# Identification of resistance to red rot in interspecific and intergeneric hybrid clones of sugarcane

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## Abstract

**The incorporation of red rot resistance in sugarcane is the foremost priority in Indian sugarcane breeding programs. An ideal variety should possess a high sucrose content, good agronomic traits, and red rot resistance. To enrich the parental pool with resistant sources having diverse backgrounds, many interspecific hybrid (ISH) clones and intergeneric hybrid (IGH) clones were identified in the previous decades from the 1960s and were utilised in the National Hybridization Programme. To further augment red rot resistance in the parental clones, we selected 27 ISH clones developed at ICAR-SBI Coimbatore and evaluated them against the prevailing *Colletotrichum falcatum* pathotypes in 10 locations representing major sugarcane growing regions of India. Among the clones, seven clones expressing more than 60% resistant or moderately resistant (R/MR) reactions in most locations against *C. falcatum* pathotypes CF06, CF07, CF08, CF09, and CF12 were identified as sources of red rot resistance. However, six clones showed more than 60% susceptible or highly susceptible (S/HS) reactions against the pathotypes across the locations. Besides *Saccharum spontaneum*, which is routinely used to transfer red rot resistance into sugarcane varieties, *S. robustum* and *Erianthus arundinaceus*, can also be used to introgress novel resistance genes to strengthen breeding for resistance. The multi-location testing of the clones identified resistance against major *C. falcatum* pathotypes in ISH/IGH clones, and such clones can be used as potential parents to obtain horizontal resistance against *C. falcatum* in sugarcane.**

**Keywords:** sugarcane, red rot, *Colletotrichum falcatum*, interspecific hybrid (ISH), resistance

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## Introduction

Globally, India is one of the top three sugarcane producing countries and sugarcane is cultivated in an area of 4.73 M ha in both subtropical and tropical regions with the production of 377 M tons (FAOSTAT, 2018). Although the country is performing well in sugar production, many biotic and abiotic constraints limit the goal of attaining the maximum potential yield of the crop. Among the biotic stresses, the century old red rot caused by the fungal pathogen *Colletotrichum falcatum* Went is a major problem in sugarcane cultivation and causes huge losses to the farmers and sugar industries. Farmers suffer directly from poor germination of setts, settling mortality, reduced cane population in the field, low cane yield, poor ratoon establishment due to death of stools, and loss of entire crop due to disease severity. Industries also suffer huge economic losses while crushing red rot affected canes, resulting in poor juice quality, poor sugar recovery, less bagasse, and increased molasses. *C. falcatum* produces an invertase enzyme which breaks the sucrose molecule into glucose and fructose, thereby affecting juice quality (Viswanathan, 2010, Viswanathan *et al.*, 2018a). Since the first report of red rot in sugarcane in the Godavari and Ganjam Districts in India, the country has witnessed a series of crop failures due to red rot epiphytotics, mainly in sugarcane belts of Uttar Pradesh and Bihar, resulting in huge economic losses to the nation (Viswanathan, 2018). Many popular varieties have been eliminated from cultivation owing to red rot susceptibility (Viswanathan 2010).

Sugarcane crops are characterised by a highly heterozygous polyploid with complex genetic constitution, and cane breeding involves a long selection period (Gupta *et al.* 2010). Modern canes are derived from recurrent backcrossing with *S. officinarum* or from complex varieties (Price, 1965). During the 1960s, one of the breeding objectives was to hybridise commercial varieties from different agro-climatic regions of the country with the *S. spontaneum* of that region to obtain parental stocks with high sucrose content and yield along with wider adaptability (Babu and Ethirajan, 1962). In addition, there are crossing programs involving *S. spontaneum* variants for obtaining genetic stocks with genes for red rot resistance, high sucrose, and tolerance to waterlogging conditions (Babu and Ethirajan, 1962). Modern varieties have been developed from the inter-specific hybridisation of *S. officianarum*, *S. spontaneum*, and *S. barberi*. To improve the productivity of sugarcane, a base broadening program was initiated through intergeneric crossing between *Saccharum* spp. and other related genera such as *Erianthus, Sclerostachya*, and *Narenga* (Nair 2007). An inter-institutional project was initiated in 1977 in India to incorporate red rot resistance into modern sugarcane clones. More than 10,000 base populations from 23 inter-cluster matings were phenotyped for red rot resistance at various centres, viz., Lauriya (Bihar), Karnal (Haryana), Etikoppaka (Andhra Pradesh), and Nellikuppam (Tamil Nadu) at the juvenile stage in humidity tents. After elimination of 6920 red rot-susceptible seedlings, 3624 red rot resistant genotypes were identified. From another set of 10,736 base populations derived from 26 crosses, 2548 seedlings were shortlisted (Alexander *et al.* 1979).

Further efforts were made at ICAR-SBI to utilise *S. spontaneum* as a source of red rot resistance in sugarcane varieties. Sreenivasan (1995) reiterated the role of *S. spontaneum* and *S. barberi* in imparting disease resistance. Alexander *et al.* (1986) identified many *S. spontaneum* clones with red rot resistance. Natarajan *et al.* (1998) speculated that *S. spontaneum* contributed to horizontal resistance against *C. falcatum*, whereas *S. officinarum* and *S. sinense* favour vertical resistance. All these studies conducted at ICAR-SBI concluded that *S. spontaneum* and other clones in germplasm collections could be exploited to impart red rot resistance in sugarcane. Inter-specific hybrids (ISH) are derived from crosses among different *Saccharum* spp., and intergeneric hybrids (IGH) are the progenies of crosses between *Saccharum* spp. and Erianthus spp. Both ISH and IGH clones have been utilised to impart red rot resistance and drought tolerance in sugarcane varieties (Viswanathan, 2021a). Recently, Viswanathan *et al.* (2018b) performed a detailed assessment of the status of resistance in wild relatives of sugarcane, especially *S. spontaneum*, ISH, and IGH, and concluded that certain cytoplasmic derivative clones and IGH clones in specific cross combinations possess disease resistance.

The search for red rot resistance in parental clones to newer pathotypes is a continuous process in which the status of red rot resistance is updated for the newly emerging pathogenic flora. Similarly, *C. falcatum* variation under field conditions is being assessed, and approximately 11 pathotypes have been characterised and designated (Viswanathan 2010). Recently, Viswanathan (2017) reported a new highly virulent pathotype, CF12, in the tropical region. Developing and identifying new parents for red rot resistance and establishing new pathogenic variations go hand in hand at ICAR-SBI and other research centres (Viswanathan, 2021b). However, such studies were conducted in different locations separately, and no effort was made to assess resistance in promising sugarcane clones across the country concurrently against the diverse *C. falcatum* pathotypes. Hence, we assembled 27 hybrid derivatives of interspecific and intergeneric crosses with various genetic backgrounds and tested them at 10 locations against five designated pathotypes that are used for red rot screening in the country.

This study allowed us to identify clones with a wide spectrum of resistance to *C. falcatum* pathotypes. The newly identified clones may serve as potential parents or donors to impart resistance to red rot in sugarcane.

## Materials and methods

In India, sugarcane growing states are distributed into five zones: north west, north central, east coast, northeast, and peninsular (Table 1). Red rot screening was carried out in 10 centres representing these agro-climatic zones, with the exception of the North East zone (Figure 1). Twenty-seven ISH and IGH clones developed at

**Table 1: List of *C. falcatum* pathotypes evaluated against hybrid clones at research centres in four agroclimatic zones in India**

| Zone | Multi location testing centres | Red rot pathotypes used |
|---|---|---|
| North West Zone | Lucknow, Shahjahanpur, Uchani, Karnal & Kapurthala | CF08 & CF09 |
| North Central Zones | PUSA & Seorahi | CF07 & CF08 |
| East Coast Zone | Anakapalle & Cuddalore | CF06 |
| Peninsular Zone | Coimbatore | CF06 & CF12 |

**Figure 1: The map showing 10 red rot testing centres in different states of India**


1. ICAR-Sugarcane Breeding Institute, Coimbatore, Tamil Nadu
2. Sugarcane Research Station, TNAU, Cuddalore, Tamil Nadu
3. Regional Agriculture Research Station, ANGRAU, Anakapalle, Andhra Pradesh
4. Sugarcane Research Institute, RAU Campus, Pusa, Bihar
5. Genda Singh Sugarcane Breeding & Research Institute, Seorahi, Uttar Pradesh
6. ICAR-Indian Institute of Sugarcane Research, Lucknow, Uttar Pradesh
7. UP Council of Sugarcane Research, Shahjahanpur, Uttar Pradesh
8. Regional Research Station, CCSHAU, Uchani, Karnal, Haryana
9. ICAR-Sugarcane Breeding Institute Regional Centre, Karnal, Haryana
10. Regional Research Centre, PAU, Kapurthala, Punjab

**Table 2: Interspecific (ISH) and intergeneric (IGH) hybrid clones evaluated for red rot resistance at different locations in India**

| Serial No | Genotype | Parents |
|---|---|---|
| **Interspecific hybrids (ISH)** | | |
| 1 | AS 04-1687 | Co 1148 X IND 84-337 |
| 2 | AS 04-1689 | CoH 114 X SH 216 |
| 3 | AS 04-2097 | Co 8371 X SH 216 |
| 4 | AS 04-245 | Co 89029 X IND 84-394 |
| 5 | AS 04-635 | CoH 114 X SH 216 |
| 6 | BM 1003143 | Co 86002//RE 132/Co 62198 |
| 7 | BM 1005149 | 81 V 48//RE 132/Co 62198 |
| 8 | BM 1009163 | 81 V 48//RE476/Co 0229 |
| 9 | BM 1010168 | Co 98010//Co 1148/SES 404 |
| 10 | BM 1022173 | 81 V 48//Co 1148/SES 404 |
| 11 | GU 07-2276 | 04(22) RE X Co 86002 |
| 12 | GU 07-3774 | PIO -88-110 X IND 00-1061 |
| 13 | GU 07-3849 | PIO -88-1703 X IND 00-1058 |
| 14 | PG 1869137 | CoC 85061////NG 77-99//28NG 51/SES 44A///28NG 210//NG 77-137/SES 538 |
| 15 | SA 04-390 | CoC 671 X (PIR 88-3350 X Co 86249) |
| 16 | SA 04-409 | CoC 671 X (PIR 88-3350 X Co 86249) |
| 17 | SA 04-454 | CoC 671 X (PIO 91-488 X SIP 93-397) |
| 18 | SA 04-458 | CoC 671 X (PIO 91-829 X SIP 93-380) |
| 19 | SA 04-472 | CoC 671 X (PIO 91-488 X SIP 93-397) |
| 20 | SA 04-496 | Co 86002 X (ISH 100 X (PIO90-188 X PIR 88-86)) |
| 21 | SA 98-13 | PIR 88-3350 X Co 86029 |
| **Inter-generic hybrids (IGH)** | | |
| 22 | CYM 07-986 | [IK 76-62 (*E. arundinaceus*) X Irrity -2 (*S. spontaneum*)] x CoC 671 |
| 23 | MA 5/22 | Co 7201 x (28NG210 x *Erianthus*) |
| 24 | MA 5/37 | Co 7201 x (28NG210 x *Erianthus*) |
| 25 | MA 5/5 | Co 7201 x (28NG210 x *Erianthus*) |
| 26 | MA 5/51 | Co 7201 x (28NG210 x *Erianthus*) |
| 27 | MA 5/99 | Co 7201 x (28NG210 x *Erianthus*) |

ICAR-SBI, Coimbatore, were selected for evaluation of resistance to red rot in each location using prevailing pathotypes of the red rot pathogen, as recommended by the All India Co-ordinated Research Project (AICRP) on Sugarcane (Table 2). In India, 12 *C. falcatum* pathotypes (CF01 to CF12) have been identified in different zones of the country based on their distinct behaviour on 14 host differentials (Viswanathan, 2010, 2017). In this study, pathotypes CF08 and CF09 were used in the North West zone, CF07 and CF08 in the North Central zone, CF06 in the East Coast zone, and CF06 and CF12 in the Peninsular zone.

In all the centres, the crop was raised following standard agronomic practices to the respective region/states. The respective cultures of *C. falcatum* pathotypes were multiplied well in advance, and the conidial suspension was made available during 6 months of crop age. Well-grown canes, free from termite or borer attacks, or from any other disease, were selected for inoculation with the pathogen. The inoculation assay was performed using an established set of procedures (Viswanathan, 2010, Mohanraj *et al.*, 2012). With the help of a specially devised red rot inoculator, an 8 mm diameter bore-hole was made at a 45° slanting angle to a depth of 0.5 cm in the 3rd internode above the ground level (Mohanraj *et al.*, 2012). About 0.5 ml of conidial suspension (1x$10^6$ conidia/ml of water) was delivered into the borehole. The core tissue that was initially removed was returned to its initial location, and the borehole was sealed with plastic clay to prevent entry of ants (Viswanathan, 2010, Mohanraj *et al.*, 2012). Eight to ten canes were inoculated per sugarcane clone and per pathotype. Immediately after inoculation, irrigation was provided to the plots to maintain the optimum humidity to facilitate disease initiation and development.

Sixty days after inoculation, the canes were cut below the point of inoculation, split open longitudinally, and disease symptoms were recorded with scores for each parameter viz., i. Condition of the top (green = 0; yellow/dry = 1), ii. Lesion width above the inoculated internode (restricted = 1, spreading = 2, or fully covered =3), iii. Presence and intensity of white spots (1 when restricted; 2 when spreading), and iv. Number of nodes crossed above the inoculated internodes (single node only = 1, up to 2 nodes = 2 or minimum 3, and more nodes = 3). Average of the total score is taken for grading the clones on a 0-9 scale, i.e. 0 to 2.0 = resistant (R), 2.1 to 4.0 = moderately resistant (MR), 4.1 to 6.0 = moderately susceptible (MS), 6.1 to 8.0 = susceptible (S) and 8.1 to 9.0 = highly susceptible (HS) (Srinivasan and Bhat, 1961). The reactions against each red rot pathotype were consolidated, and clones with resistant reactions were identified (Table 3). Disease reactions of R, MR, S, and HS were grouped under resistant and susceptible categories, respectively, along with MS to present the overall behaviour of the clones.

## Results

### Identification of clones with resistance to red rot

When the hybrid clones were screened against different pathotypes across locations, the reaction to red rot varied between locations. Among the 27 clones, seven clones were found resistant against many of the tested pathotypes across the locations showing more than 60% resistant types including 'R' or 'MR' reactions viz., GU 07-2276 (83.3 %), MA 5/99 (77.8 %), SA 04-454 (75.0 %), SA 04-390 (72.2 %), SA 98-13 (68.8 %), AS 04-1689 (66.7 %) and MA 5/37 (62.5 %) (Figures 2 and 3).

Regarding susceptible types, six clones, SA 04-458 (100.0 %), GU 07-3774 (93.8 %), AS 04-245 (83.3 %), BM 1009163 (75.0 %), PG 1869137 (62.5 %), and MA 5/5 (61.1 %) showed more than 60% susceptible types including 'S' or S /HS or 'HS' reactions against the pathotypes across the locations (Figure 2). Clone AS 04-1687, derived from the cross Co 1148 x IND 84-337, recorded the highest degree (72.2%) of MS reactions across the locations (Tables 3 and 5).

**Table 3: Reactions to red rot of 27 hybrid clones after inoculation with five pathotypes of *Colletotrichum falcatum* in 10 different testing centres**

| Sugarcane genotype | Pathotype CF06 | | | Pathotype CF07 | | Pathotype CF08 | | | | | | | Pathotype CF09 | | | | | Pathotype CF12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | CBE | Cuddalore | Anakapalle | PUSA | Seorahi | PUSA | Seorahi | Lucknow | Shahjahanpur | Uchani | Karnal | Kapurthala | Lucknow | Shahjahanpur | Uchani | Karnal | Kapurthala | CBE |
| AS 04 - 1687 | MR | MS | MS | MR | MS | MS | MS | MS | MS | MS | S | MR | MS | MS | MR | MS | MS | MS |
| AS 04-1689 | MR | MS | MS | MR | MS | MR | MR | MR | MR | MS | MR | MR | MR | MS | MS | MR | MR | MR |
| AS 04-2097 | MR | MR | MR | MR | S | MR | HS | MR | - | MS | HS | MS | MR | - | MS | HS | S | MS |
| AS 04-245 | HS | MS | HS | S | HS | S | HS | HS | HS | MS | S | HS | HS | HS | MS | S | HS | HS |
| AS 04-635 | R | MS | S | MS | MS | S | HS | MR | MR | MS | MR | MR | MS | MS | MS | MR | MS | MS |
| BM 1003143 | S | MS | S | S | S | S | MS | R | MS | MR | S | MR | R | S | MR | S | MS | HS |
| BM 1005149 | MS | MR | MS | - | MS | - | HS | MR | MS | MR | MS | MR | MR | MR | MS | HS | MR | S |
| BM 1009163 | S | HS | S | MS | HS | S | MS | S | - | MS | S | MS | S | - | S | S | S | S |
| BM 1010168 | R | MR | MS | MR | MS | MR | MS | R | MR | MS | MS | MS | R | MR | MR | MS | S | MR |
| BM 1022173 | MS | HS | HS | - | HS | - | MS | MS | - | R | S | MS | MS | - | MS | S | MS | MS |
| CYM 07-986 | MS | MS | S | MS | HS | S | MS | MS | MS | MR | S | HS | MS | MS | MS | S | HS | MS |
| GU 07-2276 | R | R | MR | MR | S | MR | MR | R | MS | MR | MR | MR | R | MR | MR | S | MR | MR |
| GU 07-3774 | HS | HS | S | S | - | S | - | HS | HS | S | HS | HS | HS | HS | MS | HS | S | HS |
| GU 07-3849 | MR | MR | MS | S | MS | S | MR | R | MS | MR | S | S | R | MS | R | S | S | MS |
| MA 5/22 | R | MR | MS | MS | HS | S | S | MR | MS | R | MS | S | MR | S | R | MS | MS | S |
| MA 5/37 | MR | MR | MR | S | HS | S | HS | MR | - | MS | MR | MR | MR | - | MR | MR | MS | MR |
| MA 5/5 | S | HS | S | S | MS | S | S | MR | S | MS | S | MS | MR | MS | MS | HS | S | S |
| MA 5/51 | HS | HS | MR | S | MR | S | HS | MS | MS | MS | S | MR | MS | MS | MR | S | MR | HS |
| MA 5/99 | R | MR | MR | S | MR | S | MR | MR | MS | MR | MR | MR | MR | MS | MR | MR | MR | MR |
| PG 1869137 | MS | MR | R | S | S | S | HS | S | MR | - | HS | MS | S | MS | - | HS | S | HS |
| SA 04-390 | MR | MR | MS | MR | S | MR | S | MR | MR | MR | MS | MR | MR | MR | MR | MR | MR | MS |
| SA 04-409 | MR | MR | S | S | S | S | S | MR | MS | MR | MS | MS | MR | MS | MS | S | MS | MR |
| SA 04-454 | MR | R | MR | MR | MS | MR | S | R | MR | - | R | MR | R | MS | - | MS | MR | MR |
| SA 04-458 | HS | HS | S | S | - | S | - | N | - | - | HS | HS | - | - | - | HS | HS | S |
| SA 04-472 | S | MS | S | MS | MS | MS | MS | MR | MS | MR | S | MS | MR | MS | R | S | MS | S |
| SA 04-496 | S | MS | MR | MR | S | MR | MS | R | MR | MR | MS | MR | R | MR | MS | S | MR | S |
| SA 98-13 | MR | MR | MS | - | MS | - | MS | R | R | MR | MS | MR | R | R | MR | S | S | R |

**Figure 2: Characterization of hybrid clones of sugarcane as resistant, moderately susceptible and susceptible and types to *C. falcatum* pathotypes**

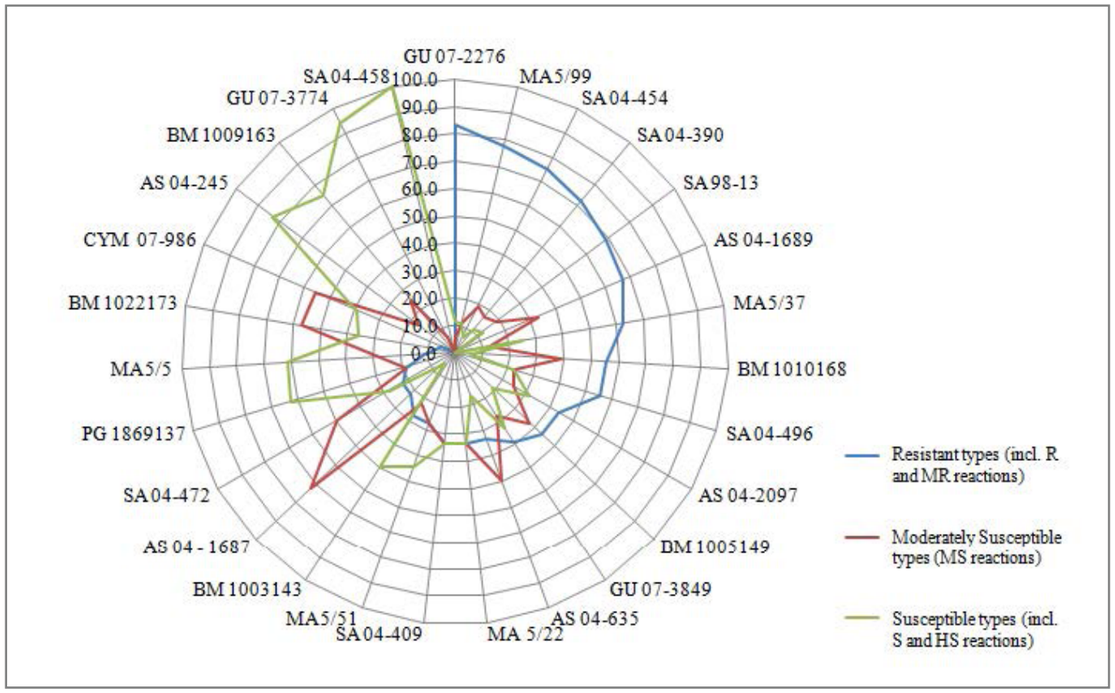
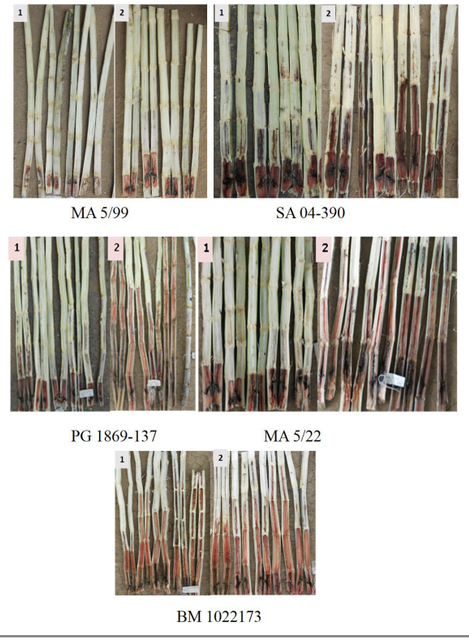



**Figure 3: Behaviour of sugarcane ISH/IGH clones to *C. falcatum* pathotypes CF06 (1) and CF12 (2) under Coimbatore conditions**




In the east coast zone, clone BM 1022173 derived from 81 V 48//RE Co 1148/SES 404 showed HS reaction against the CF06 pathotype and two clones AS 04–1687 and AS 04-1689 exhibited MS reactions, and AS 04-2097, MA 5/37, and MA 5/99 showed MR reactions at both the Cuddalore and Anakapalle locations (Table 3).

In North Central Zone, the clones BM 1003143, PG 1869137 and SA 04-409 and two clones AS 04-635 and SA 04-472 showed 'S' and 'MS' reactions respectively against CF07 pathotype in both Pusa and Seorahi centres (Table 3). Against CF08 pathotype, the clones AS 04-1689 and GU 07-2276 showed 'MR' reaction and AS 04 - 1687 and SA 04-472 behaved as MS and MA 5/22, MA 5/5 and SA 04-409 behaved as S in both Pusa and Seorahi centres (Table 3).

In the northwest zone, clones AS 04-245 and GU 07-3774 showed HS reactions against the CF08 pathotype in all four centres except Uchani, where they showed MS and S reactions, respectively. Similarly the clones BM 1009163 and PG 1869137 behaved as susceptible against CF08 pathotype but the reactions ranged from MS to HS in all the centres except the clone PG 1869137 which showed 'MR' reaction in Shahjahanpur centre (Table 3). Clones such as SA 04-454 and SA 98-13 behaved as resistant types in all the centres. The clone SA 04-496 derived from Co 86002 X (ISH 100 X (PIO90-188 X 88-86) behaved as a resistant type in all the centres except Karnal where it behaved as MS and the clones AS 04-1689, AS 04-635, MA 5/99, and SA 04-390 behaved as MR in four locations and at Karnal centre it behaved as MS (Table 3). Clones AS 04–1687 and MA 5/51 behaved as 'MS' in all the centres and in Kapurthala behaved as MR. The results clearly indicated the role of the environment in red rot expression by the pathotype in different locations. When the pathotype CF09 was used for evaluation, the clones AS 04-245, BM 1009163, BM 1022173, GU 07-3774, PG 1869137 and SA 04-458 behaved as susceptible clones in all the centres, ranging from 'MS' to HS (Table 3).

## *C. falcatum* pathotypes and red rot reactions

In total, 27 ISH/IGH clones were evaluated against five pathotypes across sugarcane growing regions. Pathotype CF07 caused 12.2% HS and 36.7% S reactions, but none of the clones showed "R" reactions against this pathotype. Although there were less than 10% clones with R reactions, there were more than 20% clones of MR reactions against all the pathotypes (Table 4). Against the pathotypes CF06 and CF08 a high level of MR reactions (33.3%) was obtained. Among the disease testing locations, seven R clones were identified in Lucknow viz., BM 1003143, BM 1010168, GU 07-2276, GU 07-3849, SA 04-454, SA 04-496, SA 98-13 against the pathotypes CF08 and CF09 (Table 3). A higher proportion of 48.1% clones with MR reactions were identified in Kapurthala against CF08, followed by 45.8% in Uchani against CF08, 42.3% in Lucknow against CF08, and 40.7% in Cuddalore against CF06. Whereas, a higher proportion of 'MS' reactions were found in Seorahi (40.0%) against CH07, Shahjahanpur (50.0%) and Uchani (41.7%) against CF08, Shahjahanpur (54.5%) and Uchani (45.8%) against CF09 (Table 4).

Two centres, namely Pusa and Karnal, recorded more S reactions than any other centre (Table 3). The Pusa centre showed 45.8% and 62.5% S reactions against CF07 and CF08, respectively, whereas the Karnal centre showed 44.4% S reactions against CF09. A high incidence of HS reactions (22.2%) was recorded in Cuddalore and Karnal against CF06 and CF09, respectively, and the centre Seorahi centre recorded 24.0% and 28.0% HS reactions against CF07 and CF08 pathotypes, respectively (Table 4).

## Discussion

The use of healthy planting material is a prerequisite for growing red rot-free sugarcane crops in farmers' fields. However, the identification of disease-free material in field-grown sugarcane is difficult because of incipient infections of the pathogen (Viswanathan 2021a). Management of red rot after crop establishment is also not feasible and cost ineffective; therefore, utilisation of red rot resistant varieties is the only alternative to curb the spread of the disease in the field. Although the identification of resistant sources in sugarcane is cumbersome and time consuming, incorporation of red rot resistance into new varieties is an integral part of the Indian sugarcane breeding program.

In India, breeding for red rot resistance is a continuous process, and many diverse sources have been identified to impart red rot resistance in the gene pool, and red rot resistance is a prerequisite to release a new variety (Viswanathan, 2018). During the 1980s, red

**Table 4: Frequency of resistance levels to red rot in each testing centre after inoculation of 27 hybrid clones with five *C. falcatum* pathotypes**

| Location | Red rot reactions | | | | | Frequency of red rot reactions under three groups | | | |
|---|---|---|---|---|---|---|---|---|---|
| | R | MR | MS | S | HS | Resistant (R + MR reactions) | Moderately Susceptible (MS reactions) | Susceptible (S+ HS reactions) | Total reactions |
| **CF06** | | | | | | | | | |
| Cuddalore | 2 (7.4) | 11 (40.7) | 8 (29.6) | 0 (0.0) | 6 (22.2) | 13 (48.1) | 8 (29.6) | 6 (22.2) | 27 |
| Anakapalle | 1 (3.7) | 7 (25.9) | 8 (29.6) | 9 (33.3) | 2 (7.4) | 8 (29.6) | 8 (29.6) | 11 (40.7) | 27 |
| Coimbatore | 5 (18.5) | 9 (33.3) | 4 (14.8) | 5 (18.5) | 4 (14.8) | 14 (51.9) | 4 (14.8) | 9 (33.3) | 27 |
| | 8 (9.9) | 27 (33.3) | 20 (24.7) | 14 (17.3) | 12 (14.8) | 35 (43.2) | 20 (24.7) | 26 (32.1) | 81 |
| **CF07** | | | | | | | | | |
| PUSA | 0 (0.0) | 8 (33.3) | 5 (20.8) | 11 (45.8) | 0 (0.0) | 8 (33.3) | 5 (20.8) | 11 (45.8) | 24 |
| Seorahi | 0 (0.0) | 2 (8.0) | 10 (40.0) | 7 (28.0) | 6 (24.0) | 2 (8.0) | 10 (40.0) | 13 (52.0) | 25 |
| | 0 (0.0) | 10 (20.4) | 15 (30.6) | 18 (36.7) | 6 (12.2) | 10 (20.4) | 15 (30.6) | 24 (49.0) | 49 |
| **CF08** | | | | | | | | | |
| Pusa | 0 (0.0) | 7 (29.2) | 2 (8.3) | 15 (62.5) | 0 (0.0) | 7 (29.2) | 2 (8.3) | 15 (62.5) | 24 |
| Seorahi | 0 (0.0) | 4 (16.0) | 9 (36.0) | 5 (20.0) | 7 (28.0) | 4 (16.0) | 9 (36.0) | 12 (48.0) | 25 |
| Lucknow | 7 (26.9) | 11 (42.3) | 4 (15.4) | 2 (7.7) | 2 (7.7) | 18 (69.2) ) | 4 (15.4) | 4 (15.4) | 26 |
| Shahjahanpur | 1 (4.5) | 7 (31.8) | 11 (50.0) | 1 (4.5) | 2 (9.1) | 8 (36.4) ) | 11 (50.0) | 3 (13.6) | 22 |
| Uchani | 2 (8.3) | 11 (45.8) | 10 (41.7) | 1 (4.2) | 0 (0.0) | 13 (54.2) | 10 (41.7) | 1 (4.2) | 24 |
| Karnal | 1 (3.7) | 6 (22.2) | 6 (22.2) | 10 (37.0) | 4 (14.8) | 7 (25.9) | 6 (22.2) | 14 (1.9) | 27 |
| Kapurthala | 0 (0.0) | 13 (48.1) | 8 (29.6) | 2 (7.4) | 4 (14.8) | 13 (48.1) | 8 (29.6) | 6 (22.2) | 27 |
| | 11 (6.3) | 59 (33.7) | 50 (28.6) | 36 (20.6) | 19 (10.9) | 70 (40.0) | 50 (28.6) | 55 (31.4) | 175 |
| **CF09** | | | | | | | | | |
| Lucknow | 7 (26.9) | 10 (38.5) | 5 (19.2) | 2 (7.7) | 2 (7.7) | 17 (65.4) | 5 (19.2) | 4 (15.4) | 26 |
| Shahjahanpur | 1 (4.5) | 5 (22.7) | 12 (54.5) | 2 (9.1) | 2 (9.1) | 6 (27.3) | 12 (54.5) | 4 (18.2) | 22 |
| Uchani | 3 (12.5) | 9 (37.5) | 11 (45.8) | 1 (4.2) | 0 (0.0) | 12 (50.0) | 11 (45.8) | 1 (4.2) | 24 |
| Karnal | 0 (0.0) | 5 (18.5) | 4 (14.8) | 12 (44.4) | 6 (22.2) | 5 (18.5) | 4 (14.8) | 18 (66.7) | 27 |
| Kapurthala | 0 (0.0) | 8 (29.6) | 8 (29.6) | 8 (29.6) | 5 (11.1) | 8 (29.6) | 8 (29.6) | 11 (40.7) | 27 |
| | 11 (8.7) | 37 (29.4) | 40 (31.7) | 25 (19.8) | 13 (10.3) | 48 (38.1) | 40 (31.7) | 38 (30.2) | 126 |
| **CBE CF12** | 1 (3.7) | 7 (25.9) | 7 (25.9) | 7 (25.9) | 5 (18.5) | 8 (29.6) | 7 (25.9) | 12 (44.4) | 27 |

rot screening was performed in various red rot endemic locations representing Tamil Nadu (zone I), Coastal Andhra Pradesh (zone II), Eastern Uttar Pradesh and North Bihar (zone III), and western Uttar Pradesh, Uttarakhand, Haryana, and Punjab (zone IV). Over 200 genotypes were screened at Nellikuppam, Tanuku, Chakia, and Karnal, representing zones I, II, III, and IV, respectively, using mixtures of three major pathotypes following the plug method in the field (Alexander *et al.* 1986). These efforts have led to the identification of many clones with horizontal resistance against diverse pathogenic variants during that period.

The 27 hybrid clones developed from crossing commercial canes and species clones or genetic stocks showed differential reactions to five *C. falcatum* pathotypes. Among the different species of *Saccharum*, *S. spontaneum* was introgressed extensively for high tillering, wider adaptability, and resistance to pests and diseases, including red rot (Chona, 1954; Baksha *et al.*, 2003). Genome analysis of the 27 hybrid clones indicated that *S. robustum* is emerging as a new candidate species as a potential donor for red rot resistance. Out of eight clones with *S. robustum* as one of the parents in the pedigree, four showed resistance in more than 50% of the testing across the pathotypes. Although *S. spontaneum* was one of the parents in the immediate pedigree in seven clones, only AS 04-1689 showed resistance in more than 50% of the locations across the pathotypes. An ISH progeny GU 07-3774, developed from the cross between *S. officinarum* and *S. spontaneum*, exhibited a susceptible reaction to all pathotypes of the pathogen, although it had 50% of the *S. spontaneum* chromosome complement, whereas GU 07-3849, selected from the same cross combination, showed only 38.9% resistance to *C. falcatum*. It was postulated that horizontal resistance in the progenies is proportional to the number of *S. spontaneum* chromosomes (Natarajan *et al.* 2001a) which is contrary to the highly susceptible reactions across the pathotypes exhibited by GU 07-3774 with 50% of the *S. spontaneum* chromosomes. Among the three BC1 clones involving *S. spontaneum* as one of the parents, only SA04-454 showed 75 % resistance across the pathotypes. This clearly indicated that *S. spontaneum* clones showed greater variability for transmitting genes for resistance to red rot (Srinivasan and Chennulu 1956; Hale *et al.*, 2010) and intensive studies are required to select specific *S. spontaneum* clone(s) as a parental material in breeding programs for red rot resistance. The inheritance of red rot resistance involving *S. spontaneum* parents is also poorly understood due to confusing segregation patterns (Natarajan, 2001b). Later, Babu *et al.* (2010) screened 1356 progenies derived from 39 biparental crosses involving *S. officinarum* and *S. spontaneum* inter-varietal crosses at Coimbatore under controlled condition testing (CCT) using the CF06 pathotype and found two crosses viz., 987032 × Co 93009 and RS 93-2182 x Co 93009 contributed more than 80.0% red rot resistant progenies.

*Erianthus arundinaceus* (EA) is an important related genus of sugarcane and a member of the *Saccharum* complex. Generating intergeneric hybrids between *Saccharum* and *Erianthus* is a difficult task because of the different genera and wide genetic distance between them. However, several successful reports have been published recently on the generation of progenies involving these two genera. *E. arundinaceus*, the only cane-forming species in the genus *Erianthus*, is of recent interest to sugarcane breeders as it is a candidate species for the incorporation of high tillering, high biomass, and resistance to pests and diseases. There have been very few targeted breeding programs involving *Erianthus* for transferring red rot resistance has been very few (Ram *et al.*, 2001, Mohanraj *et al.*, 2019). Of the 10 clones having *E. arundinaceus* as one of the parents, three clones, viz., MA 5/99, MA 5/37, and GU 07-2276, recorded more than 50% resistance across the pathotypes. This indicated the possibility of introducing novel red rot resistance genes from *E. arundinaceus* to increase horizontal resistance by combining resistant genes from genetically diverse parents. The clone GU 07-2276 had the parent 04(22) RE, an IGH selected from the segregating progenies of *E. arundinaceus* and *S. robustum*, showed the highest percentage of resistance (83.3%) across the pathotypes. Most of the 27 clones evaluated were the first-or second-generation progenies involving different species and possibly selected for cane yield and quality traits, but no selection pressure was applied for screening against red rot. It is imperative that if ISH/IGH crosses are made with different *Saccharum* species and targeted selection is made for red rot, the proportion of identifying resistant progenies may increase. However, the study demonstrated that in addition to *S. spontaneum* which is routinely used to develop red rot resistant varieties, new sources of resistance can be incorporated by involving *S. robustum* and *E. arundinaceus* in the backcrosses to achieve horizontal resistance in sugarcane varieties. A larger number of genetic stocks are available for *E. arundinaceus*, which can be screened against different pathotypes and used in breeding programs to achieve both increased biomass and horizontal resistance in modern sugarcane varieties.

In our experiment, among the 27 hybrid clones, seven clones (AS 04-1689, GU 07-2276, MA 5/37, MA 5/99, SA 04-390, SA 04-454, and SA 98-13 were found to be resistant, and six clones were found to be susceptible to all the tested pathotypes across the locations. The other clones exhibited more than 50% 'MS' reactions were, AS 04-635 (50.0%), BM 1022173 (57.1 %), CYM 07-986 (55.6 %) and SA 04-472 (50.0 %) (Tables 3 and 5). Further studies should utilise these seven resistant clones as potential resistant donors or parents. Our results indicate that the behaviour of the clones to red rot pathotypes varied across different locations, probably due to the interaction between the host and the pathogen under the influence of prevailing climatic conditions. The complexity of sugarcane genetics makes research on red rot resistance more difficult; however, the search for red rot resistance sources in the germplasm and ISH/IGH has to be continued to identify new red rot resistance parental stocks.

Of the five pathotypes, CF12 was found to be more virulent causing 18.5% 'HS' and 25.9% 'S' reactions followed by CF06 causing 14.8 % 'HS' and 17.3% 'S' reactions. Among the two tropical pathotypes, CF12 was more virulent than pathotype CF06 (Table 4). Overall, we found that both tropical pathotypes exhibited higher virulence than subtropical pathotypes. Higher virulence of pathotypes/isolates from tropical regions has been reported earlier (Padmanaban *et al.*, 1996; Viswanathan *et al.*, 1997). Previously, the senior author validated the enhanced virulence of the pathotype CF12 isolated from the cv Co 94012 in Tamil Nadu over the CF06 pathotype on 32 varieties and designated it as a new pathotype (Viswanathan, 2017). Recent studies by Viswanathan *et al.* (2017) with ~117 isolates also revealed that tropical isolates exhibited more virulence than those from the subtropical region when they tested the isolates at Coimbatore and Karnal simultaneously for five years on a susceptible cv CoC 671. Our observations indicate that the tropical pathotypes of *C. falcatum* still possess higher virulence. However, the recent studies revealed emergence of a highly virulent pathotype CF13 which broke down resistance to *C. falcatum* in the ruling variety of the subtropical region Co 0238, occupying about 53.2% cane area (2.58 million hectares) in India (Viswanathan *et al.*, 2021) This scenario warrants replacement of the ruling variety with a new variety with resistance to new pathotype CF13. Hence, search for new sources of red rot resistance and their incorporation in the commercial varieties is a continuous process in Indian sugarcane improvement program.

**Table 5: Frequency of resistance levels to red rot of 27 hybrid clones inoculated with five *C. falcatum* pathotypes in 10 locations in India**

| Genotype | Red rot reactions | | | | | Frequency of red rot reactions under three groups | | | Total reactions |
|---|---|---|---|---|---|---|---|---|---|
| | R | MR | MS | S | HS | Resistant (R + MR reactions) | Moderately Susceptible (MS reactions) | Susceptible (S+ HS reactions) | |
| AS 04 - 1687 | 0 (0.0) | 4(22.2) | 13 (72.2) | 1(5.6) | 0 (0.0) | 4(22.2) | 13(72.2) | 1(5.6) | 18 |
| AS 04-1689 | 0 (0.0) | 12(66.7) | 6 (33.3) | 0 (0.0) | 0 (0.0) | 12(66.7) | 6 (33.3) | 0 (0.0) | 18 |
| AS 04-2097 | 0 (0.0) | 7(43.8) | 4 (25.0) | 2(12.5) | 3(18.8) | 7(43.8) | 4(25.0) | 5 (31.3) | 16 |
| AS 04-245 | 0 (0.0) | 0 (0.0) | 3 (16.7) | 4(22.2) | 11(61.1) | 0 (0.0) | 3(16.7) | 15(83.3) | 18 |
| AS 04-635 | 1 (5.6) | 5 (27.8) | 9 (50.0) | 2(11.1) | 1(5.6) | 6(33.3) | 9(50.0) | 3 (16.7) | 18 |
| BM 1003143 | 2 (11.1) | 3 (16.7) | 4 (22.2) | 8(44.4) | 1(5.6) | 5(27.8) | 4 (22.2) | 9 (50.0) | 18 |
| BM 1005149 | 0 (0.0) | 7 (43.8) | 6(37.5) | 1 (6.3) | 2 (12.5) | 7 (43.8) | 6 (37.5) | 3 (18.8) | 16 |
| BM 1009163 | 0 (0.0) | 0 (0.0) | 4 (25.0) | 10 (62.5) | 2 (12.5) | 0 (0.0) | 4 (25.0) | 12 (75.0) | 16 |
| BM 1010168 | 3 (16.7) | 7 (38.9) | 7 (38.9) | 1 (5.6) | 0 (0.0) | 10 (55.6) | 7 (38.9) | 1 (5.6) | 18 |
| BM 1022173 | 1 (7.1) | 0 (0.0) | 8 (57.1) | 2 (14.3) | 3 (21.4) | 1 (7.1) | 8 (57.1) | 5 (35.7) | 14 |
| CYM 07-986 | 0 (0.0) | 1(5.6) | 10 (55.6) | 4 (22.2) | 3 (16.7) | 1 (5.6) | 10 (55.6) | 7 (38.9) | 18 |
| GU 07-2276 | 4 (22.2) | 11 (61.1) | 1 (5.6) | 2 (11.1) | 0 (0.0) | 15 (83.3) | 1 (5.6) | 2 (11.1) | 18 |
| GU 07-3774 | 0 (0.0) | 0 (0.0) | 1 (6.3) | 5 (31.3) | 10 (62.5) | 0 (0.0) | 1 (6.3) | 15 (93.8) | 16 |
| GU 07-3849 | 3 (16.7) | 4 (22.2) | 5 (27.8) | 6 (33.3) | 0 (0.0) | 7 (38.9) | 5 (27.8) | 6 (33.3) | 18 |
| MA 5/22 | 3 (16.7) | 3 (16.7) | 6 (33.3) | 5 (27.8) | 1 (5.6) | 6 (33.3) | 6 (33.3) | 6 (33.3) | 18 |
| MA 5/37 | 0 (0.0) | 10 (62.5) | 2 (12.5) | 2 (12.5) | 2 (12.5) | 10 (62.5) | 2 (12.5) | 4 (25.0) | 16 |
| MA 5/5 | 0 (0.0) | 2 (11.1) | 5 (27.8) | 9 (50.0) | 2 (11.1) | 2 (11.1) | 5 (27.8) | 11 (61.1) | 18 |
| MA 5/51 | 0 (0.0) | 5 (27.8) | 5 (27.8) | 4 (22.2) | 4 (22.2) | 5 (27.8) | 5 (27.8) | 8 (44.4) | 18 |
| MA 5/99 | 1 (5.6) | 13 (72.2) | 2 (11.1) | 2 (11.1) | 0 (0.0) | 14 (77.8) | 2 (11.1) | 2 (11.1) | 18 |
| PG 1869137 | 1 (6.3) | 2 (12.5) | 3 (18.8) | 6 (37.5) | 4 (25.0) | 3 (18.8) | 3 (18.8) | 10 (62.5) | 16 |
| SA 04-390 | 0 (0.0) | 13 (72.2) | 3 (16.7) | 2 (11.1) | 0 (0.0) | 13 (72.2) | 3(16.7) | 2 (11.1) | 18 |
| SA 04-409 | 0 (0.0) | 6 (33.3) | 6 (33.3) | 6 (33.3) | 0 (0.0) | 6 (33.3) | 6 (33.3) | 6 (33.3) | 18 |
| SA 04-454 | 4 (25.0) | 8 (50.0) | 3 (18.8) | 1 (6.3) | 0 (0.0) | 12 (75.0) | 3 (18.8) | 1 (6.3) | 16 |
| SA 04-458 | 0 (0.0) | 0 (0.0) | 0 (0.0) | 4 (40.0) | 6 (60.0) | 0 (0.0) | 0 (0.0) | 10 (100.0) | 10 |
| SA 04-472 | 1 (5.6) | 3 (16.7) | 9 (50.0) | 5 (27.8) | 0 (0.0) | 4 (22.2) | 9 (50.0) | 5 (27.8) | 18 |
| SA 04-496 | 2 (11.1) | 8 (44.4) | 4 (22.2) | 4 (22.2) | 0 (0.0) | 10 (55.6) | 4 (22.2) | 4 (22.2) | 18 |
| SA 98-13 | 5 (31.3) | 6 (37.5) | 3 (18.8) | 2 (12.5) | 0 (0.0) | 11 (68.8) | 3 (18.8) | 2 (12.5) | 16 |
| | **31** | **140** | **132** | **100** | **55** | **171** | **132** | **155** | **458** |

## Conclusion

The multilocation evaluation of ISH/IGH clones against *C. falcatum* pathotypes identified red rot resistant genotypes that could serve as genetic stocks for imparting red rot resistance. The diverse source of resistance for red rot may slow down the development of new variants of *C. falcatum* in the field, and the time taken for adaptation of the pathogen to the host variety is also prolonged, thus expanding the field life span of a variety. The introgression of new sources of resistance from the new basic germplasm of *S. spontaneum* and the development of ISH genetic stock clones have great potential in sugarcane improvement programs because they have better variability for morphological and yield traits than their parents.

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