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# SUNFLOWER HYBRIDS EVALUATION FOR CHARCOAL ROT RESISTANCE

**T. BASHARAT[1*], S. GUL[1], S. RAUF[1], S. AHMAD[2], and R. ORTIZ[3*]**

[1]Department of Plant Breeding and Genetics, College of Agriculture, University of Sargodha, Pakistan
[2]Department of Plant Pathology, College of Agriculture, University of Sargodha, Pakistan
[3]Department of Genetics and Plant Breeding, Swedish University of Agricultural Sciences, Al-Narp, Sweden
*Corresponding authors' emails: rodomiro.ortiz@slu.se, taiyyibahbasharat@gmail.com
Email addresses of co-authors: saeedbreeder@hotmail.com, samringulpbg@gmail.com, salman.ahmad@uos.edu.pk

## SUMMARY

Sunflowers (*Helianthus annuus*) play a vital role as a global oilseed crop, but biotic and abiotic factors threaten their yield, especially in the context of climate change. One significant challenge is charcoal rot, a disease prevalent in tropical and subtropical climates. This disease causes stem lesions, leading to wilting and premature lodging, resulting in severe yield losses, ranging from 20% to 50%. Combating this issue led to introducing disease-resistant genes as a primary strategy. This study investigates the outcomes of incorporating charcoal-resistant lines into a breeding program. Four resistant inbred lines—'B-208', 'B-124', 'B-224', and 'B-112'—reached crossing with male fertility restorer lines, creating 16 half-sib cross combinations. These crosses showed varied levels of resistance to charcoal rot. Notably, the combinations 'C.112' × 'RSIN.82' and 'C.208' × 'RH.344' exhibited minor infestations and displayed negative heterosis, indicating a tendency toward complete to overdominance in resistance traits. The estimates of heterosis were modest, with a significant dominance variance relative to additive variance for disease resistance. Lines 'B-112', 'B-208', and 'RSIN.82' demonstrated strong general combining ability effects, suggesting their potential usefulness in breeding programs.

**Keywords:** dominance, fertility restorers, heterosis, infestation, symptoms

**Key findings:** The cross combinations with charcoal-resistant lines, particularly 'C.112' × 'RSIN.82' and 'C.208' × 'RH.344', showed considerable negative heterosis and a clear inclination toward complete and overdominance of resistance traits. The inheritance of charcoal rot resistance showed more dominance variance characteristic, significantly greater than the additive variance, emphasizing the importance of dominant genetic factors in providing disease resistance.

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## INTRODUCTION

Sunflower (*Helianthus annuus*) is an essential source of oilseed, confectionary, and bird food. It is the fourth major oilseed crop, providing more than 10% of the total edible oil (Rauf, 2019). Its oil is a rich source of tocopherols, sterols, polyunsaturated, and saturated fatty acid crucial for human health and improves the taste of food when functioning as salad spread or cooking oil (Rauf, 2019). Sunflower yield has incurred threats from various biotic and abiotic factors that may affect the sunflower yield in the field.

Biotic stress remains a persistent threat to the survival of host plants, exerting its detrimental effects by infiltrating the fibrovascular bundles via the roots and subsequently compromising various morphological traits, including the stem. Sunflower, renowned as a host to over 30 pathogens, is particularly susceptible to a multitude of infectious diseases. Among the major afflictions are sunflower rust, downy mildew, *Phoma* black stem, charcoal rot, powdery mildew, sclerotinia head rot, stalk rot, and wilt, as well as, *Alternaria* leaf and stem spot, broomrape, bacterial stalk, and *Verticillium* wilt (Rauf *et al.*, 2020). These diseases, each caused by distinct pathogens, collectively pose significant challenges to sunflower cultivation, necessitating vigilant management strategies to mitigate their impact and ensure crop health and productivity.

*Macrophomina phaseolina,* the causal organism responsible for inducing charcoal rot disease, poses a considerable threat across a broad spectrum of plant species. This disease's documentation has covered over 500 species of both wild and domesticated plants. Its impact extends beyond mere presence, as it inflicts yield losses in more than 67 economically valuable plant species (Marquez *et al.*, 2021). The spread of the disease primarily occurs through infected seeds and soil, with the fungal pathogen capable of persisting in dry soil for over 10 months. Once established the infection progresses to the fibrovascular system of the roots and targets the basal internodes of sunflower stems. Remarkably, the sclerotia produced by *M. phaseolina* can endure in the soil for several years, thereby further complicating disease management efforts. Notably, chemical control methods are non-recommendable due to challenges associated with effectively targeting the pathogen in the soil and the persistence of sclerotia (Cotuna *et al.,* 2022). This underscores the importance of implementing integrated disease management strategies focused on prevention, cultural practices, and the utilization of resistant cultivars to mitigate the impact of charcoal rot disease in sunflower.

Worldwide, diseases represent a significant menace to sunflower harvests, exerting substantial economic repercussions. An estimate renders diseases accountable for an average annual yield loss of approximately 12% across roughly 12 million hectares of sunflower cultivation globally (Bahadur and Dutta, 2023). This substantial reduction in yield not only affects the economic viability of sunflower farming, but also highlights the critical need for effective disease management strategies to safeguard crop productivity and ensure food security. Implementing measures aimed at disease prevention, early detection, and proactive intervention are essential to mitigate the impact of diseases on sunflower yields and sustainably enhance agricultural productivity on a global scale.

The impact of charcoal rot, caused by the pathogen *M. phaseolina*, on sunflower yields is particularly prevalent in arid regions worldwide, as reported by Bahadur and Dutta (2023). In regions, such as, Spain, the USA, Uruguay, and the former USSR, yield loss attributed to charcoal rot have reached a documented high of 25%. Furthermore, under conditions conducive to the optimal growth and development of *M. phaseolina*, it recorded instances of total crop failure (Jordaan *et al.*, 2019). The detrimental effects of disease infestation extend beyond mere yield reductions, as highlighted by Cotuna *et al.* (2022). Notable declines in various yield components have been evident, including a 3% reduction in stem height, a 36% decrease in stem girth, a 73% decline in root development, and an 11% decrease in head weight (Cotuna *et al.,* 2022). These findings underscore the multifaceted impact of charcoal rot on sunflower crop health and productivity, thereby, emphasizing the urgent need for effective disease management strategies to mitigate its adverse effects and sustainably enhance sunflower yields in affected regions.

The attack of a pathogen triggers a hypersensitive reaction in the host, often leading to the activation of the antioxidative defense system. Essential enzymes, like superoxide dismutase, play a crucial role in neutralizing reactive oxygen species, such as, hydroxyl radicals, hydrogen peroxide, and superoxide radicals (Khan *et al.*, 2017). However, assessing host resistance against pathogens through morphological analyses is challenging due to the influence of environmental factors, i.e., temperature and humidity. Additionally, variation in pathogenicity among different pathogen isolates further complicates these analyses (Babu *et al.*, 2010). Overcoming these challenges require molecular markers increasing application to identify and characterize host pathogens. For instance, conserved genes like internal transcribed spacers (ITS) and 18S rRNA help identify fungus isolates (Babu *et al.*, 2010b). Moreover, advanced molecular techniques, such as, simple sequence repeats (SSR) and single nucleotide polymorphisms (SNP), facilitate the screening of thousands of loci across the entire genome. These molecular markers are instrumental in linking resistance genes or mapping quantitative trait loci associated with disease resistance (Reyes-Franco *et al.*, 2006; Rajeshwaran *et al.*, 2022). By leveraging molecular tools, researchers can gain deeper insights into host-pathogen interactions based on genes, ultimately aiding them to develop more effective disease management strategies in crops like sunflower.

A germplasm panel consisting of 46 accessions sourced from both the National Agriculture Research Council (NARC), Islamabad, and the Department of Plant Breeding and Genetics, College of Agriculture, University of Sargodha, Pakistan, underwent screening against charcoal rot. The study evaluated the disease's impact on various yield components, including head diameter (cm) and the total number of achenes per head. Results indicated breeding lines sourced from COA, specifically 'HA-259' with a yield of 2,494 kg ha$^{-1}$ and 'HA-65' with a yield of 1,858 kg ha$^{-1}$, exhibited the highest yield potential under artificial disease conditions. Head diameter across the germplasm ranged from 7.9 to 24.3 cm, which decreased to 7.2 and 23.8 cm, respectively, under disease stress. The maximum number of achenes per head resulted in 'HA-259', totaling 1121, but this count declined to 621 after artificial inoculation (Qamar *et al.*, 2018). These findings emphasize the importance of identifying and utilizing disease-resistant breeding lines to mitigate adverse effects of charcoal rot on sunflower yields, ultimately enhancing crop productivity and resilience in affected regions. Based on this background, a breeding program initiative studied the utility of charcoal rot-resistant breeding material in hybrid development. Likewise, it estimated the genetic parameters associated with the charcoal rot resistance that may further help formulate breeding strategies for carrying out further selection cycles.

The primary objective of our research was to screen sunflower germplasm against charcoal rot disease and assess its impact on its various yield components. Through meticulous experimentation and data collection, valuable insights validated the performance of different breeding lines under artificial disease conditions. This study contributes to the ongoing efforts aimed at enhancing sunflower productivity and resilience to diseases in Pakistan.

## MATERIALS AND METHODS

The study proceeded in the Department of Plant Breeding and Genetics at the College of Agriculture of the University of Sargodha, Pakistan, spanning from 2021 to 2023. During this period, researchers focused on evaluating a germplasm comprising 46 accessions sourced from both the National Agriculture Research Council and the Department of Plant Breeding and Genetics.

**Development of plant material**

Sowing all plant material commenced in the cropping season of February 2021. The breeding lines, sown in single rows, spanned 4 m each row. Selection of resistant plant material relied on previous trials conducted by the corresponding authors and the Department of Plant Pathology at the College of Agriculture, University of Sargodha, as documented in studies by Shehbaz *et al.* (2018), Qamar *et al.* (2019a), and Qamar and Ghazanfar (2019b). A total of four cytoplasmic male sterile (CMS) female lines and four male restorers were materials used to develop 16 single cross combinations. Each female line received pairing with restorers in a line × tester fashion to generate the 16 cross combinations. Preventing insect pollination had all floral capitula of selected plants covered with net bags. Manual pollination ensued by shaking the heads of the male capitula over the female capitula to facilitate hybrid seed formation. Pollen shedding continued until all stigmas withered. Subsequently, all capitula attained air-drying under the shade, then, manually harvested, and stored in kraft bags at room temperature. This meticulous process ensured the controlled pollination and collection of hybrid seeds for subsequent evaluation and analysis.

**Experimental trials**

The trial (32.13367° or 32° 8′ 1″ north; Longitude 72.68705) transpired during the spring, from February 18, 2022, to February 18, 2023. All single crosses and their parental lines' sowing in single rows spanning 4 m each employed a split-plot design with three replications. The inoculation regime served as the main plot, while the breeding material constituted the subplots. Soil type was a loam with 1.13 EC (dS $m^{-1}$), pH 7.12, organic matter of 0.96%, with initial potassium level of 193 mg $Kg^{-1}$ and phosphorus of 12.13 mg $kg^{-1}$. The soil has an optimum water holding capacity of 18% determined through the gravimetric method.

A pre-emergence pesticide containing S-metolachlor (dual gold magnum) treatment helped prevent weed growth. However, no pesticide application was necessary throughout the field trial due to the absence of significant pests. The field received periodic irrigation every 10 days using canal water to mitigate the effects of drought and heat stress on the crops. Enhancing soil fertility consisted of adding nitrogen, phosphorus (as $P_2O_5$), and potassium (as $K_2O$) at rates of 112, 80, and 50 kg $ha^{-1}$, respectively, as recommended by the Agriculture Department, Punjab, Pakistan. Field conditions' routine monitoring for the appearance of any diseases recorded as absent during the entire duration of the field trial. These meticulous cultivation practices ensured optimal growth conditions for the sunflower breeding material, facilitating accurate evaluation of their performance and disease resistance.

**Plants inoculation of charcoal rot race**

The plant material's inoculation with the charcoal rot race continued from the Sargodha Plant Fungal Lab. The collected pathogen came from the sunflower plant debris at the sunflower field of the College of Agriculture, University of Sargodha. Inoculation occurred at the base of the stem without causing any wounds, using a micro syringe with a concentration of 3 basidium $\mu L^{-1}$, 30 days after sowing. Additionally, repeating inoculation happened at the time of bud formation, utilizing a toothpick at the top of the succulent and young stem. Diligent observing of the plants went on daily, with symptoms recorded at the time of anthesis based on the severity of lesions appearing on the stem. Symptoms had the categories as follows: lodging (5), severe with no lodging (4), medium (3), minor (2), and no lesions (1) (Shehbaz *et al.*, 2018).

**Determination of morphological and yield components**

In the experimental setup, five plants bore tagging within a single row for their evaluation, by averaging the measurements of these five plants. Plant height measurement started from the base of the stem to the peduncle using a measuring tape, indicating the vertical growth of the plants. Head diameter determination began at the midpoint of the head with a measuring tape, with measurements provided in cm. This measurement offers insights into the size and development of the sunflower heads. Leaf area assessment used a leaf area meter, with the results expressed in $cm^2$. This measurement quantifies the total surface area of the leaves, providing information on leaf size and overall foliage development.

**Statistical analysis**

The data analysis employed the "R" program, with the split-plot design analyzed utilizing the "Agricolae" library. For the genetic analysis of the inoculation regime, the methodology outlined by Kempthorne (1957) was operational, as well as, the "ggbstat" library in the "R" program. The "Agricolae" library provides functions for various experimental designs, including split plot designs, allowing for robust statistical analysis of agricultural experiments. On the other hand, the "ggbstat" library facilitates genetic analysis, implementing methodologies proposed by Kempthorne (1957) to assess the genetic effects of experimental treatments.

## RESULTS AND DISCUSSION

Selected breeding lines sustained evaluation for infestation over two-year trials, as documented by Qamar *et al.* (2019a) and Qamar and Ghazanfar (2019b) and our research. Among these lines, 'B-224', 'B-12', 'B-124', 'B-112', and 'RSIN.82' exhibited minor infestation, indicating resistance to charcoal rot. Conversely, breeding lines 'RH.344' and 'RHA.459' displayed moderate resistance due to higher infestation levels, while 'RHA-456' showed severe infestation, indicating as susceptible (Table 1). Notably, breeding line 'B-224' had the topmost levels of antioxidant and chlorogenic acid, previously associated with resistance to charcoal rot. Inoculation led to elevated oxidation levels (Khan *et al*., 2017). Moreover, both 'RHA456' and 'B-224' exhibited the premiere tocopherol activity, which also possesses antioxidation properties (Rauf, 2019). Sterol contents were notably high in 'B-112'. A comparison between a resistant cultivar 'Fengkuiza No. 1' and a susceptible cultivar '7101' showed a marked increase in the antioxidant activity of enzyme, altered plant growth regulators, and increase in the chlorogenic acid in resistant cultivar when inoculated by the *Sclerotinia sclerotiorum* (Liu *et al*., 2017).

Charcoal rot poses a sizable threat to plant health and crop production, particularly in sunflower (*H. annuus*) and other crop species, amid the current scenario of global climate change. The disease is characteristic of distinctive symptoms, such as black lesions on the stem, leading to premature wilting and lodging of the plants (Shehbaz *et al.*, 2018). Susceptible accessions often exhibit severe disintegration and blackening of the stem due to the overgrowth of fungal components like mycelia, sclerotia, and pycnidia, resulting in

**Table 1.** Mean values of breeding lines for charcoal rot infestation and various biochemical traits in sunflower lines.

| Breeding lines | Charcoal infestation | Peroxide (m mol $kg^{-1}$) | Chlorogenic acid (µg $g^{-1}$) | Sterols (µg $g^{-1}$) | Tocopherol (µg $g^{-1}$) |
|---|---|---|---|---|---|
| B.208 | 2.03 ± 0.12 | 14.15 ± 0.78 | 51.77 ± 10.41 | 2446.19 ± 26.65 | 629.787 ± 13.15 |
| B.112 | 2.19 ± 0.22 | 13.93 ± 0.79 | 43.74 ± 3.98 | 2896.15 ± 55.58 | 361.823 ± 12.19 |
| B.124 | 2.12 ± 0.19 | 14.79 ± 0.15 | 30.65 ± 5.09 | 1612.70 ± 46.00 | 444.902 ± 10.21 |
| B.224 | 1.94 ± 0.16 | 16.58 ± 0.28 | 58.48 ± 4.15 | 2568.80 ± 62.25 | 897.271 ± 8.36 |
| RH.347 | 3.16 ± 0.14 | 12.37 ± 0.62 | 45.01 ± 4.44 | 1795.66 ± 33.99 | 376.149 ± 4.51 |
| RH.SIN.82 | 2.17 ± 0.09 | 14.83 ± 2.36 | 32.46 ± 5.65 | 1908.96 ± 50.51 | 408.453 ± 7.35 |
| RH.344 | 3.34 ± 0.11 | 12.07 ± 0.48 | 45.81 ± 5.03 | 918.81 ± 71.12 | 565.621 ± 8.12 |
| RHA.456 | 4.01 ± 0.13 | 6.43 ± 1.59 | 33.31 ± 2.50 | 1030.69 ± 131.46 | 1206.799 ± 29.13 |
| RHA.459 | 3.02 ± 0.12 | 12.93 ± 0.36 | 52.69 ± 1.53 | 1892.29 ± 51.46 | 997.086 ± 15.17 |

**Table 2.** Charcoal rot infestation in various single cross $F_1$ in sunflower.

| Lines | Testers | | | |
|---|---|---|---|---|
| | RH.344 | RH.456 | RH.459 | R.SIN.82 |
| C.112 | 2.33 c–e [1] | 3.33 a–d | 3.00 a–d | 1.67* e |
| C.124 | 2.67 a–e | 3.67 ab | 3.00 a–d | 2.33 c–e |
| C.208 | 2.07 de | 2.67 a–e | 3.83 a | 2.90 a–e |
| C.224 | 2.33 c–e | 3.51 a–c | 3.17 a–d | 2.50 b–e |

[1]Means followed by the same letter were not significant ($P > 0.05$), Lodging (5), severe with no lodging (4), medium (3), minor (2), and no lesions (1) (Shehbaz *et al.*, 2018).

**Table 3.** Heterosis and degree of dominance among various half sib offspring.

| Breeding lines | Heterosis | | | | Degree of dominance | | | |
|---|---|---|---|---|---|---|---|---|
| | RH.344 | RH.456 | RH.459 | R.SIN.82 | RH.344 | RH.456 | RH.459 | R.SIN.82 |
| C.112 | -15.73 | 7.42 | 15.16 | -23.39* | 0.76 | -0.25 | -0.96 | -51 |
| C.124 | -2.20 | 19.74 | 16.73 | 8.62 | 0.1 | -0.64 | -0.96 | -7.4 |
| C.208 | -22.91* | -11.59 | 51.68* | 38.10* | 0.94 | 0.35 | -2.63 | -11.43 |
| C.224 | -11.74 | 17.98 | 27.82 | 21.65 | 0.44 | -0.52 | -1.27 | -3.87 |

spacing and hollowing of the stem, whereas resistant types effectively restrict the growth of fungal cells (Siddique *et al.*, 2021). These findings underscore the magnitude of identifying and utilizing resistant breeding lines to mitigate the impact of charcoal rot on crop production.

Table 2 gives the mean values of half-sib offspring resulting from crosses between different parental lines. Among these combinations, the cross of 'C.112' × 'RSIN.82' exhibited the lowest infestation, thereby, suggesting resistance to charcoal rot. Additionally, combinations 'C.208' × 'RH.344' and 'C.224' × 'RH.344' displayed minor infestation following inoculation. However, cross combinations 'C.208' × 'RH.459' and 'C.112' × 'RH.456' displayed the highest scores post-inoculation and were illustrative with "severe" and "medium" symptoms, respectively, hence, indicating susceptibility and moderate tolerance (Table 2).

Genetic component estimation revealed a significant contribution of environmental variance to the total phenotypic variance. The heritability of charcoal resistance was low ($H^2 = 0.17$), with dominance estimates surpassing the additive variance. The selectable heritability was also low ($H^2 = 0.13$), which indicated the predominance of dominance effects in the $F_1$ generation. These findings underscore the complex genetic nature of charcoal rot resistance, with environmental factors playing a substantial role in determining phenotypic expression. Moreover, the low heritability implies the challenges associated with breeding for charcoal rot resistance and emphasizes the substance of incorporating dominance effects in breeding programs aimed at enhancing disease resistance in sunflower cultivars (Abdelsatar *et al.*, 2020). A proposed recurrent selection cycle may be effective for disease control and could enhance the resistance or performance of breeding lines under infestation.

In Table 3, cross combinations 'C.112' × 'RSIN.82' and 'C.208' × 'RH.344' exhibited significant negative heterosis, while combinations 'C.208' × 'RH.459' and 'C.208' × 'RSIN.82' displayed high positive heterosis. Cross combinations, showing negative heterosis, may be better options due to the presence of resistant alleles from the parental lines, thereby, contributing to lower infestation for charcoal rot resistance. The degree of dominance demonstrated significant deviation from the additive dominance model, with a high degree of over-dominance of resistance alleles observed for cross combinations 'C.112' × 'RSIN.82', 'C.208' × 'RSIN.82', 'C.124' × 'RSIN.82', and 'C.224' × 'RSIN.82'. Additionally, cross combinations 'C.112' ×

'RH.459' and 'C.124' × 'RH.459' showed complete dominance toward resistant parents, while C.208 × RH.344 showed complete dominance toward susceptible parents (Table 3). The gene action was dominance toward resistant parents, as indicated by the ratio for the degree of dominance. However, environmental effects significantly influenced phenotypic variance, thus, suggesting the need for confirmation of resistant genes through marker-assisted selection (MAS) and quantitative trait locus (QTL) mapping, as observed in soybeans (Ghorbanipour *et al.*, 2019). These approaches can help identify and validate key genes associated with charcoal rot resistance in sunflower, aiding in the development of more resilient cultivars.

The general combining ability (GCA) test revealed 'C.112', 'RH.344', and 'RSIN.82' exhibited negative combining ability (Table 4), thereby, suggesting they may carry resistant loci. These parental lines could be valuable resources for resistant breeding programs and the development of segregating populations aimed at enhancing charcoal rot resistance in sunflower. Furthermore, cross combinations 'C.208' × 'RH.344', 'C.208' × 'RH.456', 'C.124' × 'RH.459', and 'C.112' × 'RSIN.82' displayed negative specific combining ability effects. This result indicates these hybrids may be beneficial for exploiting heterosis, leading to improved performance for charcoal rot resistance. These findings highlight the potential of specific crossings in breeding programs focused on enhancing disease resistance and utilizing heterosis to develop high-performing sunflower hybrids. A two-step strategy may be operational, such as, selection of single genotypes showing resistance to the charcoal infestation for possible selection and further testing for their GCA. The genotype with resistance and good GCA could be options to establish segregating offspring for testing and selection (Rauf, 2019).

The leaf area under the inoculated regime ranged from 1.05 to 2.64 $cm^2$, while under the non-inoculated regime, it ranged from 1.12 to 2.44 $cm^2$. Overall, a decrease of 10.21% in stem diameter appeared when comparing between the stress and non-stress environments. When averaged across both, the parental line 'RH.447' exhibited the highest stem diameter (Table 5). 'C.124' × 'RSIN.82' also displayed the supreme stem diameter among the cross combinations (Table 5). These findings suggest both the parental line 'RH.447' and the cross combination 'C.124' × 'RSIN.82' possess favorable alleles contributing to increased stem diameter, which could be valuable for sunflower breeding programs enhancing plant vigor and resilience in stressful environments.

The chlorophyll contents ranged from 5.81 to 11.06 under the inoculated regime and from 8.13 to 11.35 under the uninoculated regime. Comparing the values of accessions under stress and non-stress regimes revealed an 11% decrease in chlorophyll contents. Among the cross combinations, 'C.112' × 'RH.447' exhibited the maximum chlorophyll contents, followed by 'C.224 × RSIN.82' and 'C.124 × RH.456' (Table 5). These findings signify 'C.112' × 'RH.447' and the other mentioned crosses possess genetic traits associated with higher chlorophyll content, indicating better photosynthetic efficiency and potential resilience to stress conditions (Markulj-Kulundžić *et al.*, 2023).

**Table 4.** Estimate of general combining ability (GCA) in female and male breeding lines of elite sunflower.

| Female | GCA | Male | GCA |
|---|---|---|---|
| C.112 | -0.23* | RH.344 | -0.46* |
| C.124 | 0.11 | RH.456 | 0.48* |
| C.208 | 0.06 | RH.459 | 0.44* |
| C.224 | 0.07 | RSIN.82 | -0.46* |
| C.D. | 0.38 | | |

**Table 5.** Mean values of hybrids averaged over contrasting inoculated regimes for traits; i.e., stem diameter (SD), chlorophyll contents (CC), head diameter (HD), leaf area (LA), plant height (PH), chlorogenic acid (CA), and peroxide value (PV).

| Accessions | SD (cm) | CC | HD | LA | PH | CA | PV |
|---|---|---|---|---|---|---|---|
| RH-447 | 2.34 a | 9.42 a–e | 10.83 fg | 281.74 bc | 112.17 hi | 22.09 ij | 12.13 c–e |
| C.124 × RSIN.82 | 2.31 a | 9.13 b-e | 12.67 ef | 249.47 cd | 120.08 e–g | 21.00 jk | 13.59 ab |
| C.124 × RH.344 | 2.09 b | 10.02 ab | 17.08 ab | 364.58 a | 134.00 b | 25.39 e–g | 11.29 d-h |
| C.224 × RH.459 | 1.99 bc | 8.71 b-e | 17.79 a | 174.18 fg | 141.83 a | 26.72 de | 8.92 j |
| C.224 × RH.344 | 1.97 b-d | 8.47 c–e | 10.25 g | 178.59 fg | 108.83 i | 27.48 cd | 9.45 ij |
| C.112 × RH.459 | 1.90 b-e | 10.22 ab | 13.18 de | 197.05 ef | 122.87 d-f | 32.97 a | 10.84 g–h |
| C.124 × RH.365 | 1.87 c-e | 8.73 b–e | 15.30 b–d | 174.26 fg | 143.22 a | 21.76 jk | 11.63 d–g |
| C.224 × RH.456 | 1.87 c-e | 8.89 b–e | 11.43 e–g | 250.23 cd | 118.67 e-h | 24.64 f–h | 10.84 f–h |
| C.124 × RH.459 | 1.78 de | 9.91 a–c | 11.83 e–g | 174.57 fg | 115.83 f-i | 22.64 h–j | 14.46 a |
| C.112 × RH.344 | 1.75 ef | 8.52 c–e | 15.30 b–d | 231.01 de | 99.33 j | 33.01 a | 10.27 hi |
| B-124 | 1.72 ef | 7.87 ef | 10.58 fg | 300.17 b | 123.08 d–f | 23.89 g–i | 11.96 c–f |
| C.124 × RH.456 | 1.58 fg | 9.58 a–d | 11.39 e–g | 201.63 ef | 142.83 a | 27.87 b–d | 10.62 g–i |
| C.112 × RSIN.82 | 1.46 gh | 6.97 f | 13.45 c–e | 248.96 cd | 129.05 b–d | 29.07 bc | 8.82 j |
| C.224 × RSIN.82 | 1.44 gh | 9.58 a–d | 15.47 b–d | 176.81 fg | 132.67bc | 29.53 b | 12.46 b–d |
| C.208 × RH.344 | 1.43 g-i | 9.24 a–e | 10.81 fg | 323.69 ab | 119.44 e–h | 26.44 d–f | 13.53 ab |
| C.208 × RH.456 | 1.38 hi | 9.00 b–e | 16.18 ab | 149.71 g | 121.78 d–f | 25.34 e–g | 11.20 e–h |
| C.208 × RH.459 | 1.32 hi | 9.00 b–e | 10.40 fg | 14271g | 90.42 k | 25.34 e–g | 12.15 c–e |
| C.208 × RSIN.82 | 1.32 hi | 8.89 b–e | 12.01 e–g | 193.33 ef | 118.33 e–h | 27.87 b–d | 11.24 e–h |
| B.208 | 1.24 ij | 8.40 d–f | 13.58 c–e | 201.04 ef | 113.58 g–i | 19.80 k | 12.99 bc |
| C.112 × RH.447 | 1.09 j | 10.54 a | 9.89 g | 87.56 h | 125.59 c–e | 29.70 b | 13.56 ab |

[1] Means followed by the same letter were not significant ($P > 0.05$).

The head diameter ranged from 8.56 to 17 cm under the inoculated regime and from 9.67 to 19.43 cm under the non-inoculated regime. Results showed a decrease of 16% in head diameter for all accessions under the inoculated regime. Among the combinations, 'C.224 × RH.459' (moderate infestation), followed by 'C.208 × RH.456' (minor infestation), exhibited the highest head diameter when averaged over both regimes (Table 5). These results indicate these combinations possess genetic traits associated with larger head diameters, which could be advantageous for sunflower breeding programs aimed at improving yield potential and overall plant vigor (Mohan and Seetharam, 2005; Kulkarni *et al*., 2015).

The leaf area varied from 85.40 to 487.89 $cm^2$ under the inoculated regime and from 89.73 to 365.60 $cm^2$ under the uninoculated regime. Largely, only a 5% decrease in leaf area emerged under the stress regime. Plant height ranged from 89.59 to 135.83 cm under the inoculated regime and from 91.26 to 154.44 cm under the uninoculated regime. A 9% decrease in plant height manifested due to inoculation with charcoal rot disease. Among the combinations, 'C.124' × 'RH.344' had the highest leaf area, while 'C.224' × 'RH.459' and 'C.124' × 'RH.365' had the maximum plant height (Table 5).

The chlorogenic acid content in sunflower accessions increased threefold due to the inoculation with charcoal rot disease. The range of chlorogenic acid content was 9.62–15.56 μg $g^{-1}$ under the uninoculated regime and 31.32–55.43 μg $g^{-1}$ under the inoculated regime. Chlorogenic acid may act as inhibitory components for the charcoal rot disease and its concentration has been evident to rise under disease infestation regime (Khan *et al.*, 2017; Liu *et al.*, 2017). Additionally, the peroxide contents in tissues ranged from 1.25 to 5.60 μg $g^{-1}$ under the uninoculated regime and from 15.87 to 26.16 μg $g^{-1}$ under the inoculated regime, giving a 9% increase in peroxide value in the inoculation regime. Among the combinations, 'C.112' × 'RH.459' exhibited the highest chlorogenic acid content and peroxide value, followed by 'C.124' × 'RH.459' and 'C.124' × 'RSIN.82' (Table 5).

**Table 6.** Genotypic correlation among traits under inoculated regime.

| Traits | SD | CC | HD | LA | PH | CA | PV | IN |
|---|---|---|---|---|---|---|---|---|
| SD | 1.00 ** | -0.17$^{NS}$ | 0.21$^{NS}$ | 0.36$^{NS}$ | 0.11$^{NS}$ | -0.22 | 0.01 | 0.11 $^{NS}$ |
| CC | -0.17 $^{NS}$ | 1.00$^{NS}$ | 0.08 $^{NS}$ | -0.12$^{NS}$ | 0.39 $^{NS}$ | 0.20 | 0.52 | 0.41 $^{NS}$ |
| HD | 0.17 $^{NS}$ | 0.09 $^{NS}$ | 1.00$^{**}$ | 0.30$^{NS}$ | 0.31 $^{NS}$ | 0.14 | -0.42 | -0.47 * |
| LA | 0.36 $^{NS}$ | -0.12 $^{NS}$ | 0.30 $^{NS}$ | 1.00* | 0.32 $^{NS}$ | -0.03 | -0.17 | -0.63 ** |
| PH | 0.11 $^{NS}$ | 0.39 $^{NS}$ | 0.31 $^{NS}$ | 0.32$^{NS}$ | 1.00 $^{NS}$ | -0.02 | 0..05 | -0.03 $^{NS}$ |
| CA | -0.22 $^{NS}$ | 0.20 $^{NS}$ | 0.14 $^{NS}$ | -0.03 $^{NS}$ | -0.02 $^{NS}$ | 1.00 ** | -0.41 $^{NS}$ | -0.28 $^{NS}$ |
| PV | 0.01 $^{NS}$ | 0.52* | -0.52* | -0.17 | -0.05 | -0.41 $^{NS}$ | 1.00 * | 0.51 $^{NS}$ |
| IN | 0.11 $^{NS}$ | 0.41$^{NS}$ | -0.47* | -0.63$^{*}$ | -0.03 $^{NS}$ | -0.28 $^{NS}$ | 0.51 * | 1.00 ** |

The significance ($P \leq 0.05$) of genotypic correlation testing used t test (two-tail) and the number of genotypes – 2 as degrees of freedom. Stem diameter (SD), Chlorophyll contents (CC), Head diameter (HD), Leaf area (LA), Plant height (PH), Chlorogenic acid (CA), Peroxide value (PV), and Infection intensity (IN).

The molecular basis of resistance against charcoal rot suggests resistant breeding lines present higher activity of chlorogenic acid, which may act as an inhibitory compound in the leaves and stem, thereby, potentially serving as an antifungal agent within resistant breeding lines. Research has also shown inoculation of disease increases oxidation, and resistant lines display higher activity of antioxidants, such as peroxidase and lipase (Khan *et al.*, 2017; Liu *et al.*, 2017).

Table 6 reveals a significant negative genotypic correlation between infection intensity and head diameter, as well as, leaf area. Conversely, a positive relationship was evident between infection intensity and peroxide value. These correlation analyses suggest accessions exhibiting higher infection intensity tend to have smaller head diameters and leaf areas. Additionally, accessions with higher infection intensity also display elevated peroxide values. This implies as the severity of infection increases, sunflower plants may experience reduced growth for head diameter and leaf area, while concurrently experiencing heightened oxidative stress as indicated by increased peroxide levels. These findings underscore the detrimental impact of charcoal rot infection on sunflower plant morphology and physiology, highlighting the relevance of developing resistant cultivars to mitigate such effects and enhance overall plant health and productivity (Saif *et al*., 2023).

## CONCLUSIONS

The presented study's design sought to develop the sunflower (*H. annuus*) hybrids with charcoal rot resistance. The hybrids 'C.112' × 'RSIN.82' and 'C.208' × 'RH.344', with minor infestation, showed significant negative heterosis and complete dominance toward resistant breeding lines. Breeding lines 'B-112', 'B-208', and 'RSIN.82' had significant GCA effects and should continue usage in the sunflower breeding program. Morphological data under an inoculated regime showed it caused about a 16% reduction in the head diameter, a 5% decrease in stem diameter, and an 11% shrinkage in leaf area, with a 9-fold increase in peroxide and a 3-fold increase in chlorogenic acid. Moreover, resistant lines demonstrated lower losses regarding morphological traits, whereas susceptible genotypes showed reduced values.

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