J Gen Plant Pathol
DOI 10.1007/s10327-017-0733-8

FUNGAL DISEASES

Node inoculation: A quick and easy technique to screen pigeonpea for resistance to Phytophthora blight

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Received: 16 April 2016 / Accepted: 3 June 2017

Abstract The petiole on pigeonpea was removed for easy, precise inoculation of node with *Phytophthora drechsleri* f. sp. *cajani*. After node inoculation, 96.0% plants were infected compared with 89.0% after stem-cut inoculation. Among various nodes inoculated on 30-day-old plants, the 5th node had the greatest relative susceptibility (90.0%), followed by the 3rd node (78.0%). This technique was validated on different cultivars (ICP 7119, Bahar, MA 6 and MAL 13), and 586 lines were successfully screened in the field, confirming the rapidity and effectiveness of the technique for resistance screening.

Keywords Phytophthora blight · *Cajanus cajan* · Node inoculation · Resistant · Techniques

Pigeonpea [*Cajanus cajan* (L.) Millspaugh] is an often cross-pollinated, perennial legume that is traditionally cultivated as an annual crop in Asia, Africa, Latin America, and the Caribbean region (Saxena 2008). India alone contributes 72.5% of world's cultivated area and 62.5% of world production (Sharma et al. 2015).

The greater susceptibility of pigeonpea to Phytophthora blight (PB) on the Indian subcontinent is one of the main causes for declining pigeonpea productivity (Sharma et al. 2015). PB of pigeonpea caused by *Phytophthora drechsleri* Tucker f. sp. *cajani* (PDC) (Kannaiyan et al. 1980; Pal et al. 1970) was first reported in 1966 by Williams et al. (1968). PB may appear at any growth stage; however, the seedling stage is most vulnerable. PB incidence data from the last decade clearly determined the most favorable conditions for an outbreak are ≥300 mm rainfall in 1 week with maximum temperature of 28–35 °C, minimum of 12–24 °C and relative humidity >75% (Pande et al. 2010; Sharma et al. 2006).

Management of PB is important for optimum yield of pigeonpea. Among the management practices, growing resistance cultivars is cheapest and ecologically sound. However, identification of resistant genotypes is only possible when the germplasm screening procedure mimic the natural infection process. Therefore, here we developed a new node inoculation technique and compared its efficiency and precision with that of the present standard inoculation to validate its use on pigeonpea germplasm.

Cultivar Bahar, a PB-susceptible genotype, was used to standardize a node inoculation technique (Fig. 1) on different nodes of plants of different ages under controlled conditions. The petiole was detached from the stem, exposing the leaf scar, and a mycelial disk (5 mm in diameter) from a 7-day-old culture of *P. drechsleri* f. sp. *cajani* isolate PDC 013-1 (accession KJ412453) grown on potato dextrose agar (PDA) was pressed into the leaf scar and surrounding node tissue.

The effect of plant age on resistance levels after node inoculation was tested in a complete randomized block design in three replications each using 30-, 40- or 50-day-old plants with 90 plants in each age group and 30 plants per replication. The spacing between rows and between plants was 30 × 15 cm. Fifth nodes were inoculated, then percentage of plants infected and lesion area at 4 days after inoculation and the days to 75% plant mortality was recorded (Table 1).

Effect of inoculating different nodes (3rd, 5th, 7th, 9th and 11th) on 30-day-old plants of cultivar Bahar on lesion

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Published online: 08 September 2017

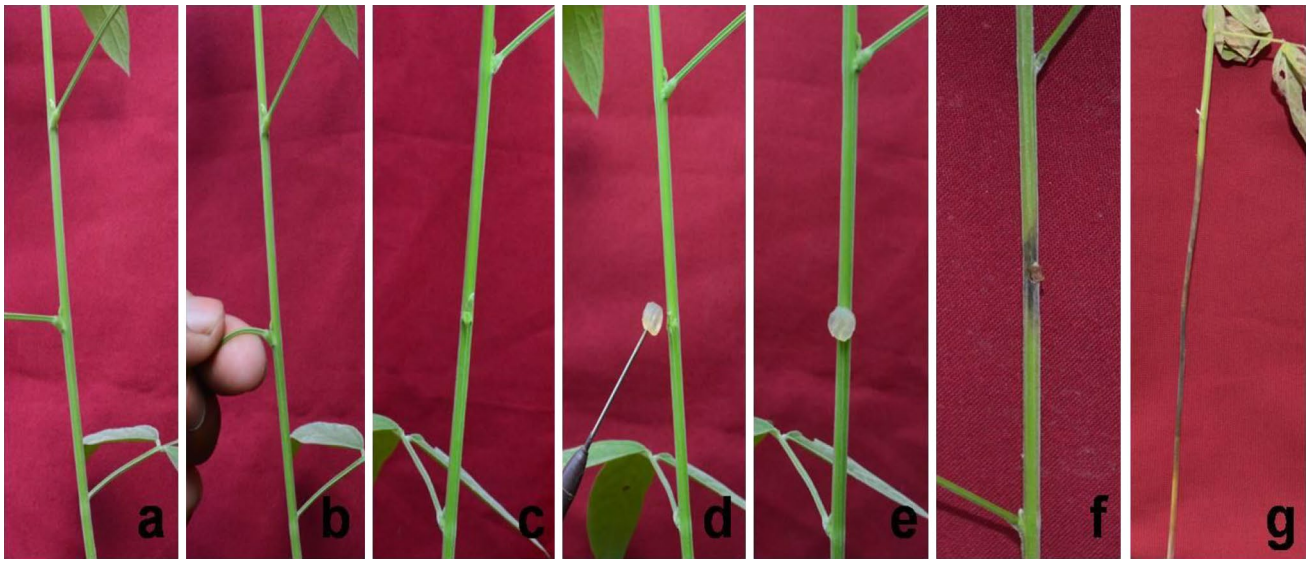

Fig. 1 Node inoculation of pigeonpea with *Phytophthora drechsleri* f. sp. *cajani* isolate PDC 013-1. **a** Node is selected; **b** petiole is gently pulled upward to remove the leaf and petiole; **c** leaf scar at node is exposed; **d** mycelial disk is placed on the leaf scar and node tissue; **e** node with inoculum; **f** dark brown to black lesion extending out from inoculated site; **g** plants dead

Table 1 Effect of plant age on susceptibility at the inoculated 5th node assessed by percentage of plants infected, lesion area at 4 days after inoculation, and number of days until 75% plant mortality

Age (days from sowing)	% Plants infected	Mean lesion area ± SD (cm^2)	Days to 75% plant mortality
30	96.0 (1.28)	2.2 ± 0.2	13.7
40	91.0 (1.14)	1.8 ± 0.4	18.3
50	89.0 (1.09)	1.6 ± 0.3	22.0
MSD		0.33	4.72

Lesion area = $2\pi rh$, where r = radius of infected portion of stem, h = length of lesion. Values in parentheses were arcsine-transformed

MSD minimum significant difference ($P \leq 0.05$); *SD* standard deviation; 90 plants were tested in each age group

Table 2 Incidence and severity of Phytophthora blight after inoculation of different nodes on 30-day-old plants of pigeonpea cultivar Bahar

Inoculated node	% Plants infected	Mean lesion area ± SD (cm^2)
3	78.0 (0.89)	2.5 ± 0.1
5	90.0 (1.11)	2.5 ± 0.5
7	74.0 (0.83)	2.4 ± 0.1
9	72.0 (0.80)	2.4 ± 0.2
11	40.0 (0.41)	0.5 ± 0.1
MSD		0.57

Values in parentheses were arcsine-transformed. Lesion area = $2\pi rh$, where r = radius of infected portion of stem, h = length of lesion

MSD minimum significant difference ($P \leq 0.05$), *SD* standard deviation

area and days to 75% plant mortality was tested using a randomized block design with three replications and 90 plants for each node group. Lesion area was measured at 4 days after inoculation (Table 2).

The new node inoculation technique compared with four inoculation techniques already in use (Table 3). The 5th node on 30-day-old plants in the field was inoculated as described in Fig. 1. A randomized block design was used with 3 replications and 150 plants for each technique. A 9-point disease rating scale (Reddy et al. 1989) was modified to a 10-point scale (Fig. 2) to rate the severity of PB lesions on the stem and classify severity into four disease reaction groups: 1–3, resistant (R); 4–5, moderately resistant (MR); 6–7, susceptible (S); 8–10 as highly susceptible (HS).

Data related to mean percentage infection and days to 75% mortality were analyzed using PROC GLM in the program SAS (SAS 2010). The means were compared using Dunnett's minimum significant difference (MSD) test at $P \leq 0.05$.

The mean percentage infection differed significantly ($P = 0.05$) among the inoculation techniques (Table 3). Node inoculation after petiole detachment resulted in the highest percentage of infection (96.0%) followed by the stem-cut inoculation at 89.0%. Tray, spray and leaf scar inoculation led to 31.0, 11.0 and 5.0% infection, respectively. The 30-day-old plants were most susceptible (Table 1) with maximum 96.0% infection of nodes and 75% plant mortality at

Table 3 Summary of inoculation methods tested, time that lesion appeared, percentage infection and number of days for 75% mortality of plants

Method	Plant age (days from sowing)	Lesion visible (days after inoculation)	No. of plants inoculated	Mean % of plants infected	No. of days for 75% plant mortality	References
Node	30	4	150	96.0 (1.28)	7.0	Present study
Stem cut	60	8	150	89.0 (1.09)	11.4	Chauhan et al. (2002) and Nene et al. (1981)
Tray	30	10	150	31.0 (0.31)	17.7	Pande et al. 2012
Spray	30	10	150	11.0 (0.11)	23.0	Gupta et al. (1997), Mallikarjuna et al. (2005) and Nene et al. (1981)
Leaf scar	30	12	150	5.0 (0.52)	32.0	Pal et al. (1970) and Reddy et al. (1990)
MSD				2.23	3.25	

Values in parentheses are arcsine-transformed means

MSD minimum significant difference ($P \leq 0.05$)

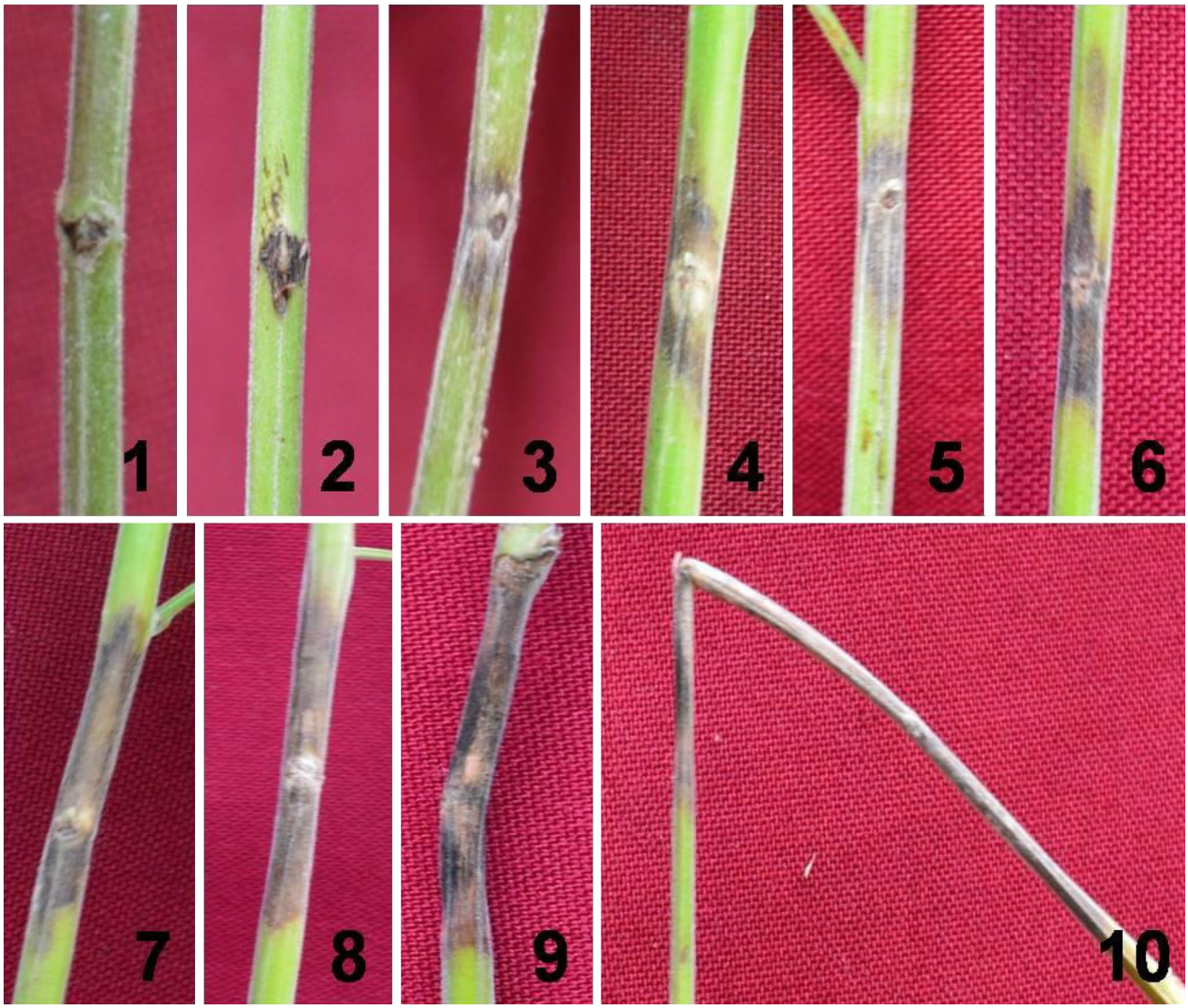

Fig. 2 Phytophthora disease rating scale (1–10) for screening pigeonpea germplasm. *1* Lesions restricted to inoculation point on node, typical growth of plant; *2* minute lesion around infection point, typical plant growth; *3* lesion irregular, ca. 0.5 cm long at inoculation site, typical plant growth; *4* dark brown lesion, ca. 1 cm long, typical plant growth; *5* brown to black lesion extending on infected stem, plants alive; *6* lesion extending several centimeters, dark brown in color and plants alive; *7* dark brown to black lesion, stem girdling started, some plants alive; *8* infected stem is dry, weak and prone to topple; *9* slightly blackish stem is dry, upper leaves dried; *10* infected stem completely dry, plant dead

Table 4 Validation of different pigeonpea cultivars by node inoculation technique

Cultivars	Mean infection (%)	Rating scale (1–10) Mean ± SD	Days to 75% plant mortality
ICP 7119	90.0 (1.11)	2.4 ± 0.4	5.0
Bahar	82.0 (0.96)	2.2 ± 0.2	7.0
MA 6	30.0 (0.30)	2.1 ± 0.1	12.0
MAL 13	29.0 (0.29)	1.9 ± 0.4	11.0
MSD		NS	2.26

Values in parentheses were arcsine-transformed

MSD minimum significant difference ($P \leq 0.05$), *SD* standard deviation, *NS* non-significant

>13 days after inoculation compared with 89.0% infection of 50-day-old plants and 75% plant mortality at >22 days after inoculation. When the 3rd, 5th, 7th, 9th and 11th nodes were inoculated, infection (90.0%) was greatest on the 5th node at 4 days after inoculation.

Node inoculation technique was further validated on 30-day-old seedlings of PB-susceptible cultivars ICP 7119, Bahar and moderately resistant MA 6 and MAL 13 (Chauhan et al. 2002; Pande et al. 2011), with 50 seedlings of each cultivar for each replication (150 plants of each cultivar). The 5th node was inoculated, and typical PB symptoms were observed at 4 days after inoculation (Table 4). The mean percentage infection and days to 75% plant mortality differed significantly among the genotypes.

Inoculated by the node technique, 586 lines of pigeonpea, collected from eastern Uttar Pradesh were then screened for resistance in the field. At 4 days after inoculation, lesion area on 10 plants was measured, and disease rated on the 1–10 scale. Among the 586 lines, 58 lines were found to be R, 206 were MR and 322 were S to HS.

When Mishra and Shukla (1986) inoculated leaves of 15-day-old seedlings, they obtained 100% incidence of PB, and incidence declined with increasing age of the inoculated plant to a minimum incidence of 25% on 120-day-old plants. In the present study, 96.0% of the seedlings developed PB after node inoculation compared with 89.0% after stem-cut inoculation and the other tested methods.

Because the stem-cut inoculation requires an I-shaped cut in the bark for inserting the mycelia and wrapping with cellophane tape (Chauhan et al. 2002; Nene et al. 1981), the stem thickness must be >1 cm, which is reached at 60 days after sowing, so plants will then be tested for adult plant resistance (Mishra and Shukla 1986). For node inoculation, removal of the petiole creates a very small, uniform opening, minimizing the possibility of variation in the lesion size. The method is fast and easy and requires no special skills. Node inoculation proved sensitive enough to detect variation in PB resistance among the pigeonpea cultivars/genotypes and thus can be used for rapid screening pigeonpea germplasm for resistance against PB.

Acknowledgements Authors thank the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) for developing the project and the Ministry of Agriculture, Government of India for financial support.

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