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Unveiling the mechanistic regulation of host genetic resistance in oilseed brassica against *Sclerotinia sclerotiorum*: A comprehensive study across developmental growth stages

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Abstract

Sclerotinia sclerotiorum, causing Sclerotinia stem rot, poses a significant threat to oilseed Brassica crops, necessitating the identification of resistant genotypes. Previous studies often used single pathogen isolates or plant-developmental stages to identify resistance. Our recent study revealed diverse resistance mechanisms in *Brassicaceae* genotypes against *S. sclerotiorum*, emphasizing the need for understanding these mechanisms. We evaluated ten *Brassicaceae* genotypes at four plant developmental stages against six diverse *S. sclerotiorum* isolates from India, assessing both structural and physiological resistance traits. Results showed significant ($P \leq 0.05$) effects of host genotype, developmental stage, pathogen isolate, and their interactions on resistance components. *S. alba* SA1 consistently displayed resistance, making it an ideal candidate for resistance breeding. Other genotypes exhibited age and isolate dependency, and resistance varied among developmental stages. The study identified nine suitable host genotypes as differentials for characterizing *S. sclerotiorum* pathotypes and delineated six distinct pathotypes. Stem physical strength and physio-biochemical attributes were genotype-specific and dependent on plant developmental stage. *S. alba* genotypes exhibited higher stem physical strength, while other genotypes displayed superior physio-biochemical traits. Disease pressure was negatively correlated with stem lignin content, indicating its role in durable resistance, while a positive correlation was observed between disease severity and total soluble sugar. The study emphasizes the importance of combining structural and physiological resistance for developing cultivars with durable resistance to *S. sclerotiorum* in oilseed Brassica.

Keywords: *Brassicaceae, plant developmental stages, pathogen isolates, sclerotinia Sclerotiorum, structural and physiological resistance*

Introduction

The *Brassicaceae* family, comprising 3709 diverse species, holds paramount significance in food, fodder, pharmaceuticals, research, industry, and agriculture. Notably, oilseed Brassica species, such as *Brassica napus*, *B. juncea*, *B. carinata*, and *B. rapa*, dominate the oilseed market, providing high-quality edible oil and economic value. *B. nigra* and *Sinapis alba* serve as minor oilseed and condiment crops, respectively (Singh *et al.,* 2024). Globally, Brassica oilseeds cover 41.96 million hectares, yielding 88.34 million tonnes with a productivity of 2110 kg/ha in 2022-23, ranking as the third-largest source of vegetable oils. The production and export of Brassica oilseeds significantly contribute to agricultural profitability and global food security (Singh *et al.,* 2023a).

Sclerotinia stem rot, caused by the fungus *Sclerotinia sclerotiorum* (Lib.) De Bary, poses a significant threat to Brassica oilseed cultivation globally. This economically damaging pathogen, with adaptability and aggressiveness attributed to oxalic acid release, cell wall-degrading enzymes, and a bimodal infection mode, affects over 600 plant species. Major oilseed Brassica-producing countries, including China, Canada, India, Australia, and the European Union, are particularly vulnerable to its impact (Singh *et al.,* 2020). In China, Sclerotinia stem rot has led to oilseed rape yield losses ranging from 50% to complete crop failure, while Canada experiences up to a 50% reduction in Prairie Provinces. India and Australia report significant losses of 24 to 90% (Singh *et al.,* 2022a), and the European Union faces varying challenges (Singh *et al.,* 2023). The extended survival of *S. sclerotiorum* as sclerotia in soil for up to a decade, combined with issues related to thick crop canopies and the lack of reliable fungicide forecasting methods, hampers disease management efforts. This prolonged survival period limits crop rotation options, increases sclerotia accumulation risk, and renders control methods highly ineffective, resulting in an estimated annual global loss of US$ 200 million (Singh *et al.,* 2023b).

Efforts to combat Sclerotinia stem rot in Brassica oilseeds through plant breeding have been extensive, yet literature analysis indicates a lack of complete and enduring resistance. Notably, recent studies in India have identified limited but promising sources of resistance within *Brassicaceae* crops (Garg *et al.,* 2010; Singh *et al.,* 2021a). Genetic variability in *S. sclerotiorum* pathogenicity across the Indian subcontinent has been established in previous studies (Sharma *et al.,* 2018a). Furthermore, resistance expression in *Brassicaceae* is influenced by developmental growth stages. Recent findings by Khan *et al.,* (2022) highlight genetic resistance variations throughout various growth stages, emphasizing the need for targeted breeding programs. While earlier studies explored interactions between growth stages, *Brassicaceae* genotypes, and *S. sclerotiorum* isolates, they were often limited to a single pathogen isolate or specific developmental stages (Singh *et al.,* 2022a; Singh *et al.,* 2021b; Garg *et al.,* 2010). A more comprehensive investigation involving diverse *S. sclerotiorum* isolates and various plant developmental stages is essential to effectively identify and characterize resistant genotypes. Understanding these aspects is crucial for developing sustainable and durable strategies to mitigate the impact of Sclerotinia stem rot, ensuring the long-term productivity and stability of *Brassicaceae* crops worldwide.

Plants employ various strategies to combat pathogen attacks, encompassing pre-existing physical barriers, biochemical obstacles, and the activation of physiological and metabolic responses. Within a given plant species, it is reasonable to assume that resistant and susceptible genotypes may exhibit distinct structures and responses to the same pathogen. A comparative investigation of resistant genotypes with varying disease resistance can illuminate the structural and physiological elements underlying these differences, aiding the development of preventive methods and novel germplasms. The mechanisms conferring durable resistance against the broad-spectrum pathogen *S. sclerotiorum* remain incompletely understood. Resistance sources have been identified in *Brassicaceae* species (Garg *et al.,* 2010; Singh *et al.,* 2021b, 2023b), categorized as incomplete or complete based on infection process stages. Our recent research suggests that Brassica oilseed resistance to *S. sclerotiorum* may involve both physiological and structural factors (Singh *et al.,* 2023b). However, the specific sources and resistance mechanisms critical for durability remain unclear. Distinguishing between structural and physiological resistance in crop breeding offers advantages such as targeted trait selection, strategic trait combination, adaptability in diverse environments, informed disease management, efficient genetic resource utilization, and enhanced resistance durability through stacking.

To address knowledge gaps, this study focuses on four main objectives. Firstly, it assesses aggressiveness and pathogenicity attributes of *S. sclerotiorum* isolates from diverse Indian regions, crucial for understanding *Brassicaceae-S. sclerotiorum* compatibility. The study also evaluates durable resistance in varied Brassicaceae genotypes. Secondly, it explores the interplay of host-genotype, pathogen-isolate, and plant growth stage in Sclerotinia stem rot resistance. The aim is to illuminate their individual and combined contributions to resistance and identify potential sources of durable resistance for breeding. The third objective examines interactions between resistant *Brassicaceae* genotypes and *S. sclerotiorum* isolates to uncover pathotypes and establish host differentials. Lastly, the study differentiates structural and physiological resistance phenotypes across various plant developmental stages and in response to diverse pathogen isolates. This analysis aims to deepen understanding of resistance dynamics in *Brassicaceae* crops, particularly in Sclerotinia stem rot, contributing to effective disease management approaches.

Materials and Methods

Isolate collection, pure culture preparation, and assessment of pathogenicity and aggressiveness traits in *Sclerotinia sclerotiorum*

Six isolates of *S. sclerotiorum*, namely SsHsi, SsLdi, SsDli, SsBhi, SsPni, and SsVni, were collected from diverse regions in India representing significant mustard cultivation areas. These isolates originated from Hisar, Ludhiana, New Delhi, Bharatpur, Pantnagar, and Varanasi. Sclerotia from Brassica plant samples affected by stem rot underwent preparation steps involving surface sterilization and ethanol treatment. Following a protocol by Singh *et al.* (2022b), each sclerotium was halved and placed on potato dextrose agar (PDA) supplemented with tetracycline antibiotics to prevent contamination. Incubation at 20°C ± 2°C in darkness was carried out in six replicate PDA plates for each *S. sclerotiorum* isolate, ensuring statistical reliability. Various traits related to aggressiveness and pathogenicity were recorded on PDA, including colony diameter, time for sclerotial appearance, number of sclerotia, sclerotial diameter, and 100-sclerotial weight. Oxalic acid production was quantified following the method followed by Singh *et al.* (2022a) using vacuum-filtered cultures. Results were reported as micrograms of oxalic acid equivalent per gram of dried mycelium (µg

OA/g dried mycelium) after measuring absorbance at 600 nm with a UV-vis spectrophotometer (Shimadzu, model UV-1800).

Plant materials

In this study, a collection of ten *Brassicaceae* genotypes was specifically chosen based on their varying responses to Sclerotinia stem rot, as demonstrated in previous research (Garg *et al.,* 2010; Sharma *et al.,* 2018b; Singh *et al.,* 2021b; 2022a). These genotypes exhibit distinct and diverse reactions to Sclerotinia stem rot, highlighting their potential for investigating resistance mechanisms. Among the selected genotypes, eight have previously displayed resistance against the disease, indicating promising resistance traits. These resistant genotypes include *B. juncea* RH1222-28 (Sharma et al., 2018b; Singh *et al.,* 2021b; Singh *et al.,* 2022b), *B. juncea* IC589660 (Singh *et al.,* 2023b), *B. fruticulosa* BF1 (Garg *et al.,* 2010a), *Diplotaxis tenuisiliqua* (Garg et al., 2010; Singh *et al.,* 2023b), *Erucastrum abyssinicum* (Garg *et al.,* 2010), and *Sinapis alba* SA1, *S. alba* SA2, and *S. alba* SA3 (Singh *et al.,* 2023a; Singh *et al.,* 2023b). In addition, two genotypes, B. *tournefortii* BT1 and *B. juncea* IC401678 (Singh *et al.,* 2023b), were included as susceptible checks to serve as reference points for assessing disease susceptibility. The careful selection of these genotypes provides a comprehensive range of responses, allowing for an in-depth exploration and evaluation of the underlying mechanisms involved in resistance to Sclerotinia stem rot in *Brassicaceae* crops.

Plant developmental growth stages

In order to comprehensively evaluate the responses of the selected genotypes regarding resistance components, stem structural and physiological resistance-associated traits, a meticulous experimental design involving four consecutive plantings was implemented, with a 30-day interval between each planting. The initial planting was executed on September 15th, followed by subsequent plantings on October 15th, November 15th, and December 15th. In a sequential manner, all genotypes were subjected to inoculation at four distinct plant-developmental growth stages: the bolting stage (approximately 30-days old crop), flowering stage (approximately 60-days old crop), podding stage (approximately 90-days old crop), and ripening stage (approximately 120-days old crop). This systematic approach allowed for a comprehensive assessment of the genotypes' performance across different plant-developmental growth stages, enabling us to unravel their resistance mechanisms and gain valuable insights into the intricate dynamics of resistance expression.

Artificial stem inoculation and comprehensive assessment of quantitative disease resistance components in *Brassicaceae* genotypes

In the artificial stem inoculation process, twenty representative plants were chosen from the center of each plot for every genotype and replicate. Inoculation occurred at the second internode of the primary stem during the appropriate developmental growth stage. Using a parafilm strip, agar discs (5 mm^2) from a four-day-old *S. sclerotiorum* pure culture were affixed, along with a moist cotton swab (Singh *et al.,* 2022b). To maintain high humidity, frequent irrigation was conducted with a water sprinkler. Quantitative disease resistance parameters encompassed the incubation period, stem lesion length, stem breakage percentage, and the number of sclerotia per plant. Measurements, including stem lesion length (cm), stem breakage (%), and sclerotia count, were taken 20 days' post-inoculation for each plant. These values were averaged for each plot, serving as the experimental unit. Genotypes were categorized based on stem lesion length (SLL): highly resistant (SLL $\geq$ 2.5 cm), resistant (SLL = 2.6 - 5.0 cm), moderately resistant (SLL = 5.1 - 7.5 cm), susceptible (SLL = 7.6 - 10.0 cm), and highly susceptible (SLL > 10.0 cm), following the protocol outlined by Singh *et al.* (2020).

Assessment of stem physical strength attributing traits

Stem physical strength traits were evaluated in this study using 20 cm stem sections obtained from the base of 20 randomly selected plants (genotype/replicate) at different developmental stages (bolting, flowering, podding, and ripening). Stem diameter (mm) was measured at the mid-point of each segment using a precise digital Vernier caliper (Aerospace 300 mm digimatic Vernier caliper with 0.01 mm resolution) to ensure consistency. Individual stem sections were weighed with an electronic precision balance to determine initial fresh weight (FW). After controlled drying at 80°C until a constant weight was achieved, followed by cooling in a desiccator, final dry weight (DW) was obtained. Stem water content (%) and stem dry matter content (%) were calculated as [(FW - DW) / FW] × 100 and [(DW / FW) × 100], respectively. Stem-specific density (SSD) was determined using the dimensional method as discussed in our previous paper (Singh *et al.,* 2023b), calculating volume based on the mid-point diameter measured with the Vernier caliper. Stem-specific density was computed by dividing the oven-dry mass (g) by the fresh volume, expressed in g/cm^3. Stem breaking force (SBF) was measured using a three-point bending test with a digital stem strength tester (Plant Stem Strength Tester, Parisa Technology Co., Mumbai, India), recording the maximum force (N) required to break the 20 cm stem section. Stem breaking strength (SBS) was calculated as stem breaking strength

(N/mm^2) = Stem breaking force (N) / [$\pi(SD/2)^2$], where SD represents the stem diameter (mm).

Assessment of physio-biochemical analysis

Physio-biochemical parameters were assessed in 20 cm stem sections (middle portion) from 15 randomly chosen plants at different developmental stages (bolting, flowering, podding, and ripening). A 1.0 g portion of powdered stem was refluxed with 10 mL of 80% methanol at room temperature. After filtration, the volume was adjusted to 10 mL through repeated methanol washing. This extract was utilized for physio-biochemical assays. For total antioxidant capacity determination, the extract was mixed with a reagent solution containing 0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate, followed by incubation at 90°C for 80 minutes. Absorbance at 695 nm was measured against a blank, and total antioxidant capacity was calculated using a standard curve with known concentrations of ascorbic acid equivalents (Umamaheswari and Chatterjee, 2008). DPPH-free radical scavenging activity was measured by adding 5 mL of 0.1 mM DPPH reagent to 1.5 mL of the methanolic extract. After 30 minutes of incubation in the dark, decolorization of DPPH was measured at 517 nm against a reagent blank (Park *et al.,* 2008). Scavenging activity was calculated as a percentage reduction in DPPH radicals compared to the control. The total phenol content was assessed by combining 0.5 mL of methanolic extract with 1.5 mL of diluted Folin–Ciocalteu's reagent and 7.5 mL of saturated sodium carbonate solution, followed by a 1-hour incubation. Absorbance at 725 nm was measured (Singleton and Rossi, 1965), and quantification utilized a gallic acid calibration curve, expressed as milligrams of gallic acid equivalent per gram of dry weight (mg GAE/g DW). For total flavonoid content determination, 0.5 mL of methanolic extract was mixed with 0.1 mL of 10% aluminum chloride, 0.2 mL of 1 M potassium acetate, and 2.8 mL of distilled water, with absorbance at 415 nm measured using a UV-vis spectrophotometer (Chang *et al.,* 2002). The total flavonoid content was quantified using a quercetin equivalents standard curve, expressed as quercetin equivalents per gram of dry weight (QE/g DW). Total soluble sugar content was determined by combining 1.0 mL of methanolic extract with 2 mL of 5.0% phenol and 5 mL of concentrated sulfuric acid. Absorbance at 490 nm was measured (DuBois *et al.,* 1956), and quantification used a glucose standard curve, expressed as milligrams of glucose equivalent per gram of tissue on a dry weight basis (mg GE/g DW). The stem samples (200 mg) were analyzed for total lignin content following a modified acetyl bromide protocol (Fukushima and Hatfield, 2001). The samples were dispersed in 20.0 mL distilled water, underwent ethanol washes, and were incubated in a 2:1 chloroform-ethanol mixture. After air-drying and weighing, 10 mL of the stored sample was digested in a solution containing 25% acetyl bromide and 70% perchloric acid. The digestion occurred at 70 ºC for 30 min. The lignin concentration was calculated using the formula: Total Lignin Content (%) = [100(As-Ab) ×V]/(a × w), where As is the sample's absorbance at 280 nm, Ab is the blank absorbance at 280 nm, V is the final volume, w is the dry weight of the pellet, and a is the extinction coefficient (20.09/nm/cm).

Experimental design and data analysis

The experiment was conducted at the Research Farm of the Oilseeds Section, Department of Genetics and Plant Breeding, CCS Haryana Agricultural University, Hisar, India, during the 2022-23 cropping season. A split-split plot arrangement with randomized complete blocks was employed, organizing experimental units into whole-plot units representing plant developmental growth stages. Split-plots were assigned to different *S. sclerotiorum* isolates, while split-split plots were assigned to different host genotypes, allowing for randomization and replication across three blocks. Each split-split plot comprised paired rows of five-meter length, with a row-to-row distance of 45 cm and a plant-to-plant distance of 10 cm. The experiment was conducted in triplicate, and all the recommended agricultural practices were implemented to cultivate a healthy crop. Disease assessment parameters were analyzed through a three-way ANOVA (4 × 6 × 10 factorial design), and stem structural and physiological resistance traits were examined using a two-factorial ANOVA. Pearson correlation coefficients explored relationships between experiments. Graphs were generated using Microsoft Excel (2016), and the DMRT assessed statistical significance ($P \leq 0.05$). Statistical analyses were conducted with STAR version 2.0.1 Statistical Software developed by International Rice Research Institute, Manila, Philippines.

Results and Discussion

Assessment of various pathogenicity related attributes of *Sclerotinia sclerotiorum* isolates collected from different geographical locations of India

Six isolates of *S. sclerotiorum* collected from different regions in India exhibited significant ($P \leq 0.05$) variations in pathogenicity and aggressiveness-related traits (Table 1). The Hisar isolate (SsHsi) showed the fastest growth rate, with a significantly larger colony diameter (6.96 mm at 72 hours) compared to other isolates, followed by the Delhi isolate (SsDli) and the Ludhiana isolate (SsLdi). SsHsi also formed sclerotia in the shortest time (135 hours), while the Varanasi isolate (SsVni) took the longest (188 hours) (Fig. 1A–B). SsHsi produced the significantly highest number of sclerotia per plate (36.6 sclerotia/plate), followed by SsDli and SsLdi. The isolates with the most sclerotia also had the smallest sclerotial diameter and weight, while those with fewer sclerotia had larger sizes (Fig. 1D-E). Statistically significant differences in mean sclerotial diameter and 100-sclerotial weight were observed among *S. sclerotiorum* isolates, with SsHsi displaying the smallest diameter (2.79 mm) and SsVni the largest (4.17 mm) (Fig. 1D-E). The total oxalic acid content varied significantly among isolates, with SsVni having the lowest concentration (34.36 g OA/g dried mycelium) and SsHsi the highest (76.18 g OA/g dried mycelium) (Fig. 1F). These findings highlight significant genetic diversity among isolates in terms of aggressiveness and pathogenicity traits. Previous studies have emphasized the need for a comprehensive approach in evaluating resistance against multiple isolates due to genetic variation in *S. sclerotiorum* isolates (Attanayake *et al.,* 2013; Sharma *et al.,* 2018a). The observed variations have important implications for tailored control measures (Hossain *et al.,* 2023).

Table 1: Analysis of variance for various pathogenicity and aggressiveness-related attributes of different *Sclerotinia sclerotiorum* isolates used in the study

Source of variation	df	P values
Mean colony diameter at 72h	5	<0.00001
Mean time for sclerotial appearance	5	0.02997
Mean number of sclerotia per plate	5	0.00004
Mean sclerotial diameter	5	0.02078
100-sclerotial weight	5	<0.00001
Total oxalic acid produced	5	<0.00001

Pearson product-moment correlation analyses revealed highly significant ($P \leq 0.01$) negative correlations between mean colony diameter at 72 hours and total oxalic content with incubation period, and positive correlations with stem lesion length, stem breakage, and number of sclerotia per plant (Table 2). Significant negative associations were found between mean time for sclerotial appearance, mean sclerotial diameter, 100-sclerotial weight, and these disease assessment parameters on host plants. Additionally, a significant positive correlation was observed between the average number of sclerotia per plate and the number of sclerotia per plant. These findings underscore the importance of isolate-specific traits in disease management, consistent with previous research (Hossain *et al.,* 2023; Sharma *et al.,* 2018a).

Table 2: Pearson's product moment correlation matrix between pathogenicity related attributes of *S. sclerotiorum* and various disease assessment parameters

Variables	IP	SLL	SB	NSPP
Mean colony diameter at 72h	-0.90*	0.94**	0.93**	0.98**
Mean time for sclerotial appearance	0.79NS	-0.84*	-0.83*	-0.92*
Mean number of sclerotia per plate	-0.66NS	0.69NS	0.70NS	0.80*
Mean sclerotial diameter	0.77NS	-0.86*	-0.83*	-0.89*
100-sclerotial weight	0.80NS	-0.88*	-0.86*	-0.89*
Total oxalic acid produced	-0.95**	0.96**	0.97**	0.98**

NS-Non significant, *Significant at $P \leq 0.05$ and **Significant at $P \leq 0.01$; IP-Incubation period (days), SLL-Stem lesion length (cm), SB-Stem breakage (%), NSPP-Number of sclerotia produced/plant

Assessment of the impact of plant developmental stages, pathogen isolates, host genotypes, and their interactions on disease resistance components

A comprehensive three-way analysis of variance was conducted to investigate the influence of plant developmental stages (GS), pathogen isolates (PI), and host genotypes (HG) on various quantitative disease resistance components. The results revealed highly significant effects ($P \leq 0.01$) for all these factors, as well as their interactions, with respect to the examined resistance components, including incubation period, stem lesion length, stem breakage, and number of sclerotia produced per plant (Table 3). This study addresses the gap in knowledge regarding *Brassicaceae-S. sclerotiorum* interactions, emphasizing the need for a comprehensive approach evaluating resistance against multiple isolates and developmental stages (Khan *et al.,* 2022). The significant influence of plant developmental stage on resistance, termed developmental resistance, is

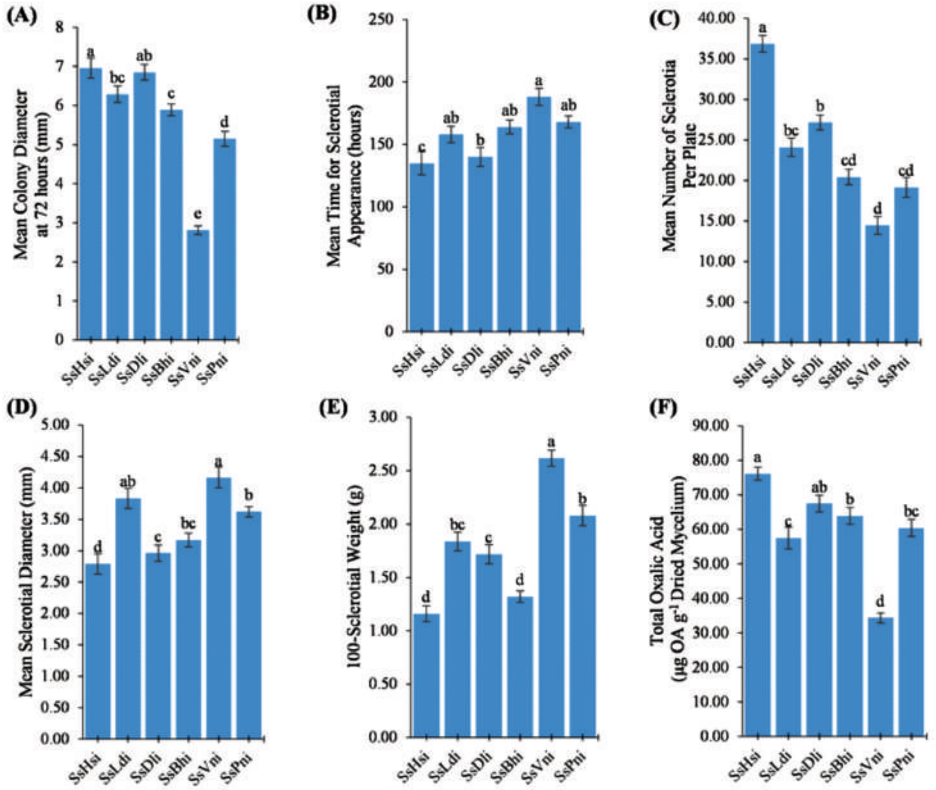

Fig. 1: Comparison of six different isolates of *Sclerotinia sclerotiorum* collected from different geographical locations of India for various pathogenic and aggressiveness attributing traits viz., (A) mean colony diameter (mm) at 72 hours after inoculation on PDA plate (B) mean time for sclerotial appearance (hours) (C) mean number of sclerotia per plate (D) mean sclerotial diameter (mm) (E) 100-sclerotial weight (g) and (F) total oxalic acid (µg OA/g dried mycelium). Column and bar represent mean and standard error, respectively. Different letters on different bars indicate significant differences ($P \leq 0.05$) based on DMRT. SsHsi *S. sclerotiorum* Hisar isolate, SSLdi *S. sclerotiorum* Ludhiana isolate, SsDli *S. sclerotiorum* Delhi isolate, SsBhi *S. sclerotiorum* Bharatpur isolate, SsVni *S. sclerotiorum* Varanasi isolate, SsPni *S. sclerotiorum* Pantnagar isolate.

Table 3: Three-way analysis of variance of the effect of plant-developmental stages (GS), pathogen isolates (PI), and *Brassicaceae* genotypes with differential response to *S. sclerotiorum* (HG) and their interactions on the incubation period (IP), stem lesion length (SLL), stem breakage (SB) and number of sclerotia produced/plant (NSPP)

Source of variation	df	P values			
		IP	SLL	SB	NSPP
Growth stage (GS)	3	<0.00001	0.00484	0.00045	<0.00001
Pathogen isolate (PI)	5	0.00784	<0.00001	<0.00001	<0.00001
Host genotype (HG)	9	0.00006	<0.00001	<0.00001	<0.00001
GS × PI	15	<0.00001	<0.00001	0.00026	<0.00001
GS × HG	27	<0.00001	0.00027	0.00032	0.00052
PI × HG	45	<0.00001	0.00546	<0.00001	0.00087
GS × PI × HG	135	<0.00001	<0.00001	<0.00001	0.00018

IP-Incubation period (days), SLL-Stem lesion length (cm), SB-Stem breakage (%), NSPP-Number of Sclerotia Produced/Plant

particularly noteworthy and has been observed in various pathosystems (Ando *et al.,* 2009; Chongo and Gossen, 2001).

Brassicaceae genotypes exhibited diverse resistance traits against six *S. sclerotiorum* isolates across four plant growth stages. Variations in incubation period, stem lesion length, stem breakage, and sclerotia production were evident. On average, bolting stage plants (~30 days old) displayed greater disease severity compared to ripening stage plants (~120 days old), showing prolonged incubation, increased stem lesion length, higher stem breakage percentage, and an elevated number of sclerotia per plant. During plant development from bolting to maturity, an intriguing observation emerged- overall disease severity declined. Certain genotypes (*B. fruticulosa* BF1, *D. tenuisiliqua* DT1, and *E. abyssinicum* EA1) consistently displayed disease resistance across developmental stages, unlike other genotypes. Pathogen isolates exhibited variable virulence, with *S. sclerotiorum* Hisar isolate (SsHsi) displaying the highest aggressiveness and *S. sclerotiorum* Varanasi isolate (SsVni) the lowest. SsHsi consistently caused larger stem lesions, higher stem breakage, and more sclerotia per plant. In contrast, SsVni demonstrated reduced virulence with smaller lesions, lower breakage, and fewer sclerotia. *S. alba* SA1 consistently had the shortest stem lesion, while *B. juncea* IC401578 had the longest across all isolates and growth stages (Fig. 2). These findings underscore the importance of considering plant developmental stages when evaluating disease resistance, consistent with previous studies on genotype-pathogen isolate interactions (Willbur *et al.,* 2017; Michael *et al.,* 2023). The variability in resistance across different growth stages highlights the complexity of disease resistance mechanisms in *Brassicaceae* and the need for a comprehensive evaluation across multiple stages and isolates.

Host-pathogen interaction and identification of host differential and pathotype

The *Brassicaceae* genotypes exhibited diverse responses to *S. sclerotiorum*, ranging from resistance to susceptibility. Stem lesion length (SLL) measurements after 20 days of inoculation facilitated the classification of genotypes into distinct resistance groups. *S. alba* SA1 consistently displayed resistance (SLL $\geq$ 5.0 cm) against all isolates and developmental stages. Conversely, *B. tournefortii* BT1 and *B. juncea* IC401578 consistently showed susceptibility (SLL < 7.5) across all conditions. Other genotypes exhibited varying responses to *S. sclerotiorum*, depending on both plant development stage and pathogen isolate. The majority displayed resistance against the Varanasi isolate (SsVni) across all growth stages, with exceptions like *B. juncea* RH1222-28, which showed resistance during podding and ripening but moderate response (SLL = 5.0-7.5 cm) to other isolates. Notably, *B. juncea* RH1222-28 was susceptible to Ludhiana (SsLdi) and Delhi (SsDli) isolates. SsVni demonstrated resistance to *E. abyssinicum* EA1, while other isolates showed high susceptibility. *D. tenuisiliqua* DT1 consistently resisted Hisar (SsHsi), Ludhiana (SsLdi), and Varanasi (SsVni) isolates, and *B. fruticulosa* BF1 resisted Ludhiana (SsLdi) and Varanasi (SsVni) isolates, regardless of plant development stage. These findings align with previous research highlighting the complexity of host-pathogen interactions (Martín *et al.,* 2021). The identification of genotypes exhibiting consistent resistance across developmental stages and isolates, such as *S. alba* SA1, is a significant outcome, suggesting intrinsic resistance mechanisms (Hu and Yang, 2019; Calonnec *et al.,* 2021). The diverse responses observed in genotypes like *B. juncea* RH1222-28 and *B. fruticulosa* BF1 indicate the presence of unique resistance mechanisms that warrant further investigation.

Utilizing an octal classification system, we identified nine host genotypes (excluding *B. juncea* IC401578) suitable for characterizing *S. sclerotiorum* pathotypes. Through octal triplet codes, six distinct pathotypes and nine host differentials were delineated. Each of the six *S. sclerotiorum* isolates and selected host genotypes exhibited unique octal codes. *S. alba* SA1 displayed resistance with a 00 octal code, while *B. juncea* IC401578 indicated susceptibility with a 77 octal code. The Varanasi isolate (SsVni) was classified as the least virulent pathotype with the 001 octal code (virulent against one host differential), while the Delhi isolate (SsDli) received the 761 octal code, marking it as the most virulent pathotype (virulent against six host differentials). Efficient identification of plant host differentials and pathogen pathotypes is crucial for targeted breeding strategies, enhancing breeding efficiency, and developing crop cultivars resistant to pathogens. This approach has been successful in various pathosystems and is essential for the *Brassicaceae-S. sclerotiorum* system (Ge *et al.,* 2012). The delineation of host differentials and pathotypes in this study offers valuable insights into the pathosystem dynamics, aiding in the selection of breeding lines with resistance traits across diverse pathotypes.

Assessment of stem physical strength and physio-biochemical attributes at different plant-developmental stages

A comprehensive analysis was conducted to assess the stem physical strength and physio-biochemical attributes across different developmental stages and host genotypes. Two-way ANOVA revealed significant variations ($P \leq 0.01$) in stem physical strength attributes (stem diameter, stem specific density, breaking force, breaking strength) and physio-biochemical parameters (stem water content, dry matter content, lignin concentration, total antioxidant capacity, free-radical scavenging activity, total phenol content, and total flavonoid content) (Table 4). Stem diameter generally

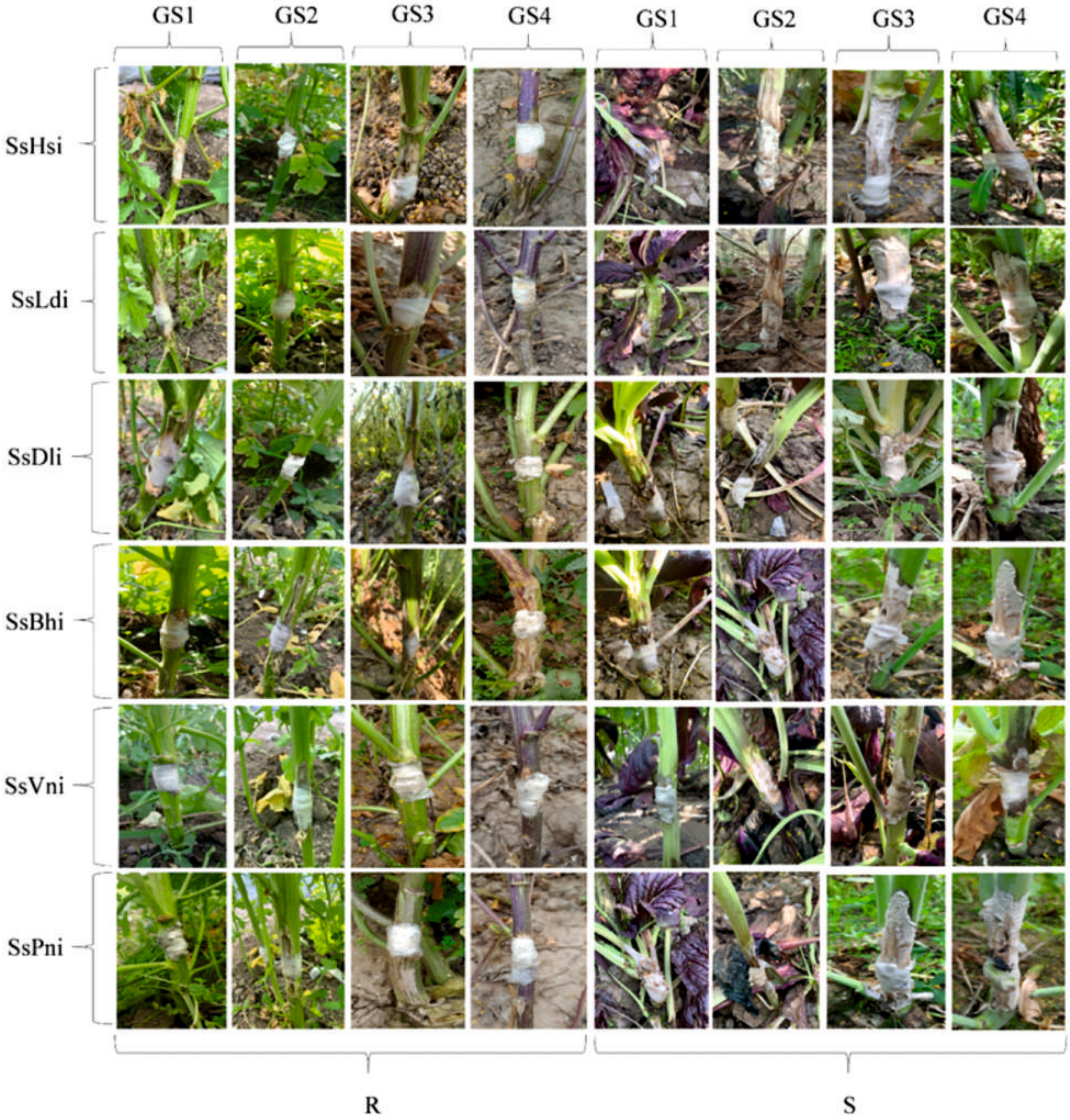

Fig. 2: Comparison of disease responses exhibited by the most resistant (R) and most susceptible (S) *Brassicaceae* genotypes inoculated with six different isolates of *S. sclerotiorum* at four different plant-developmental growth stages. R stands for resistant genotype *S. alba* SA1 while S stands for susceptible genotype *B. juncea* IC401578 (from left to right) that were inoculated with six different isolates of *S. sclerotiorum* (from top to bottom, SsHsi-*S. sclerotiorum* Hisar isolate, SsLdi-*S. sclerotiorum* Ludhiana isolate, SsDli-*S. sclerotiorum* Delhi isolate, SsBhi-*S. sclerotiorum* Bharatpur isolate, SsVni-*S. sclerotiorum* Varanasi isolate, SsPni-*S. sclerotiorum* Pantnagar isolate) at four different plant-developmental stages (from left to right, GS1-bolting stage (~30-days old crop), GS2-flowering stage (~60-day old crop), GS3-podding stage (~90-days old crop), GS4-ripening stage (~120-days old crop)

increased with plant maturity, with *B. juncea* IC401578 exhibiting the largest stem diameter (>15.0 mm) and *E. abyssinicum* EA1 the smallest (Fig. 3A). Stem water content showed a declining trend from bolting to ripening stages, with corresponding increases in dry matter content. *S. alba* SA1 consistently displayed the highest dry matter content (Fig. 3B-C). Significant ($P \leq 0.05$) differences in stem physical strength-related variables were observed among the genotypes. *S. alba* SA1 exhibited superior traits, including higher stem specific density, breaking force, and breaking strength throughout development (Fig. 4A-C).

Table 4: Two-way analysis of variance of the effect of plant-developmental growth stages (GS) and *Brassicaceae* genotypes with differential response to *S. sclerotiorum* (HG) as well as their interactions on various stem physical strength attributes and various physio-biochemical parameters

Source of variation	P values		
	GS	HG	GS × HG
df	3	9	27
SD	<0.00001	0.00055	0.00034
SWC	0.00045	0.00023	<0.00001
SDMC	<0.00001	<0.00001	0.00075
SSD	<0.00001	<0.00001	0.00326
SBF	0.00182	0.00025	<0.00001
SBS	<0.00001	0.00047	<0.00001
TLC	0.00063	<0.00001	<0.00001
TAC	<0.00001	<0.00001	0.00058
FRSA	<0.00001	<0.00001	0.00472
TPC	0.00487	0.00014	<0.00001
TFC	<0.00001	<0.00001	0.00238
TSS	<0.00001	<0.00001	0.00964

SD stem diameter (mm), SWC stem water content (%), SDMC stem dry matter content (%), SSD stem specific density (g cm^{-3}), SBF stem breaking force (N), SBS stem breaking strength (N/mm^2), TLC total lignin content (%), TAC total antioxidant capacity (µg AAE/g FW), FRSA DPPH-free radical scavenging activity (%), TPC total phenol content (mg GAE/g DW), TFC total flavonoid content (µg QE/g DW), TSS total soluble sugar (mg GE/g DW).

Lignin concentrations varied significantly ($P \leq 0.05$) among the genotypes and increased with plant maturity. *S. alba* SA1 exhibited the highest lignin content at the ripening stage (18.94%), while *B. juncea* IC401578 had the lowest lignin content (3.9%) (Fig. 5A). Total antioxidant capacity (TAC) and free-radical scavenging activity (FRSA) generally peaked at the bolting stage and decreased with maturity, with *D. tenuisiliqua* DT1 exhibiting the highest values (Fig. 5B-C). Total phenol content (TPC) and total flavonoid content (TFC) increased from bolting to ripening stages, with *B. juncea* RH1222-28 consistently showing the highest content (Fig. 6A-C). These findings highlight the importance of structural and biochemical traits in disease resistance. Higher lignin content, stem specific density, breaking force, and strength were associated with greater resistance, as indicated by significant negative correlations with stem lesion length. The role of lignin in reinforcing cell walls and inhibiting pathogen invasion is well-documented (Vanholme *et al.,* 2010). The observed trends in TAC, FRSA, TPC, and TFC suggest their involvement in defense responses, corroborating studies linking antioxidant and phenolic compounds to enhanced disease resistance (Sharma *et al.,* 2018b).

Conclusion

Sclerotinia stem rot, caused by *S. sclerotiorum*, poses a significant threat to *Brassicaceae* crops, necessitating the identification of resistant genotypes for effective disease management. Previous studies have explored physiological and structural resistance mechanisms independently, leading to challenges in distinguishing between these phenotypes due to overlapping characteristics. This study aimed to differentiate and compare structurally and physiologically resistant genotypes of *Brassicaceae*. Results revealed genotype-specific, isolate-specific, and developmental stage-dependent resistance to Sclerotinia stem rot. Notably, the genotype *S. alba* SA1 consistently exhibited resistance across all stages and isolates, making it a promising candidate for developing resistant varieties. Stem physical strength and various physio-biochemical attributes varied among genotypes and developmental stages. Stem hardness, woodiness, and total lignin

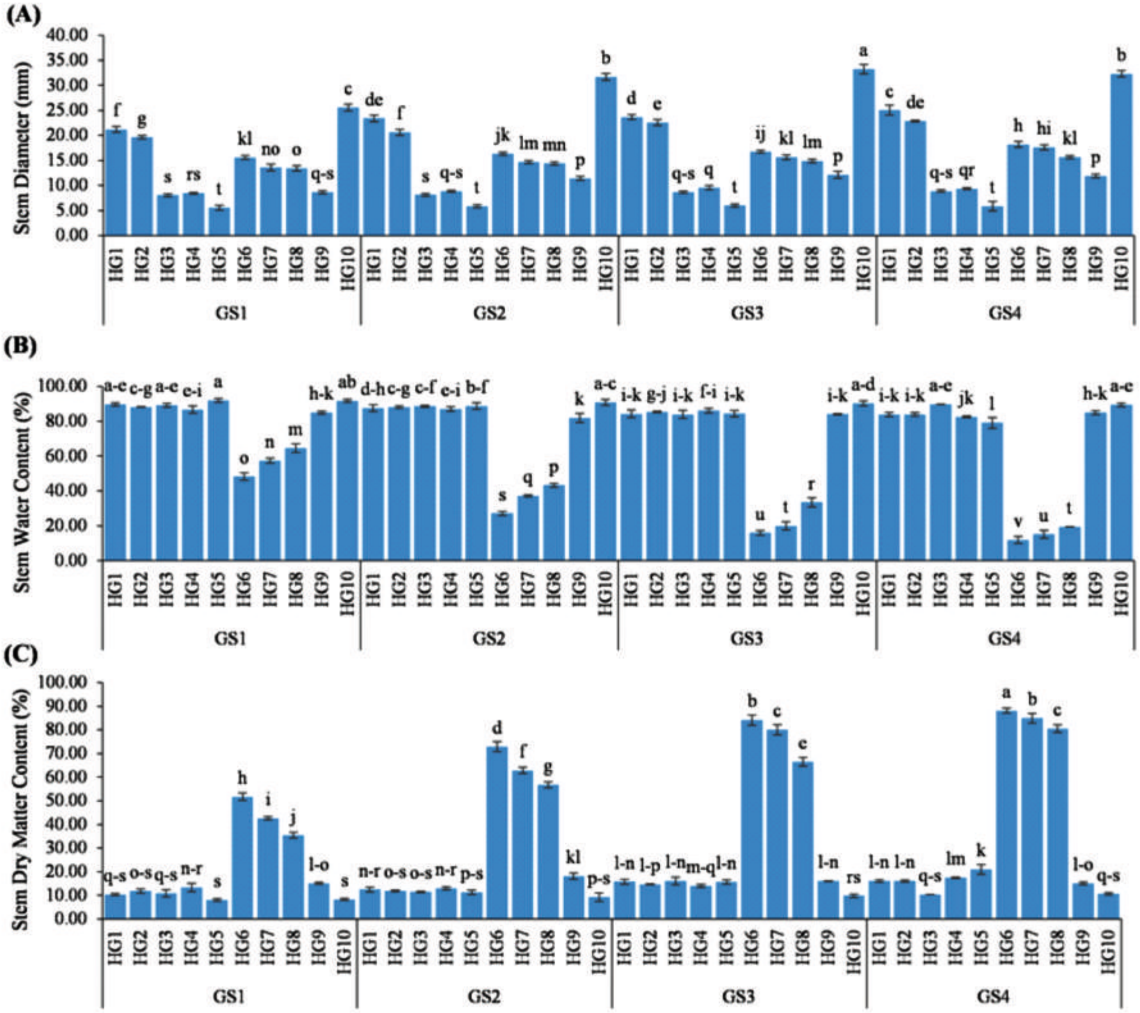

Fig. 3: Comparison of ten *Brassicaceae* genotypes with differential response to *S. sclerotiorum* (HG1-HG10) for various stem physical strength attributing traits viz. (A) stem diameter (mm), (B) stem water content (%) and (C) stem dry matter content (%) over different plant-developmental growth stages (GS1-GS4). Column and bar represent mean and standard error, respectively. Different letters on different bars indicate significant differences ($P \leq 0.05$) based on DMRT. HG1-*Brassica juncea* RH1222-28, HG2-*B. juncea* IC589660, HG3-*B. fruticulosa* BF1, HG4-*Diplotaxis tenuisiliqua* DT1, HG5-*Erucastrum abyssinicum* EA1, HG6-*Sinapis alba* SA1, HG7-*S. alba* SA2, HG8-*S. alba* SA3, HG9-*B. tournefortii* BT1, HG10-*B. juncea* IC401578; GS1-Bolting stage (~30-days old crop), GS2-Flowering stage (~60-days old crop), GS3-Podding stage (~90-days old crop), GS4-Ripening stage (~120-days old crop)

content were associated with durable resistance, while total soluble sugar content correlated with susceptibility. The interplay between physiological and structural resistance mechanisms is crucial for comprehensive resistance management strategies and breeding cultivars with durable resistance. By understanding and harnessing these mechanisms, breeders can select traits for crop breeding programs and combine them through resistance stacking to develop cultivars resistant to Sclerotinia stem rot. Further research is needed to explore the genetic factors and pathways involved in these resistance mechanisms and identify additional genotypes with diverse resistance profiles against different isolates and developmental stages.

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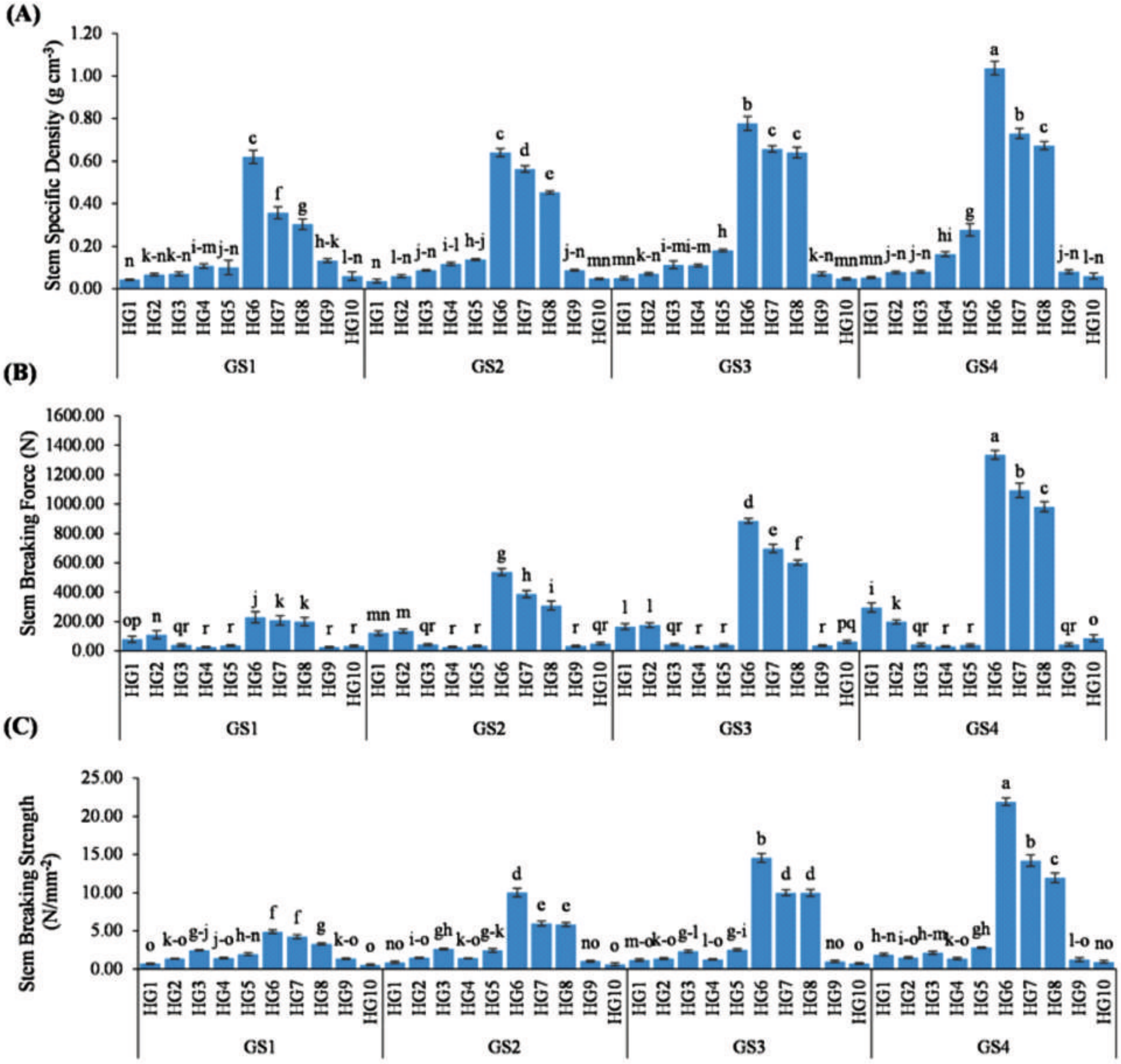

Fig. 4: Comparison of ten *Brassicaceae* genotypes with differential response to *S. sclerotiorum* (HG1-HG10) for various stem physical strength attributing traits viz., (A) stem specific density (g/cm^3), (B) stem breaking force (N) and (C) stem breaking strength (N/mm^2) over different plant-developmental stages (GS1-GS4). Column and bar represent mean and standard error, respectively. Different letters on different bars indicate significant differences ($P \leq 0.05$) based on DMRT. HG1-*Brassica juncea* RH1222-28, HG2-*B. juncea* IC589660, HG3-*B. fruticulosa* BF1, HG4-*Diplotaxis tenuisiliqua* DT1, HG5-*Erucastrum abyssinicum* EA1, HG6-*Sinapis alba* SA1, HG7-*S. alba* SA2, HG8-*S. alba* SA3, HG9-*B. tournefortii* BT1, HG10-*B. juncea* IC401578; GS1-Bolting stage (~30-days old crop), GS2-Flowering stage (~60-days old crop), GS3-Podding stage (~90-days old crop), GS4-Ripening stage (~120-days old crop)

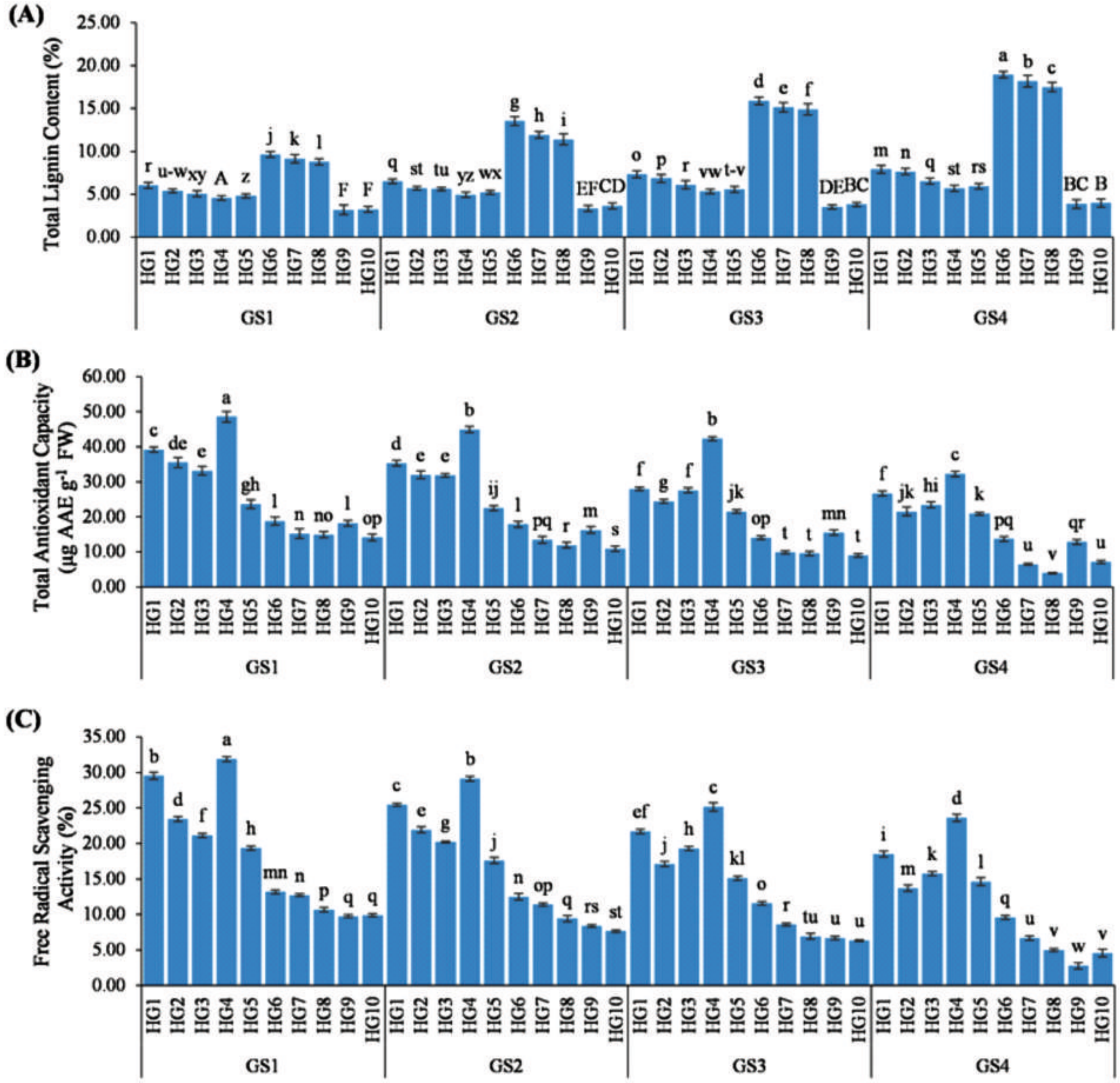

Fig. 5: Comparison of ten *Brassicaceae* genotypes with differential response to *S. sclerotiorum* (HG1-HG10) for various physio-biochemical traits viz., (A) total lignin content (%), (B) total antioxidant capacity (µg AAE/g FW) and (C) DPPH-based free radical scavenging activity (%) over different plant-developmental growth stages (GS1-GS4). Column and bar represent mean and standard error, respectively. Different letters on different bars indicate significant differences (P ≤0.05) based on DMRT. HG1-*Brassica juncea* RH1222-28, HG2-*B. juncea* IC589660, HG3-*B. fruticulosa* BF1, HG4-*Diplotaxis tenuisiliqua* DT1, HG5-*Erucastrum abyssinicum* EB1, HG6-*Sinapis alba* SA1, HG7-*S. alba* SA2, HG8-*S. alba* SA3, HG9-*B. tournefortii* BT1, HG10-*B. juncea* IC401578; GS1-Bolting stage (~30-days old crop), GS2-Flowering stage (~60-days old crop), GS3-Podding stage (~90-days old crop), GS4-Ripening stage (~120-days old crop)

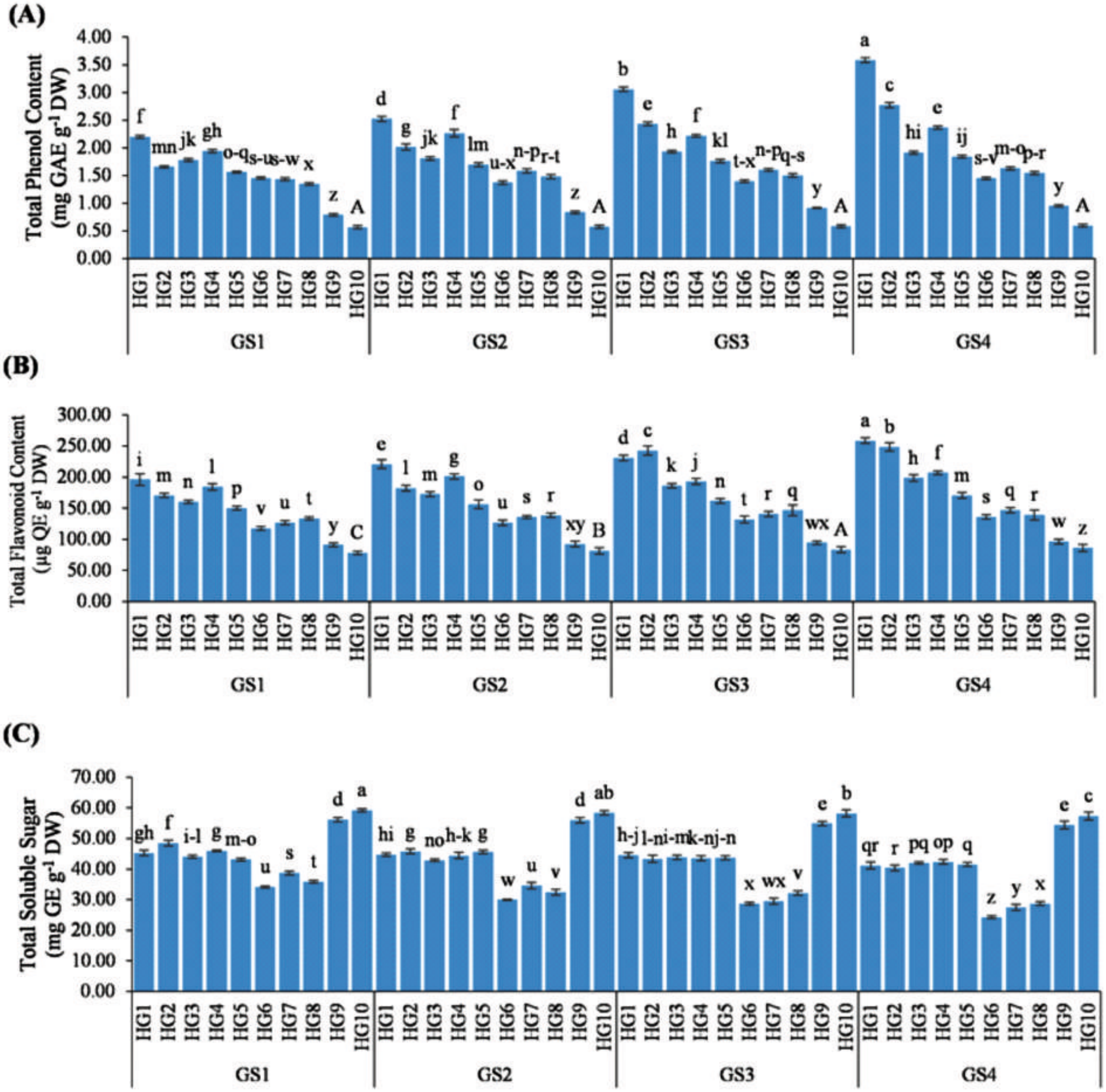

Fig. 6: Comparison of ten *Brassicaceae* genotypes with differential response to *S. sclerotiorum* (HG1-HG10) for various physio-biochemical traits viz., (A) total phenol content (mg GAE/g DW), (B) total flavonoid content (µg QE/ gDW) and total soluble sugar (mg GE/g DW) over different plant-developmental growth stages (GS1-GS4). Column and bar represent mean and standard error, respectively. Different letters on different bars indicate significant differences ($P \leq 0.05$) based on DMRT. HG1-*Brassica juncea* RH1222-28, HG2-*B. juncea* IC589660, HG3-*B. fruticulosa* BF1, HG4-*Diplotaxis tenuisiliqua* DT1, HG5-*Erucastrum abyssinicum* EB1, HG6-*Sinapis alba* SA1, HG7-*S. alba* SA2, HG8-*S. alba* SA3, HG9-*B. tournefortii* BT1, HG10-*B. juncea* IC401578; GS1-Bolting stage (~30-days old crop), GS2-Flowering stage (~60-days old crop), GS3-Podding stage (~90-days old crop), GS4-Ripening stage (~120-days old crop)

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