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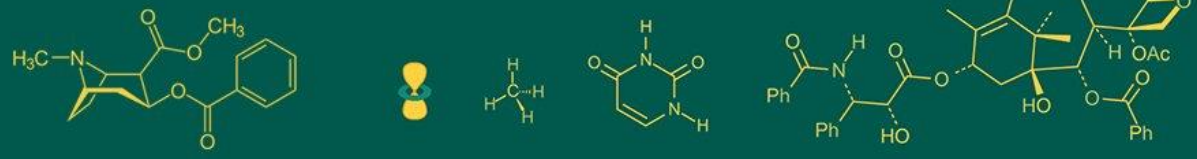

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Evaluation of commercial varieties and hybrids for resistance to gummy stem blight in watermelon

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Abstract
Gummy stem blight (GSB), caused by *Stagonosporopsis cucurbitacearum*, is a major constraint in watermelon production, necessitating the identification of resistant cultivars for effective disease management. In the present study, twenty commercial watermelon varieties and hybrids sourced from public and private seed agencies were screened under controlled polyhouse conditions using artificial inoculation techniques. Multiple inoculation methods—leaf spray, root inoculation, leaf disk placement, and stem injection—were evaluated for their reliability in symptom expression. Among these, the stem injection method consistently produced uniform, systemic, and scorable symptoms and was therefore selected for detailed screening. Ten-to fifteen-day-old seedlings were inoculated with the SC-W10 isolate, and disease severity was assessed using 0-9 disease scale with Percent Disease Index (PDI) calculated using McKinney's formula. Disease progression was strongly influenced by humidity, with >90% relative humidity promoting rapid symptom development. Based on PDI values, nine hybrids—IB-20, Melody, Arka Shyam, Arka Aishwarya, AFA-205, AFA-306, AFA-505, SS-345, and SS-906—exhibited lower disease severity and were categorized as moderately susceptible. Five hybrids (NS-295, Ayush, Arka Manik, Arka Muttu, and Black Beauty). The findings highlight significant variability in host response and identify promising genotypes for use in resistance breeding and integrated management of gummy stem blight in watermelon.

Keywords: *Citrullus lanatus*, potato dextrose agar, percent disease index, Pathogenecity

Introduction
Watermelon (*Citrullus lanatus* [Thunb.] Matsum. & Nakai) is an economically important cucurbit cultivated extensively across tropical and subtropical regions for its nutritional and commercial value (Wehner, 2008) [18]. India is one of the major producers of watermelon, and the crop contributes significantly to farm income, particularly in arid and semi-arid regions. However, watermelon production is constrained by several fungal, bacterial, and viral diseases, among which gummy stem blight (GSB) is considered one of the most destructive (Keinath, 2014) [7].

Gummy stem blight is caused by a species complex within the genus Stagonosporopsis (formerly *Didymella bryoniae*), primarily *S. cucurbitacearum, S. citrulli, and S. caricae* (Stewart *et al.*, 2015) [13]. These pathogens infect stems, leaves, petioles, and fruits, producing characteristic water-soaked lesions, necrosis, stem cankers, and gummy exudates (Keinath & DuBose, 2004) [8]. Severe infections can lead to rapid vine collapse, premature drying, and economic losses in both field and greenhouse-grown crops.

Environmental conditions play a critical role in disease development. High relative humidity (>90%), extended leaf wetness, and moderate temperatures (20-28 °C) favor infection, sporulation, and disease spread (Zitter, Hopkins & Thomas, 1996) [7]. The pathogen is seedborne and survives in crop debris, allowing it to persist between seasons and initiate new infections (Schenck, 1968) [11]. Due to its polycyclic nature and multiple infection cycles within a growing season, GSB is difficult to manage using fungicides alone.

Chemical control, although widely practiced, is increasingly challenged by fungicide resistance. Resistance to QoI (strobilurin) and MBC (benzimidazole) fungicides in *Stagonosporopsis* spp. has been reported from several watermelon-growing regions worldwide (Avenot *et al.*, 2012; Keinath, 2011) [1, 6].

In addition, reliance on repeated fungicide applications raises concerns related to cost, environmental impact, and residue accumulation. Consequently, host plant resistance is considered the most sustainable and effective management strategy for GSB (Boyhan *et al.*, 2014) [2].

Despite the importance of resistant cultivars, information on the resistance status of commercially cultivated watermelon hybrids in India remains limited. Previous studies have highlighted inconsistencies in resistance assessment due to variability across inoculation methods such as leaf spray, root inoculation, and leaf disk placement (Chiu and Walker, 1949; Van Der Meer *et al.*, 1978; Wyszogrodzka *et al.*, 1986) [4, 14, 20]. Therefore, identifying a reliable inoculation method and systematically evaluating available hybrids are essential for accurate identification of resistance sources.

The present study was undertaken to compare different artificial inoculation methods for their reliability in producing uniform symptoms of gummy stem blight, and screen twenty commercial varieties and hybrids of watermelon for resistance to *Stagonosporopsis cucurbitacearum* under controlled polyhouse conditions. The findings aim to identify promising genotypes for resistance breeding and integrated management of gummy stem blight in watermelon.

Materials and Methods

Plant material and experimental setup

Twenty commercial watermelon (*Citrullus lanatus*) varieties and hybrids were obtained from public and private seed companies and research institutes for resistance screening against gummy stem blight (Table 1). Seeds were sown in plastic pots containing sterilized soil, with five seeds per pot and one pot considered as a single replication; each variety/hybrid was replicated three times under greenhouse conditions (25-30 °C) without application of any fungicides or other chemical sprays.

For seedling-stage pathogenicity and method comparison experiments, surface-sterilized seeds of each entry were sown in protrays (98 cells) filled with cocopeat and maintained in a greenhouse at ambient temperature until use.

Table 1: List of watermelon varieties/hybrids used for screening against *Stagnosporopsis cucurbitacearum*.

Sl. No.	Varieties/hybrids	Sources
1	Max	Nunhems India Private Limited
2	IB-20	Namadhari seeds Private Limited
3	Sugar Queen	Syngenta India Private Limited
4	Sugar King	Syngenta India Private Limited
5	Melody	Kalash seeds Private Limited
6	Augustha	Syngenta India Private Limited
7	Arka Muttu	Indian Institute of Horticultural Research Station-Hesaragatta, Bengaluru
8	Arka Shyam	Indian Institute of Horticultural Research Station-Hesaragatta, Bengaluru
9	Arka Manik	Indian Institute of Horticultural Research Station-Hesaragatta, Bengaluru
10	Arka Aishwarya	Indian Institute of Horticultural Research Station-Hesaragatta, Bengaluru
11	NS-295	Namadhari seeds Private Limited
12	AFA-205	Ashoka seeds Private Limited
13	AFA-306	Ashoka seeds Private Limited
14	AFA-505	Ashoka seeds Private Limited
15	SS-345	Solar Seeds Private Limited
16	Ayush	Pahuja Seeds Private Limited
17	Surya	Sakura Seeds Private Limited
18	SS-906	Samrudh seeds Private Limited
19	Chirag	Known-You Seeds India Private Limited
20	Black Beauty	Multiplex India Private Limited

Pathogen isolation and inoculum preparation

Watermelon plants showing typical gummy stem blight symptoms were collected from naturally infected fields. Symptomatic tissues were surface sterilized and plated on potato dextrose agar (PDA) to obtain pure cultures of *Stagonosporopsis cucurbitacearum*. A representative isolate, designated SC-W10, was selected and maintained on full-strength PDA. For inoculum production, SC-W10 was grown on PDA plates for 7 days to obtain abundant mycelial growth and spores.

For spore suspensions, spores were dislodged from 7-day-old cultures into sterile distilled water using a sterile loop. The resulting suspension was filtered through sterile muslin cloth to remove mycelial fragments, and the concentration was adjusted (e.g., with a hemocytometer) to the desired level prior to inoculation (Plate 1).

Plate 1: Artificial inoculation of spore suspension of *Stagnosporopsis cucurbitaecearum* on watermelon

Inoculation methods

Four inoculation methods were evaluated for screening: (i) root inoculation, (ii) leaf inoculation using mycelial discs, (iii) foliar spray of spore suspension, and (iv) stem injection of spore suspension.

Root inoculation

Ten-day-old seedlings raised in cocopeat were carefully uprooted, and roots were gently washed under running tap water to remove adhering substrate. Root systems were trimmed to approximately 2 cm length and dipped for 3 min in the fungal inoculum (mycelial or spore suspension), after which seedlings were transplanted into pots containing sterilized soil. Control seedlings were treated similarly but dipped in sterile distilled water. Plants were maintained in the greenhouse under ambient conditions.

Leaf inoculation with mycelial discs

For leaf inoculation, 15-day-old seedlings were used. Prior to inoculation, plants were irrigated to ensure full turgor. One mycelial disc (cut from the actively growing margin of a 7-day-old SC-W10 culture) was placed on each of two fully expanded true leaves per seedling using a sterile toothpick, ensuring close contact with the leaf surface. Cotyledons were not inoculated because cotyledon inoculation has been reported to be unreliable for assessing resistance to gummy stem blight. Inoculated seedlings were immediately covered with transparent polyethylene sheets to maintain high humidity (relative humidity >90% for the first 24 h and >70% for the following 7 days), using misting/fogging facilities as required. The average temperature in the polyhouse was maintained at 25-28 °C.

Foliar spray and stem injection inoculation

Pathogenicity tests and screening of entries were also performed on 15-day-old healthy seedlings using a spore suspension prepared from SC-W10 cultures. Seedlings were inoculated by:

- Spraying the spore suspension onto the foliage with a hand sprayer until runoff.
- Injecting the spore suspension into the stem using a sterile hypodermic syringe (stem injection method).

Control plants were sprayed and injected with sterile distilled water only. Following inoculation, plants were placed in a humidity chamber and maintained at relative humidity >90% until symptom development. The spore suspension was reapplied at two-day intervals until characteristic gummy stem blight symptoms appeared.

To confirm pathogenicity and fulfill Koch's postulates, the pathogen was re-isolated from symptomatic tissues of inoculated plants onto PDA and compared morphologically with the original SC-W10 culture.

Disease assessment and percent disease index

Disease severity on individual seedlings was recorded using the 0-9 categorical scale described by Gusmini *et al.* (2005) [5]:

Disease assessment and percent disease index

Disease scale	Symptoms
0	no symptoms
1	yellowing on leaves (suspect of disease only),
2	moderate symptoms (<20 percent necrosis) on leaves only
3	slight necrosis (21-45 percent necrosis) on leaves only
4	severe symptoms (>45 percent necrosis) on leaves only
5	some leaves dead, no symptoms on stem
6	moderate symptoms (<20 percent necrosis) on leaves, with necrosis also on petioles and stem (<3 mm long),
7	slight symptoms (21-45 percent necrosis) on leaves, with necrosis also on petioles and stem (3-5 mm long),
8	severe symptoms (>45 percent necrosis) on leaves, with necrosis also on petioles and stem (>5mm long)
9	Dead plant

The percent disease index (PDI) was calculated from disease ratings using McKinney's (1923) [9] formula:

$$\text{PDI} = \frac{\text{Sum of all numerical ratings}}{\text{Number of observations} \times \text{Maximum disease rating}} \times 100$$

The mean PDI for each variety/hybrid and inoculation method was used to categorize the reaction to gummy stem blight.

Results and Disussion

Disease development increased markedly with increasing relative humidity, and plants maintained at >90% RH exhibited greater pathogenicity and higher infection rates than those maintained at 70 ± 5% RH. Under these favorable conditions, all three inoculation methods tested foliar spraying of mycelial suspension, root inoculation, and placement of mycelial discs on true leaves were capable of causing infection, but they differed in reliability and symptom expression. Spraying a mycelial suspension onto the leaf surface produced erratic symptom expression and highly variable disease progression, whereas root inoculation frequently caused rapid plant collapse, limiting its usefulness for differential scoring. Inoculation by placing mycelial discs on true leaves at 95 ± 5% RH resulted in relatively slow, localized symptom development, while the stem injection method produced a uniform, progressive infection along the stem with clear, scorable lesions and no non-specific symptom development among the twenty genotypes tested. On this basis, disease assessment was most convenient and reproducible using the stem injection method, which was therefore adopted for subsequent screening.

Table 18: Screening of watermelon varieties/hybrids for resistance against *Stagnosporopsis cucurbitacearum*

Sl. No.	Varieties/hybrids	Mean PDI	Disease reaction
1	Max	88.3	Susceptible
2	IB-20	76.9	Moderately susceptible
3	Sugar Queen	86.2	Susceptible
4	Sugar King	87.1	Susceptible
5	Melody	73.7	Moderately susceptible
6	Augustha	89.6	Susceptible
7	Arka Muttu	94.8	Highly susceptible
8	Arka Shyam	76.8	Moderately susceptible
9	Arka Manik	96.4	Highly susceptible
10	Arka Aishwarya	75.2	Moderately susceptible
11	NS-295	98.3	Highly susceptible
12	AFA-205	79.2	Moderately susceptible
13	AFA-306	47.8	Moderately susceptible
14	AFA-505	50.5	Moderately susceptible
15	SS-345	59.3	Moderately susceptible
16	Ayush	96.7	Highly susceptible
17	Surya	88.5	Susceptible
18	SS-906	72.3	Moderately susceptible
19	Chirag	85.4	Susceptible
20	Black Beauty	94.5	Highly susceptible

The details of disease incidence among the various hybrids/varieties during screenings under polyhouse conditions are presented in Table (2). After stem injection, all the hybrids/varieties started showing disease symptoms like a water-soaked lesion on leaves turning light brown in colour on the third day (Plate 2). Within seven days, lesions spread and started entering the leaf petiole in susceptible varieties. With the progress of time, the pathogen was able to penetrate the stem causing stem lesions. These lesions slowly dried up. The progress of infection was found to spread towards the bottom rather than the top of the plant. The disease score was recorded at seven-day intervals after inoculation. Observations were continued up to the 7th day. Based on the recorded disease score, percent Disease Index (PDI) was calculated.

Among the 20 hybrids/varieties evaluated under polyhouse conditions, nine hybrids IB-20, Melody, Arka Shyam, Arka Aishwarya, AFA-205, AFA-306, AFA-505, SS-345 and SS-906 recorded the lowest PDI values and were classified as moderately susceptible to gummy stem blight. Five hybrids, NS-295, Ayush, Arka Manik, Arka Muttu and Black Beauty, exhibited the highest PDI values and were categorized as highly susceptible. The remaining hybrids, Augustha, Max, Sugar King, Sugar Queen, Surya and Chirag, showed intermediate PDI values and were rated as susceptible.

Worldwide several attempts have been made to identify the sources of resistance against this pathogen (Sowell and Pointer 1962; Boyhan *et al.*, 1994; Gusmini *et al.*, 2005) [12, 3, 5]. The genotypes PI 279461 and PI 526233 reported as the best sources of resistance by Gusmini *et al.* (2005) [5]. GSB has been earlier reported to be exhibiting significant variability in terms of disease expression depending upon the conditions of screening in cucumber (Wehner and Shetty, 2000) [19], melon (Zhang *et al.*, 1997) and watermelon (Boyhan *et al.*, 1994) [3]. This is because GSB infections are highly influenced by environmental conditions such as temperature, relative humidity and ventilation (Van Steekelenburg, 1984, 1985a; Van Steekelenburg and Vooren, 1980) [15, 17, 16]. The aggressiveness of a pathogen, its ability to infect disease scoring method along with environmental factors such as temperature and humidity can influence the resistance evaluation (Ren *et al.*, 2020) [10]. Similarly, a decrease in the level of resistance of "PI 189225" was observed by Ren *et al.* (2020) [10] when kept under 100% RH after inoculation for a period of 48 h compared to 24 h. Therefore, to minimize the error and generate robust screening data, testing in multiple environments will be needed.

Plate 2: Disease incidence among the various hybrids/varieties during screenings under polyhouse conditions

Conclusion

These findings underline the ongoing need to identify and deploy cultivars with improved resistance for sustainable management of GSB, particularly due to the limitations and risks associated with chemical control. The work also highlights the crucial influence of environmental conditions, especially humidity, on disease expression and the accuracy of resistance evaluation. Ultimately, the results provide valuable insights for breeding programs, suggesting that screening under high-humidity, polyhouse conditions using

stem injection can efficiently identify genotypes with moderate resistance, which can be vital components in integrated disease management strategies and resistance breeding for gummy stem blight in watermelon.

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