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# DEVELOPMENT OF *IN VITRO* SCREENING TECHNIQUE IN PIGEONPEA AGAINST DRY ROOT ROT DISEASE CAUSED BY *MACROPHOMINA PHASEOLINA* (TASSI) GOID

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**ABSTRACT**

The fungus *Macrophomina phaseolina*, which causes dry root rot, is a relatively recent danger to the global supply of pigeonpea. Because this pathogen is a polyphagic necrotroph, it remains viable in the soil for several years, making disease management challenging. Using resistant cultivars in an integrated approach is one of the most cost-effective ways to manage dry root rot in pigeonpea. Having a reliable and repeatable screening protocol is pre requisite for resistance breeding programme. Availability of an *in vitro* screening protocol further reduces the time, efforts, space and expenditure in screening large population against the disease. The present investigation was undertaken to standardise the *in vitro* screening method for pigeonpea to identify the resistance against *M. phaseolina* causing dry root rot (DRR) and screening of 27 pigeonpea genotypes and entries against the pathogen. The results were compared with the field screening of all these entries and genotypes against the DRR disease in sick plot. Out of 27 genotypes screened *in vitro* following paper towel technique, only four genotypes (GRG-811, KRG-33, GRG-152 and ICP-8863) were found moderately resistant. Where as in sick plot, five genotypes were found moderately resistant: GRG-811, KRG-33, GRG-152, ICP-8863, and NAM-2282. The remaining genotypes showed susceptible reaction. Results showed maximum reliability and repeatability of *in vitro* paper towel method standardised in the study for screening against *M. phaseolina*. The method shall be used for screening large number of pigeonpea populations in short time, space and cost with high accuracy and repeatability. Further the entries identified moderately resistant to DRR in the present study shall be used for commercial cultivation in disease prone areas and may be used in breeding programmes also.

***Key words :*** *Macrophomina phaseolina*, Pigeonpea, Genotypes, Dry root rot.

## Introduction

Pigeon pea is one of the important grain legume crop endowed with several features *viz*., high nutritive value, potential to fix atmospheric nitrogen, capacity to thrive under adverse environmental condition, suitable for intercropping and enhance the net income of small and marginal farmers. Globally pigeonpea is grown in an area of 6.35 Mha with a production of 5.47 Mt and productivity of 861.25 Kg/ha. India ranks first in global pigeonpea production with 4.3 Mt, cultivated on 4.98 Mha with productivity of 871 Kg/ha in 2021-22. Among the major pigeonpea-producing states, Maharashtra (1.34 Mha and producing 1.37 Mt) tops in production (Anonymous, 2023), followed by Karnataka (1.72 Mha and producing 1.14 Mt) and Uttar Pradesh (0.272 Mt.). Other states producing pigeonpea include Madhya Pradesh, Andhra Pradesh, Odisha, Bihar, Tamil Nadu, and Gujarat. In North Karnataka, due to its multiple benefits at low cost, pigeonpea has become an ideal, nutritionally rich, and drought-resilient crop for sustainable agriculture systems in rain-fed areas (Hemavathy *et al*., 2023).

Ever since, the effect of climate change has started visible in field crops, pigeonpea has also been experiencing the ill effects. There has been an increase in the incidence

of minor diseases of pigeonpea in to major epidemics. Over the past three years, the incidence of Dry Root Rot (DRR) of pigeonpea caused by *Macrophomina phaseolina* (Tassi) Goid (Scelerotial stage: *Rhizoctonia bataticola*) is drastically increasing and causing huge yield losses. In pigeonpea field, the onset of the dry root rot disease appears as scattered, drying of leaves and entire plants is most frequently observed. Affected plants dry and are generally straw coloured, but in some cases the lower leaves and affected stems shows brown to black discolouration. Favourable weather during the vegetative stage, coupled with frequent dry spells are predisposing pigeonpea for severe infection by DRR disease. The tap root system appears black, rotten and devoid of most of the lateral, terminal and fine roots. The dead root becomes quite brittle and shows bark shredding. Dark and minute sclerotial bodies can be seen on the roots exposed or inside the wood. When the dry stem of the collar region of infected plant is split vertically, very minute sclerotia can be seen in the pith of the root system (Nene *et al*., 1981). The vascular system allows *M. phaseolina* to spread within infected plants (Win and Oo, 2017). When the roots become infected, they start rotting, which eventually causes wilting. The microsclerotia survive in the soil for many years, allowing it to thrive in hot climates and making it difficult to control. Gadde *et al*. (2023) reported that *M. phaseolina* can grow in wide range of pH levels (6-8) and higher temperature levels (30-35$^0$C). To control *M. phaseolina*, synthetic fungicides are often applied, but their use is limited and not advocated because of environmental and health concerns as well as the potential emergence of resistance in pathogen (Iqbal and Mukhtar 2020; Lokesh *et al*., 2020).

Being soil borne in nature the disease management has become serious concern for the growers and there are no cultivated cultivars resistant to this disease. Though, many bio-control agents are tested and found effective *in vitro*, but their commercial application on large scale has remained far from reality due to their non availability everywhere and quality concerns. Under these circumstances, host resistance holds most promising and shall be the best option compared to all other opportunities of disease management. It is also one of the most sought, cost effective, long lasting and ecofriendly options for disease control.

The screening of pigeonpea for resistance against DRR in volves screening under epiphytotic conditions in a sick plot. This exercise helps in identifying the resistant genotypes or entries but for only one generation of the crop cycle in a year and any identified resistant genotypes or entries further require confirmation of the resistance by one or two more cycles before its release for commercial cultivation or use in breeding programmes. In view of these obstacles in identifying host resistance in pigeonpea against DRR, an effort was made to develop an *in vitro* screening technique to speed up or hasten the process of breeding for disease resistance and screen large number of entries in short time and space. Thus, the present investigation was undertaken.

## Materials and Methods

### Field screening of pigeonpea against dry root rot disease in sick plot

The field screening of pigeonpea entries and genotypes along with susceptible check ICP7119 were sown in separate DRR sick plot maintained at ZARS, Kalaburagi. The entries were sown in two rows at spacing of 60×20cm in two replications. Other agronomical practices were followed as per package of practices. Observations were recorded on incidence of dry root rot following the standard procedure and disease reaction was noted based on the rating of AICRP on *Kharif* Pulses Pigeonpea at flowering and pod maturation stage.

### Disease rating scale used for assessment of resistance by pigeonpea against dry root rot disease (AICRP on pigeonpea, 2022)

| S. no. | Description (wilting %) | Reaction | Grade |
|---|---|---|---|
| 1. | 0-10 | Resistant | R |
| 2. | 10-30 | Moderately resistant | MR |
| 3. | 30-100 | Susceptible | S |

### *In vitro* screening of pigeonpea entries and genotypes against dry root rot pathogen through paper towel technique

**Preparation of pathogen culture :** The DRR infected pigeonpea roots were used for isolation of pure culture of *Macrophomina phaseolina* on Potato Dextrose Agar (PDA) media. After growing on PDA medium, a pure pathogen mycelial culture disc of 5 mm was placed in 250 ml flasks holding 100 ml Potato Dextrose Broth (PDB). Mycelial mats were carefully separated in 500ml sterile beakers after a 7-day incubation period at 30°C. Each mat was mixed with 100 ml of sterilized distilled water and homogeneous suspension was prepared a warring blender for 30 seconds. The pathogen mycelium suspension was further used for screening of pigeonpea.

**Preparation of pigeonpea seedlings :** Pigeonpea seeds of different genotypes and entries were surface

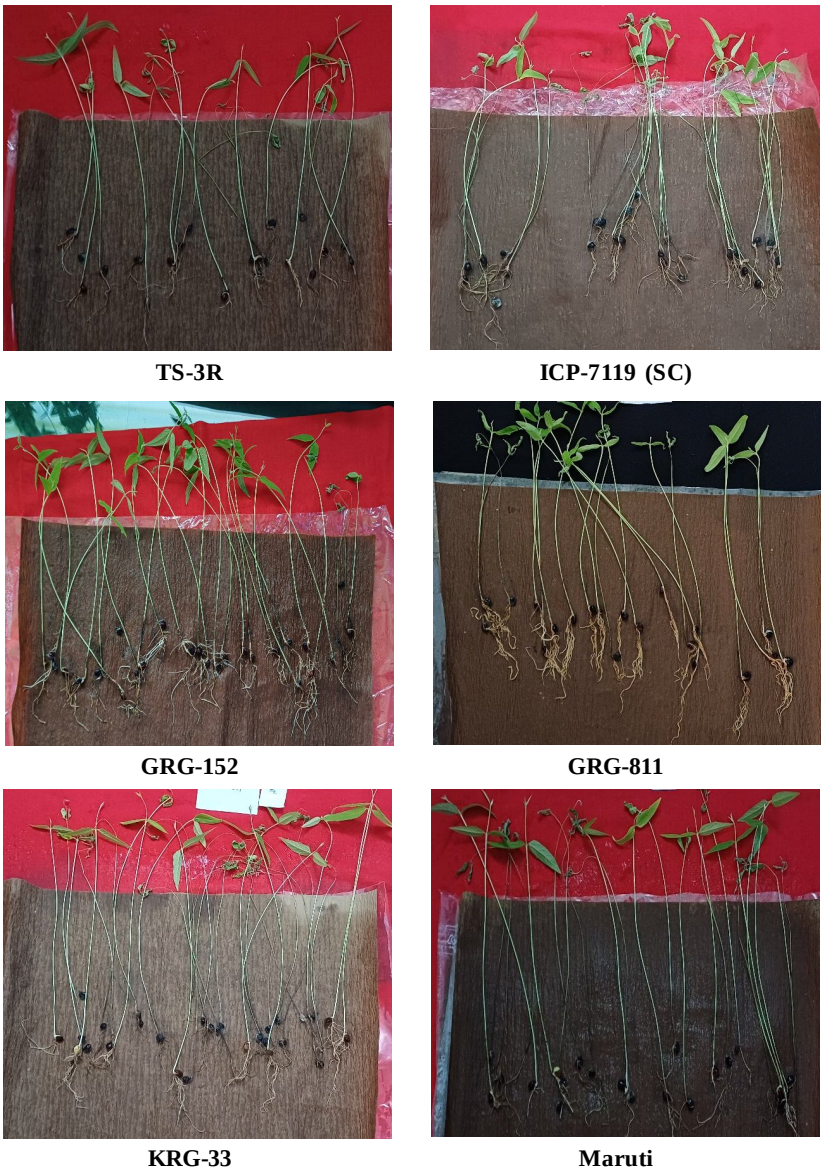


**Fig. 1 :** Paper towel screening of pigeonpea genotypes against *Macrophomina phaseolina*.

sterilized for one minute with 0.1% mercuric chloride solution and rinsed three times in sterile water. Seeds were sown in plastic trays containing sterilized sand. The seedlings of 7, 10, 12 & 15 days old after germination were carefully removed from trays to avoid root damage. They were cleaned with water to remove any sand adhered and used for inoculation.

**Inoculation of seedlings with pathogen and incubation :** Pigeonpea seedlings were dipped in the inoculum prepared for about 30 seconds. Excess inoculum was removed by touching the roots to the edge of the beaker. These treated 25 seedlings were placed on a moistened (with sterile water) blotter paper (size 45 cm × 25 cm with one-fold) in a line side by side in such a way that cotyledons and roots remained covered inside the blotter paper and the green tops of the seedlings remained outside the blotter paper after it is folded. The blotter papers containing seedlings were placed in tray in slanting position and incubated at 35°C with 12-hour light and 12-hour dark period in an incubator.

For each entry and genotype three replications were maintained following completely randomized block design (CRBD). Seedlings dipped in sterile water served as control with same number of replications as that of inoculated. The disease severity was categorized at 10 days after inoculation using AICRP on Kharif Pulses Pigeonpea scale.

## Results and Discussion

**Determination of age of seedlings and incubation period :** The seedlings age and incubation period were determined in the *in vitro* screening using most popular cultivar TS-3R. Among the four different aged seedlings (7, 10, 12 and 15 days), the stems of 12 and 15-day-old were lanky and their average shoot and root lengths were 25 and 10 cm and 28 and 12 cm respectively and the seedling's roots were larger than the standard blotter paper used for seedling assays (standard size 30cm × 60cm). Further, when blotter paper with seedlings was folded, seedling's root and shoot got damaged due to their excessive length extending too much outside the paper, with these reasons seedlings of 12 and 15 days old were difficult to handle compared to seedlings of 7 and 10 days old.

In terms of 7-day-old seedlings, the average shoot length was 20 cm and root length were 6 cm, but for the manifestation of symptoms, these seedlings took more time, *i.e.*, an incubation period of 10-12 days was necessary. Thus, seedlings of 10 days old (shoot length 22cm and root length 8 cm) were found very ideal and suitable for pathogen inoculation. The seedlings showed consistently same results for five times after an incubation period of 7 to 10 days. Thus, it was concluded that 10 days old seedlings with an incubation period of 7 days is optimal for symptom manifestation by the pathogen after inoculation. These findings were taken into consideration for screening of pigeonpea genotypes with varying genetic background.

***In vitro* screening through paper towel :** Out of

**Table 1 :** Response of pigeonpea genotypes/entries against *Macrophomina phaseolina* causing stem blight disease of pigeonpea under *in vitro* conditions.

| S. no. | Entries | Wilt incidence (PDI) | | | | Reaction |
|---|---|---|---|---|---|---|
| | | RI (%) | RII (%) | RIII (%) | Mean (%) | |
| 1 | WRG-128 | 33.15 | 38.53 | 36.9 | 36.19 | S |
| 2 | NAM-2284 | 38.13 | 42.35 | 41.35 | 40.61 | S |
| 3 | GC-11-39 | 35.35 | 38.36 | 36.75 | 36.82 | S |
| 4 | NAM-2282 | 50.12 | 52.35 | 48.36 | 50.27 | S |
| 5 | CORG-9701 | 45.38 | 44.89 | 45.39 | 45.22 | S |
| 6 | GRG-811 | 30.12 | 28.56 | 25.65 | 28.11 | MR |
| 7 | NAM-2435 | 55.35 | 54.25 | 55.75 | 55.11 | S |
| 8 | GRG-152 | 25.86 | 28.35 | 21.35 | 25.18 | MR |
| 9 | KRG-33 | 25.35 | 28.54 | 29.35 | 27.74 | MR |
| 10 | ICP-8863 | 25.65 | 28.53 | 24.35 | 26.17 | MR |
| 11 | ICP-2376 | 42.35 | 44.32 | 48.36 | 45.01 | S |
| 12 | TS-3R | 39.13 | 38.52 | 35.35 | 37.66 | S |
| 13 | ICP 7119 (SC) | 51.48 | 45.35 | 48.23 | 48.35 | S |
| 14 | NAM-2292 | 42.35 | 40.35 | 39.83 | 40.84 | S |
| 15 | NAM-2088 | 45.63 | 52.35 | 59.53 | 52.50 | S |
| 16 | NAM-2294 | 38.53 | 40.48 | 31.35 | 36.78 | S |
| 17 | NAM-2151 | 52.35 | 51.65 | 48.46 | 50.82 | S |
| 18 | NAM-2329 | 52.13 | 45.35 | 44.35 | 47.27 | S |
| 19 | NAM-2314 | 56.35 | 52.35 | 60.12 | 56.27 | S |
| 20 | NAM-2290 | 38.36 | 31.45 | 32.3 | 34.03 | S |
| 21 | NAM-2150 | 44.13 | 46.35 | 33.65 | 41.376 | S |
| 22 | NAM-2545 | 44.35 | 52.35 | 38.36 | 45.02 | S |
| 23 | BRG-1 | 44.34 | 49.35 | 35.65 | 43.11 | S |
| 24 | BRG-2 | 55.35 | 42.36 | 44.13 | 47.28 | S |
| 25 | BRG-3 | 41.53 | 44.86 | 48.32 | 44.90 | S |
| 26 | BRG-4 | 38.53 | 37.35 | 45.23 | 40.37 | S |
| 27 | BRG-5 | 51.36 | 55.35 | 44.35 | 50.35 | S |

*Macrophomina phaseolina* indicating the aggressiveness of the pathogen and virulence nature towards these cultivated genotypes.

The genotypes screened for dry root rot showed discolouration, necrosis of root tissues and finally wilting of seedlings (>30%) due to the infection by the *M. phaseolina*. Entries with moderate resistance reaction were screened for second time and recorded same trend of reaction. Higher activity of Pectin trans-eliminate and polygalacturonase trans-eliminate were observed in pigeonpea genotypes that are susceptible to *Rhizoctonia bataticola* (Srivatsava, 1987; Lokesha and Benagi, 2010) and their reduced activity might be reason for resistance in pigeonpea genotypes which showed moderately resistant reaction for dry root rot disease.

In a similar study Pandey *et al*. (2021), evaluated 296 mini core accessions of mungbean against *Macrophomina phaseolina* isolate MP1 causing dry root rot by paper towel method and identified 29 accessions resistant to *Macrophomina phaseolina* with less than 3 score and out of them 18 were consistently resistant over repeated evaluation. Similar attempt done in mungbean by Anupriya and Nitin (2022) using fifty-two mungbean genotypes and varieties identified only two genotypes resistant and one moderately resistant to *M. phaseolina*. In case of urdbean also 41, genotypes were evaluated against *M. phaseolina* by paper towel method and only two genotypes CO-5

**Table 2 :** Grouping of pigeonpea genotypes/entries in to different categories based on their resistance expression against *Macrophomina phaseolina* causing stem blight of pigeonpea under *in vitro* conditions.

| Reaction | Wilt incidence | Genotypes/ entries |
|---|---|---|
| **Resistant** | 0-10% | Nil |
| **Moderately resistant** | 11-30% | GRG-811, GRG-152, KRG-33 and ICP-8863 |
| **Susceptible** | More than 30% | ICP-2376, TS-3R, BRG-1ICP 7119 (SC), NAM-2292, NAM-2088, NAM-2294, NAM-2151, NAM-2329, NAM-2314, NAM-2290, NAM-2150, NAM-2545, BRG-2, BRG-3, BRG-4, BRG-5, WRG-128, NAM-2284, GC-11-39, NAM-2282, NAM-2435 and CORG-9701 |

27 genotypes screened, only four genotypes *viz*., GRG-811, GRG-152, Maruti (ICP-8863) and KRG-33 were found moderately resistant with mean disease incidence ranging from 25.18 to 28.11 per cent (Tables 1 and 2). None of the entries were either immune or resistant to and IPU-07-3 were found resistant to DRR pathogen. These entries with another (MASH1-1) were screened again and were found moderately resistant (Elmerich *et al*., 2022). The repetitive findings of screening against DRR pathogen reported in these crops are similar to the

**Table 3 :** Response of pigeonpea genotypes/entries against wilt incidence due to dry root rot caused by *Macrophomina pahesolina* under sick plot conditions at ZARS, Kalaburagi during *kharif* 2022.

| S. no. | Entries | Wilt incidence (PDI) | | | Reaction |
|---|---|---|---|---|---|
| | | RI (%) | RII (%) | Mean (%) | |
| 1 | WRG-128 | 32.23 | 38.31 | 35.27 | S |
| 2 | NAM-2284 | 45.35 | 39.26 | 42.30 | S |
| 3 | GC-11-39 | 48.00 | 43.48 | 45.74 | S |
| 4 | NAM-2282 | 22.03 | 27.31 | 24.70 | MR |
| 5 | CORG-9701 | 35..89 | 31.35 | 33.62 | S |
| 6 | GRG-811 | 24.53 | 23.40 | 23.97 | MR |
| 7 | NAM-2435 | 32.53 | 36.53 | 34.53 | S |
| 8 | GRG-152 | 20.00 | 25.00 | 22.50 | MR |
| 9 | KRG-33 | 27.08 | 30.00 | 28.54 | MR |
| 10 | ICP-8863 | 28.5 | 23.40 | 25.95 | MR |
| 11 | ICP-2376 | 39.31 | 35.75 | 37.53 | S |
| 12 | TS-3R | 51.11 | 55.77 | 53.44 | S |
| 13 | ICP 7119 (SC) | 65.12 | 62.50 | 63.81 | S |
| 14 | NAM-2292 | 38.35 | 42.55 | 40.44 | S |
| 15 | NAM-2088 | 31.91 | 35.42 | 33.67 | S |
| 16 | NAM-2294 | 47.35 | 42.36 | 44.85 | S |
| 17 | NAM-2151 | 32.22 | 36.09 | 34.15 | S |
| 18 | NAM-2329 | 38.35 | 36.58 | 37.46 | S |
| 19 | NAM-2314 | 40.68 | 45.23 | 42.95 | S |
| 20 | NAM-2290 | 48.53 | 51.11 | 49.82 | S |
| 21 | NAM-2150 | 36.00 | 31.28 | 33.64 | S |
| 22 | NAM-2545 | 31.37 | 35.67 | 33.52 | S |
| 23 | BRG-1 | 38.22 | 52.01 | 45.12 | S |
| 24 | BRG-2 | 61.00 | 57.41 | 59.21 | S |
| 25 | BRG-3 | 41.53 | 50.73 | 46.13 | S |
| 26 | BRG-4 | 37.25 | 45.37 | 41.31 | S |
| 27 | BRG-5 | 48.54 | 41.22 | 44.88 | S |

observations made in the current investigation.

**Screening of pigeonpea genotypes against dry root rot under sick plot conditions :** Among the twenty-seven pigeonpea entries and genotypes (27) screened, only 5 genotypes were found moderately resistant *viz*., GRG-811 (23.97%), GRG-152 (22.50%), KRG-33 (28.54%), ICPL-8863(25.95) and NAM-2282 (24.70). Other entries shown susceptible reaction and wilt incidence ranged between 22.50 to 63.81 per cent among the entries due to infection by the DRR pathogen (Tables 3 and 4). Studies on identifying the resistance against *M. phaseolina* in pigeonpea were very limited, however in different crops they were done more often. Like in pigeonpea, in other crops also success of getting resistance is very limited due to polyphagous nature of the pathogen and its wide adaptability to different ecosystem. In sorghum, Chattannavar and Bannur (2020) concluded that no genotype was resistant to charcoal rot incited by *M. phaseolina* among twenty-seven genotypes tested. In groundnut fifteen varieties were screened against the pathogen and none was found resistant but three (GG-7, GG-8 and GG-3) were found moderately resistant to the pathogen (Badana *et al*., 2021). Manjunatha and Saifulla (2021) screened 212 chickpea genotypes and concluded that PBG-5 was the lone entry showed moderately resistant reaction and rest were susceptible. Similarly, Talekar *et al*. (2021) screened 520 chickpea genotypes and concluded that only three were resistant and 21 were moderately resistant to *Rhizoctinia bataticola*.

**Confirmation of *in vitro* screening results with *in vivo* screening :** The field results obtained after screening of pigeonpea genotypes and entries in the sick plot showed five genotypes and entries expressing moderately resistant (GRG-811, GRG-152, KRG-33, ICP-8863 and NAM-2282) reaction to dry root rot disease. In conformity to these results under *in vitro* screening, only four genotypes (KRG-33, GRG-811, ICP-8863 and GRG-152) were found moderately resistant. There is possibility of an entry or genotype getting escaped from pathogen infections or due to variability in pathogen population in the sick plot at

**Table 4 :** Grouping of pigeonpea genotypes/entries in to different categories based on their resistance expression against *Macrophomina phaseolina* causing dry root rot of pigeonpea under sick plot conditions.

| Reaction | Wilt incidence | Genotypes/ entries |
|---|---|---|
| **Resistant** | 0-10% | Nil |
| **Moderately resistant** | 11-30% | GRG-811, GRG-152, KRG-33, NAM-2282, and ICP-8863 |
| **Susceptible** | More than 30% | ICP-2376, TS-3R, ICP 7119 (SC), NAM-2292, NAM-2088, NAM-2294, NAM-2151, NAM-2329, NAM-2314, NAM-2290, NAM-2150, NAM-2545, NAM-2435, WRG-128, NAM-2284, GC-11-39, BRG-1, BRG-2, BRG-3, BRG-4, BRG-5 and CORG-9701 |

particular site or location and also variation in infection level itself. However, the *in vitro* screening method offers uniform infection by the pathogen on all the test plants and equal opportunity for infection by the pathogen on all the host plants evaluated. The use of the paper towel method was found to be an efficient and consistent method to study the disease reactions between different pigeonpea genotypes and *M. phaseolina*. In addition, it is simple to execute, cost-effective because no culture medium is required, reproducible, and rapid because the whole process takes no longer than 3 weeks (inoculating 10-days-old seedlings and evaluating them after 7 days for disease response). This method can also be used to assess large population of pigeonpea entries and genotypes against DRR pathogen in short time and small space. In the present study, four genotypes exhibited moderate resistance against dry root rot. GRG-811, KRG-33, GRG-152 and ICP-8863 had low disease scores in the paper towel method and exhibited high plant survival through sick pot assay. These entries shall be used as donors in resistance breeding programme in future.

## Conclusion

The paper towel method standardised in the present investigation, its accuracy and reliability over the field screening for screening against *M. phaseolina* pathogen proved it beyond doubt that the method can be employed for screening large pigeonpea population against the DRR pathogen in short time and is highly reliable. The results can be further confirmed at filed screening also for confirmation of the true behaviour of the entries and genotypes before they are used for commercial cultivation.

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**Interest of conflict :** Authors have declared that no competing interests exist.

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