37 |

1. Mean of two replications based on the modified Cobb scale.
HR = highly resistant; R = resistant; MR = moderately resistant; S = susceptible; HS = highly susceptible.

Eight lines (1 B-line and 7 R-lines) identified highly resistant or resistant (≤10% rust severity) under field screen were evaluated in the greenhouse to confirm their field resistance. Since urediniospores for artificial inoculation were collected from infected leaves of ICMB 89111 from the field, this B-line was included as a susceptible check. Two lines (ICMP 83506 and ICML 11) reported to be rust resistant (Singh et al. 1987, Morgan et al. 1998) were included as resistant checks. Resistance was observed only in four of the eight lines in the greenhouse screen (Table 2). Four lines that were resistant in the field at flowering and seed-set stage showed susceptible reaction in the greenhouse at the seedling stage indicating the expression of adult plant resistance in these lines under field conditions. Such adult plant resistance has already been reported in wheat (*Triticum aestivum*) to yellow rust infection (Van Silfhout and Gerechter-Amitai 1988) and similar mechanism might be working with pearl millet rust. This, however, needs to be validated. ICMB 96222 had only 2% rust severity in the greenhouse screen, whereas hypersensitive response (highly resistant reaction) was observed in 3 R-lines (ICMR 06999, ICMP 451-P8 and ICMP 451-P6). Resistant check ICMP 83506 showed susceptible reaction, whereas resistance in ICML 11 was still effective against Patancheru isolate of *P. substriata* var *indica*. Rust resistance gene $Rpp_1$ in ICML 11 has been reported to be knocked down by all 11 races of *P. substriata* var *indica* in USA (Tapsoba and Wilson 1996), indicating race differentiation of pearl millet rust pathogen in India and USA. Morgan et al. (1998) identified a RFLP (restriction fragment length polymorphism) marker *Xpsm716* closely linked to rust resistance gene in ICMP 83506 effective against race PS92-1, but the gene was not found effective against Patancheru isolate of *P. substriata* var *indica* in India further supporting the existence of races in this pathogen.

Since physiologic races in *P. substriata* var *indica* have been reported from USA (Wilson 1991, Tapsoba and Wilson 1996), it would be useful to initiate similar studies in India to understand variability in the pathogen population. This will then lead to identification of diverse sources of rust resistance in pearl millet and would help prevent rust outbreaks in future. In this study, resistance sources identified in advanced breeding lines will be useful for breeding hybrid parental lines with rust resistance that could be effectively used for developing rust resistant hybrids. It would also be desirable to test the

**Table 2. Rust severity in the selected B- and R-lines of pearl millet.**

| Lines | Pedigree | Rust severity in field screen (%)[1] | | Rust severity in greenhouse screen (%)[1] |
|---|---|---|---|---|
| | | Lower leaves | Top four leaves | |
| ICMB 96222 | [{26B × (81B × SRL 50-1)}-1-1-2 × 852B]-69-1-1 | 10 | 3 | 2 |
| ICMR 01007 | Selected from a single selfed $BC_4F_2$ plant derived from backcrossing of donor parent ICMP 451-P6 with a pure line selection from H 77/833-2 | 5 | 0 | 49 |
| ICMR 06999 | MRC S1-4-1-3-B-B-B-B | 3 | 0 | HSR[2] |
| ICMP 451-P8 | Downy mildew resistant restorer selection from ICMP 451 (LCSN 72-1-2-1-1) | 10 | 0 | HSR |
| ICMP 451-P6 | Downy mildew resistant restorer selection from ICMP 451 (LCSN 72-1-2-1-1) | 10 | 0 | HSR |
| K-08-18395 | MRC HS-130-2-2-1-B-B-1-B-B-B | 0 | 0 | 33 |
| K-08-18399 | MRC S1-155-4-3-B-B-B-B-1-B-B | 3 | 0 | 57 |
| K-08-18400 | MRC S1-191-2-1-5-B-B-B-B-B | 5 | 0 | 36 |
| ICMP 83506 (Resistant check) | (B 282 × S10B-38)-3-1-3-2 | – | – | 34 |
| ICML 11 (Resistant check) | IP 2696-1-4 | – | – | HSR |
| ICMB 89111 (Susceptible check) | {843B × (GNS × SS-48-40-4)-1-9-8}-30-B-B-1 | 58 | 21 | 51 |
| Trial SE(m)± | | 4.9 | 6.1 | 3.18 |
| Trial LSD ($P$ <0.05) | | 13.6 | 17.1 | 9.23 |

1. Mean of two replications based on the modified Cobb scale.
   Resistant = 1–10% severity; moderately resistant = 11–20% severity; susceptible = 21–30% severity; highly susceptible = >30% severity.
2. HSR = Hypersensitive response (chlorotic specks without rust pustules).

resistance stability of these lines through multilocational testing in India and elsewhere.

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