RESEARCH ARTICLE

Genetic and phyto-morphological diversity analysis in the advanced breeding lines of chickpea (*Cicer arietinum* L.) for pod borer resistance

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Abstract

The pod borer *Helicoverpa armigera* is a major constraint to chickpea (*Cicer arietinum* L.) production worldwide, affecting the crop most severely during the pod formation stage, leading to reduced crop yield by approximately upto 90 to 95%. The present study was conducted to find characteristics associated with resistance to pod borer infestation by analyzing several morphological and phytochemical attributes in 200 advanced breeding lines of chickpeas and four checks. ANOVA elucidated the presence of significant differences among genotypes for all quantitative as well as qualitative traits. The number of pods per plant, plant height, total antioxidant activity and presence of trichomes were identified as key contributors to resistance against pod borer. Cluster analysis based on Euclidean Distance revealed the categorization of genotypes into distinct groups based on their traits, recommending the specific groups for targeted breeding efforts. The study also emphasized the significance of phytochemical features in improving resistance to pod borer, such as trichome density, flavonoid concentration and tannin content. The present findings advocate exploring the wild progenitors and advanced progeny that may help in increasing the diversity among cultivated chickpeas and help in developing resistant varieties in the future.

Keywords: Correlation, chickpea, diversity, pod borer, phytochemical, variability.

Introduction

The chickpea (*Cicer arietinum* L.) is an important legume that has been cultivated and consumed in various regions of the world since ancient times. It is a self-pollinating diploid (2n = 16) crop belonging to the Fabaceae family. Due to its superior nutrition and health advantages, it is currently being grown in 57 countries across the continents (Merga and Haji 2019). India produced 13.75 mt of chickpeas in 2021–2022 (fourth estimate), using 10.91 million ha of land and 12.6 q/ha of productivity (Indian Institute of Pulses Research, n.d.). Even though it is the world's largest producer of chickpeas, India has had to rely on other countries to meet demand. This dependence can be linked to the long-term, continuous farming of the same chickpea cultivars in the same geographic area. As a result, this technique has increased the vulnerability of chickpea crops to several abiotic stresses, diseases, and pests (Whitehead et al. 2017). One particularly destructive pest, the pod borer (*Helicoverpa armigera*), has caused severe yield losses for farmers worldwide, ranging up to 80 to 95% or complete losses if the infection occurred during the blooming or pod formation stage (Rahman 1989). This polyphagous pest significantly affects the field and horticultural crops of more than 82 families (Sarwar et al. 2013). A typical adult female moth lays around 500 to 1000 eggs (Mironidis and Savopoulou-Soultani 2014), contributing to an estimated economical loss exceeding \$2 billion USD throughout the world annually. According to Dhaliwal et al. (2010) crop devastation in India is caused by pod borer ranging from 40 to 95% and yield losses of up to 400 kg/ha, respectively. This causes an annual loss of more than Rs. 35,000 million. Currently, there is a lack of chickpea cultivar(s) that are resistant to pod borers. Several studies have demonstrated the capacity of wild *Cicer* progenitors to cope well with a range of biotic and abiotic stresses (Warschefsky et al. 2014). Exploring wild relatives might

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How to cite this article: Lagoriya D.S., Saharia N., Sharmah B.K. and Sarma R.N. 2024. Genetic and phyto-morphological diversity analysis in the advanced breeding lines of chickpea for pod borer resistance. Indian J. Genet. Plant Breed., **84**(3): 362-373.

Source of support: Department of Biotechnology, Govt. of India **Conflict of interest:** None.

Received: Sept. 2023 **Revised:** May 2024 **Accepted:** June 2024

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reveal important phyto-morphological characteristics that may be helpful in breeding for pod borer resistance (Von Wettberg et al. 2018) to be incorporated in suitable chickpea genetic background.

Phyto-morphological characteristics, including plant height, leaf dimensions and morphology, flower pigmentation, pod shape, pod size, thickness, and foliage color, as well as trichome size and density on the pod wall, collectively contribute to the resistance or susceptibility of plant variety from the pod borer damage (Vanambathina et al. 2021; Awol et al. 2018; Sreelatha et al. 2018; Sharma et al. 2009). Furthermore, glandular trichomes offer a combination of structural and chemical defensive mechanisms by releasing secondary metabolites such as flavonoids, terpenoids, and alkaloids. These compounds have the ability to intoxicate, deter, or capture insects and other organisms. (Golla et al. 2020; War et al. 2012).

The development of new chickpea varieties with enhanced resistance to pod borer damage will require a detailed understanding of the variability with regards to above mentioned traits conferring resistance among chickpea germplasm but studies on genetic variability among chickpeas lines derived from wild sources are limited. Therefore, the present study was undertaken to identify the nature of genetic diversity in advanced breeding lines having useful traits from wild relatives of chickpeas for diverse phyto-morphological features conferring resistance against pod borer.

Materials and methods

Experimental material

A total of 200 F_{s} and F_{6} breeding lines (genotypes) and four checks were used in the present study. These genotypes were developed by crossing of cultivated parents (ICCV 96029, Counsul, Habru, and Minjar) with their wild relatives (Sirna 60, Karab 81 and Karab 92) at the University of California, Davis, USA, and obtained by the help of NBPGR New Delhi (Supplementary Table S1). To understand the morphology and phytochemical aspects, these genotypes were categorized based on their pedigree information, *viz.* Group A (*ICCV 96029 X Karab_81),* group B (*ICCV 96029 X Sirna_60*)*,* group C (*Habru X Sirna_60*), group D (*ICCV 96029 X Karab_92*)*,* group E (*Minjar X Sirna_60*), and group F (*Consul X Sirna_60*). The evaluation of genotypes was done in the net house of DBT-NECAB, Assam Agricultural University, Jorhat by sowing seeds in polythene bags filled with uniform potting material *viz*. soil, cow dung and vermicompost in the ratio of 2:1:1 during 2021-22. The experiment was conducted in two replications with two pots for each genotype. Data on quantitative traits *viz.,* days to first flowering (DAF), plant Height (PH), number of primary branches (NPB), number of pods per plant (NPP), seed test weight (STW), grain weight (GrW) were collected

along with some biochemical parameters as well *viz.,* total phenolic content (TPC), flavonoid (FLAV), tannin (TANN), total antioxidant capacity (TAA), protein content (PC), α-amylase inhibition percent (AI%) and trypsin inhibition percent (TI%). The 30 days plants were used for conducting whole plantinsect bioassay as well as detached leaf bioassay inside the laboratory conditions and data on glandular trichome (GT), non-glandular trichome (NGT), whole Plant Assay larval weight (WLW), whole plant assay plant damage (WPD), detached leaf larval weight (DLW) and detached leaf plant damage (DLW) were obtained.

Whole plant insect bioassay

The whole-plant insect bioassay was done in two replications inside the net house with plants by releasing three first instar larvae to 30 days plant as described by Sharma et al. (2005). The plant was monitored and kept in the net house until the insect completely damaged the susceptible genotypes. The larvae were weighed and the leaf damage percentage was calculated visually.

Detached leaf insect bioassay

A detached leaf insect bioassay was conducted under laboratory conditions using rearing trays and the trays were kept in a completely randomized manner with two replications of leaf samples (Sharma *et al.*, 2005). Visible damage system of each sample was recorded by counting the number of damaged and undamaged leaflets, and the percent damage was recorded according to the scale 1 to 9 $(1 = 10\%$ and $9 = 90\%$ leaf area/ pod damaged) from the three replications (Sharma, 2005).

Determination of trichome density

With a few minor adjustments, the technique of Talebi et al. (2018) was used to measure the density of leaf trichomes. The cut leaves were dipped into FAA (formalin 7.5%, acetic acid 7.5%, and ethanol 85%) solution for 48 hrs followed by bleaching with 5% NaOCl, washing and dehydrating. The samples were then stained with the carmine dye and number of trichomes on the leaf surface was counted under the microscope from three leaf sections (base, middle and apex).

Phytochemical analysis of genotypes against pod borer

The collected leaf sample were homogenized using a MagNAlyser (Roche) and then extracted with 95% methanol (Ainsworth and Gillespie 2007) for estimation of phenol, flavonoid, tannin and total antioxidant activity (TAA). A separate extract from the collected leaf sample of 50 mg and protein extraction buffer (10 mM tris HCl, 500 mM NaCl, Triton-X 100, and 1% -mercapto ethanol) was used to estimate protein, α-amylase, and trypsin inhibition (Gupta et al. 2014).

The total phenolic content in the extract was assessed using

the method described by Ainsworth and Gillespie (2007) with slight modifications. The 100µl extract was put to Eppendorf tubes with 200 µL Folin-Ciocalteu reagent with an addition of 800 µL of Na2CO3. A Varioskan LUX microplate reader was used to record absorbance at 765 nm after 1-hour of incubation at room temperature. Gallic acid (1-mg/mL) was used to calculate the total phenolic compound using methanol as a blank. The total flavonoid content of the extract was determined by spectrophotometry following the method essentially described by Chang et al. (2002). The total tannin content was estimated following the method of Attarde et al*.* (2010) and the total antioxidant content was estimated based on the phospho-molybdenum assay as described by Prieto et al*.* (1999). The protein content was estimated by using the Bradford method (1976). The α-amylase inhibitory assay was carried out by following the standard method and the trypsin inhibition percentage was measured according to Kakade et al. (1969).

Statistical analysis

Out of 204 genotypes, replicated mean data of 202 genotypes for nineteen quantitative traits were used for analysis of variance (ANOVA) using Fisher's method (Panse and Sukhatme 1985). R-package "variability" in R version 3.6.3 was used for analysing the genetic variability parameters (Popat et al. 2020). The genetic diversity analysis based on $D²$ statistics was done according to the methodology given by Mahalanobis (1936) with the R packages Metan (Olivoto and Lucio 2020) and Biotools (da Silva et al. 2017). The unweighted pair group method of the average linkage (UPGMA) was used to classify the 202 accessions into groups (clusters). A circular dendrogram was created using the Circlize package visualization of hierarchical clustering trees (Galili 2015; Gu et al. 2014).

Results and discussion

Analysis of variance and genetic variability

Out of 204 genotypes, two genotypes failed to establish themselves in pot and were rejected. A significant difference was observed for all the morpho-phytochemical traits, indicating sufficient genetic variation among 202 genotypes of chickpea (Table 1) and the potential of these lines to exploit in breeding for pod borer resistance. Box plots (Figs 1a to 1p) illustrate how traits vary within and between groups. The genotypes of group A were early in flowering with an average of 72.15 days, but genotype G91 from group B was earliest for first flowering with 40 days. The lowest average height of 123.41 cm was observed in the B group and the genotypes of group E had the highest average height of 162.54 cm. The mean number of branches per plant was comparable across the groups. The genotypes of group B had the highest number of pods per plant, with an average of 57.56. The genotypes of group A had the highest average seed test weight of 17.54 g, and the highest average seed yield per plant of 17.91 g was noticed in group C. The genotypes of group D showed the highest total phenol content of an average of 0.16 mg GAE/100 mg fresh weight. The maximum average of 0.165 mg of flavonoid content in terms of quercetin/100 mg fresh weight was observed in group A. Amongst groups, genotypes of group D had a maximum average of 0.608 mg tannin content. Group D genotypes exhibited a higher average of 14.85 mg total antioxidant activity. Though there is not many differences in protein content among the groups, genotypes of group C showed a maximum average protein content of 0.555 mg/100 mg fresh weight. The genotypes of groups A and E recorded higher average percent of alpha-amylase inhibition of 4.44 and 4.43%, respectively. The maximum trypsin inhibitor was observed from group A, with a mean of 6.20%. The highest mean of 17.37 glandular trichomes was recorded in group D, while the genotypes of group C had a greater mean of 26 non-glandular trichomes. In detected leaf insect assay (DLA) the genotypes of group E had the highest average per cent damage of 69.44%, while that of group F had the lowest average percent damage of 49.15%. In whole plant insect bioassay WPA) out of the six groups, group C had the highest average percent damage of 74.47% and group A had the lowest average percent damage of 47.70.

The phenotypic co-efficient of Variation (PCV) was greater than the genotypic co-efficient of Variation (GCV) for all traits under study, indicating the influence of environment warranting a more replicated evaluation of these traits (Table 2). The GCV and PCV values ranged from 1.21 to 39.73 and 1.62 to 96.50, respectively. In support of variability parameters for the selection of important traits, similar reports are also reported (Chandana et al. 2023). The breeding programme might face inconsistency in the selection of certain traits, as evidenced by the low estimates of GCV and PCV observed for a few traits (Nikita and Lal 2022; Yaqoob et al. 2010).

In the present study, higher heritability was recorded for the number of primary branches (h^2 = 97.32%) followed by glandular trichome (*h2 =* 96.57%), days to first flowering (*h2 =* 95.82%), α-amylase inhibition% (*h2 =* 95.57) and the tannin content (h^2 =95.51). Because of their higher heritability, these traits could be used to develop chickpeas that may show resistant to pod borer (Khumukcham et al. 2022). The breeder can employ these traits to develop resistant varieties of chickpeas against pod borer. The percentage of trypsin inhibitors and the number of glandular trichomes have been used against pod borer as reported earlier (Handley et al. 2005; Brar and Singh 2017; Golla et al. 2018b).

Correlation and path analyses

The genotypic correlation coefficient was found to be higher than the phenotypic correlation coefficient (Table 3). Traits showing positive associations with grain yield were number of pods per plant, plant height, and total antioxidant activity. Meena et al. (2021) estimated the genotypic coefficient of correlation over the phenotypic correlation coefficient, indicated an inherent association among the traits. The prominent number of pods and plant height greatly influence grain production in plants. The present analysis reflected that the correlations among traits were complex, warranting path coefficient analysis.

The results of path coefficient analysis based on genotypic correlation are presented in Table 4. The highest

Fig. 1a. Box plots displaying chickpea genotypes for the days of first flowering in parental groups Fig. 1b. Box plots displaying chickpea genotypes for plant height

Fig. 1c. Box plots displaying chickpea genotypes for the number of primary branches

Fig. 1e. Box plots displaying chickpea genotypes for the seed test weight

positive direct effect was observed for the number of pods per plant on grain yield. Plant height and total antioxidant capacity showed maximum positive indirect effect on yield *via* the number of pods per plant. The indirect effects of flavonoid content, glandular trichome and non-glandular trichome *via* other traits were low, but the positive association could account the positive indirect effect of these traits on yield. Aarif et al. (2014) also reported a significant positive interrelation between pods per plant and seed yield per plant. The low residual effect (0.057) implies that the causative traits of pod borer resistance explained about 99% of the variability for yield per plant.

Fig. 1d. Box plots displaying chickpea genotypes for the number of pods

Fig. 1f. Box plots displaying chickpea genotypes for the grain weight

Fig. 1g. Box plots displaying chickpea genotypes for the total phenolic content

Fig. 1i. Box plots displaying chickpea genotypes for the tannin content

Fig. 1k. Box plots displaying chickpea genotypes for the protein content

ig. 1m. Box plots displaying chickpea genotypes for the trypsin inhibition %

Fig. 1h. Box plots displaying chickpea genotypes for the flavonoid content

Fig. 1j. Box plots displaying chickpea genotypes for the total antioxidant content

Fig. 1l. Box plots displaying chickpea genotypes for the α- amylase inhibition %

Fig. 1n. Box plots displaying chickpea genotypes for glandular (light green), non-glandular (Blue) trichome density

Fig. 1o. Box plots displaying chickpea genotypes for the detached leaf insect bioassay

Fig 1p. Box plots displaying chickpea genotypes for the whole plant insect bioassay insect

Genetic diversity analysis

The results of MANOVA based on Wilk's (1946) criteria revealed a significant difference among the genotypes, providing room for the diversity analysis. Nineteen quantitative characters of 202 genotypes were used to determine diversity using Mahalanobis distance (pairwise) among all the genotypes. A maximum distance of 12.88 between genotypes G139 and G26 indicated maximum diversity between two genotypes for many traits; whereas the minimum distance of 3.42 between G55 and G2 indicated a close proximity between these two genotypes. Overall, the average Mahalanobis distance between the genotypes was recorded to be 6.04. Using quantitative trait analysis, the relationship between all genotypes and their clustering pattern was graphically depicted in Fig 2. The 202 chickpea genotypes were grouped into four clusters. Different researchers have reported that the different set of materials, which was used in their analysis, was grouped into different clusters. Temesgen et al*.* (2015) evaluated the genetic diversity of 49 Kabuli chickpea genotypes categorized into eight genetic divergence classes, whereas the study carried out by Aarif et al. (2017) using D^2 divergence analysis of 22 genotypes grouped into three clusters and Thakur et al. (2018) categorized 100 genotypes into 12 clusters. These studies indicated that wild progenitors of chickpea possesses a tremendous amount of genetic diversity for utilization in the improvement of chickpeas.

The cluster composition has been provided below in Table 5. These clusters were further analyzed based on the mean performance of all the quantitative traits and results presented in Table 6. Cluster 1 is the largest, comprising of 108 genotypes, followed by cluster 3 with 13 genotypes and cluster 2 with 8 genotypes. Cluster 4 is a solitary cluster comprising only one genotype, which had late flowering genotypes (139 days) whereas cluster 1 had genotypes with early flowering (78.86 days) ability. The tallest (129.11 cm) height genotypes were grouped in cluster 1 compared to genotypes of cluster 4 (57.50 cm) with a higher number of primary branches (3 per plant). Cluster 2 had an average of 2.50 primary branches per plant. The maximum number of pods per plant (58.63) was recorded from cluster 2 and the minimum number of pods/plants (24) was recorded in cluster 4. The minimum seed test weight (15.49 g) was recorded from cluster 2, whereas the maximum seed test weight (17.55 g) was from cluster 3. A higher grain yield of 17.67g was recorded from cluster 2 and a lower grain yield of 7.62 g was recorded from cluster 4. Total antioxidant activity was recorded to be higher (14.71 mg/100 mg of fresh weight) in cluster 3 and the lower antioxidant activity of 10 mg/100 mg fresh weight from cluster 4. The maximum protein content of 0.56 mg/100 mg of fresh weight was recorded from cluster 2 and the minimum content of 0.52 mg/100 mg of fresh weight from cluster 3. There was a diminutive difference in the content of total phenolic content, the flavonoid content, the tannin content, the α-amylase inhibition% and the trypsin inhibition% in different clusters. However, a noteworthy difference was noted in the number of trichomes based on the cluster analysis. The highest glandular trichomes were noticed from cluster 2; the maximum non-glandular trichomes was recorded from cluster 4 . Cluster analysis of whole plant insect bioassay revealed that cluster 4 has

Fig. 2. Circular dendrogram depicting genetic diversity of 202 chickpea genotypes based on the quantitative traits

less percent damage 53.95% with a higher larval weight of 95.85 mg while cluster 2 had a maximum percent damage (71.91%) with 70.06 mg of larval weight. The data of detached leaf bioassay showed cluster 4 has less percent damage (45.15% with 31.82 mg of larval weight) and higher, *i.e.,* 54.69% damage was recorded from cluster 2 along with higher 44.52 mg of larval weight.

Relative contribution of different quantitative traits towards divergence

The highest contribution towards divergence in terms of Singh's (1981) criteria was noticed from the number of pods per plant (21.05%), which also showed high variability and heritability, followed by protein content (12.75%) with low variability and with high heritability. Likewise, α-amylase inhibition contributed 10.50% with a low variability and high heritability (Fig. 3). Though contribution from phytochemical was recorded to be lesser than morphological traits, they showed a high heritability offering scope of their use in breeding for plant resistance against pod borer. Saeed et al. (2011) reported weight of 100 seeds contributed to genetic divergence, followed by pods per plant, protein content and primary branching. It was observed that three traits such as 100 seed weight, number of pods per plant, and days to 50% flowering, contributed the most to genetic divergence across 51 genotypes of chickpeas.

The current experiment demonstrated that the population of chickpeas developed by combining several genotypes was successful in creating adequate genetic diversity for breeders to utilize in selecting insect-resistant lines with suitable agronomic traits. The findings shed light into the intricate, multifaceted interplay

Table 5. Clusters for quantitative trait performance for 202 genotypes of chickpea

Parental groups Cluster 1 Cluster 2 Cluster 3 Cluster 4				
Α	16			
B	76	3	4	
	10	3		
	42	\mathcal{P}		
F	5			
F	27	٠		
Checks	4	-		

manipulation, marker-assisted selection, and breeding in chickpeas. The study also highlighted the importance of phytochemical features like trichome density and flavonoid concentration in improving resistance. This research aims to increase chickpea diversity and develop resistant varieties.

Supplimentary material

Supplimentary Table S1 can be accessed at www.isgpb.org.

Author's contribution

Conceptualization of research (BKS, RNS, DKL); Designing of the experiments (DKL, RNS); Contribution of experimental

Table 6. Cluster wise means data of quantitative traits for the 202 genotypes of chickpea

Fig. 3. Percentage contributing traits to the genetic divergence in the 202 genotypes of chickpeas

between genetic attributes, morphological characteristics and biochemical composition. The heritability analysis revealed key traits with substantial heritable potential, such as the number of primary branches, glandular trichomes, days to first flowering, α-amylase inhibition, and tannin content. These characteristics hold great potential for creating chickpea varieties that are naturally resistant to the pod borer, providing an effective means to increase crop resilience. This population might be the best one for mapping insect resistance genes using an association mapping approach to identify novel resistance genes. This could open the door for their exploitation in transgenic

materials (BKS, DKL); Execution of field/lab experiments and data collection (DKL); Analysis of data and interpretation (RNS, DKL); Preparation of the manuscript (NS, DKL).

Acknowledgment

The authors are thankful to Dr. Douglas R. Cook, Professor of Plant Pathology UC Davis, USA, for providing the seed materials. The authors also express their gratitude to the Department of Biotechnology, Govt of India, for funding the research works and Ph.D. fellowship to the first author through DBT-North East Center for Agricultural Biotechnology, AAU.

References

- Aarif M., Rastogi N.K., Johnson P.L. and Chandrakar P.K. 2014. Genetic analysis of seed yield and its attributing traits in kabuli chickpea (*Cicer arietinum* L.). J. Food Legumes, **27**(1): 24-27.
- Aarif M., Rastogi N.K., Johnson P.L. and Yadav S.K. 2017. Genetic divergence analysis in kabuli chickpea (*Cicer arietinum* L.). J. Pharm. Phytochem*.*, **6**(4): 1775-1778.
- Ainsworth E.A. and Gillespie K.M. 2007. Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin–Ciocalteu reagent. Nature Protocols*,* **2**(4): 875-877
- Attarde D.L., Patil M.B., Chaudhari B.J. and Pal S.C. 2010. Estimation of tannin content in some marketed Hardechurna (*Terminalia chebula* Retz. family-Combretaceae). Int. J. Pharmacy and Technol., **2**(3): 750-756.
- Awol M. 2018. Characterization and Assessment of Genetic Diversity for Agro-Morphological Traits of Ethiopian Chickpea (*Cicer arietinum* L.) Landraces. Uganda J. Agril. Sci., **18**(1): 1-13.
- Bradford M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochem., **72**(1-2): 248-254
- Chandana B. S., Mahto R. K., Singh R. K., Singh K. K., Kushwah S., Lavanya G. R. and Kumar R. 2023. Unclenching the potentials of global core germplasm for root nodulation traits for increased biological nitrogen fixation and productivity in chickpea (*Cicer arietinum* L.). Indian J. Genet. Pl. Breed., **83**(04): 526-534.
- Chang C.C., Yang M.H., Wen H.M. and Chern J.C. 2002. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J. Food and Drug Anal., **10**(3): 178-182.
- Da Silva A.R., Malafaia G. and Menezes I.P.P. 2017. Biotools: an R function to predict spatial gene diversity via an individualbased approach. Genetics and Molecular Res., **16**(2): 1-6.
- Dhaliwal G.S., Jindal V. and Dhawan A.K. 20. Insect pest problems and crop losses: changing trends. Indian J. Ecol.*,* **37**(1): 1-7.
- Galili T. 2015. Dendextend: An R package for visualizing, adjusting and comparing trees of hierarchical clustering. Bioinformatics, **31**(22): 3718-3720.
- Golla S.K., Rajasekhar P., Sharma S.P., Hari Prasad K.V. and Sharma H.C. 2018b. Antixenosis and antibiosis mechanisms of resistance to pod borer, *Helicoverpa armigera* in wild relatives of chickpea, *Cicer arietinum.* Euphytica, **214**(5): 1-16
- Golla S.K., Sharma H.C., Rajasekhar P., Mishra S.P. and Jaba J. 2020. Biochemical components of wild relatives of chickpea confer resistance to pod borer, *Helicoverpa armigera*. Arthropod Plant Interact*.*, **14**(5): 623-639
- Gu Z., Gu L., Eils R., Schlesner M. and Brors B. 2014. Circlize implements and enhances circular visualization in R. Bioinformatics, **30**(19): 2811-2812.
- Gupta M., Sharma P. and Nath A.K. 2014. Purification of a novel α-amylase inhibitor from local Himalayan bean (*Phaseolus vulgaris*) seeds with activity towards bruchid pests and human salivary amylase. J. Food Sci. Ttechnol., **51**(7): 1286- 1293.
- Hammer, K. (1984). Das domestications syndrom. *Die Kulturpflanze*, **32**(1): 11-34.
- Handley R., Ekbom B. and Agren J. 2005. Variation in trichome density and resistance against a specialist insect herbivore

in natural populations of *Arabidopsis thaliana*. Ecological Entomol.*,* **30**(3): 284-292.

- Hasan M.T. and Deb A.C. 2013. Inheritance study of flower colour in chickpea (*Cicer arietinum* L.). Indian J. Agric. Res., **47**(5): 445-448.
- Indiastat agri. 2022. Economic-survey-2020-2021-part-I, statistics and growth figures year-wise of g-20– Indiastat. Retrieved September 12, 2022, from https://www.indiastatagri.com/g-20-state/data/economy/economic-survey-2020-2021-part-i.
- Indian Institute of Pulses Research. (n.d.). https://iipr.icar.gov.in/ chickpea-crop/
- Kakade M.L., Simons N. and Liener I.E. 1969. An evaluation of natural vs synthetic substrates for measuring the antitryptic activity of soybean samples. Cereal Chem., **46**: 518–528.
- Kassambara A. and Mundt F. 2020. Factoextra: extract and visualize the results of multivariate data analyses. R package version Daria Alfimova 220 1.0.7. Available at: https://CRAN.R-project. org/package=factoextra (accessed on 03.07.2022).
- Kumar R., Yadav R., Soi S., Srinivasan S., Yadav S. S., Yadav A., Mishra J. P., Mittal N., Yadav N. and Kumar A. 2017. Morpho-molecular characterization of landraces/wild genotypes of *Cicer* for biotic/abiotic stresses*.* Legume Res.*,* **40**(6): 974-984.
- Le S., Josse J. and Husson F. 2008. FactoMineR: An R package for multivariate analysis. J. Statistical software, **25**(1): 1-18
- Mahalanobis P.C. 1936. On the generalized distance in statistics. National Institute of Science of India, 2, **49-55**
- Merga B. and Haji J. 2019. Economic importance of chickpea: Production, value, and world trade. Cogent Food & Agricul., **5**(1): 1615718.
- Mironidis G.K. and Savopoulou-Soultani M. 2014. Development, survivorship, and reproduction of *Helicoverpa armigera* (Lepidoptera: Noctuidae) under constant and alternating temperatures. Environmental Entomol., **37**(1): 16-28.
- Olivoto T. and Lúcio A.D.C. 2020. Metan: An R package for multi‐ environment trial analysis. Methods in Ecology and Evol., **11**(6): 783-789.
- Panse V.G. and Sukhatme P.V. 1985. Statistical methods for agricultural workers. ICAR Publication (2nd Ed.), New Delhi.
- Pogue M.G. 2004. A new synonym of *Helicoverpa zea* (Boddie) and differentiation of adult males of *H. zea* and *H. armigera* (Hübner) (Lepidoptera: Noctuidae: Heliothinae). Annals Entomological Society of America, **97**(6): 1222-1226.
- Popat R., Patel R. and Parmar D. 2020. Variability: Genetic Variability Analysis for Plant Breeding Research. R package version 0.1.0
- Prieto P., Pineda M. and Aguilar M. 1999. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. Analytical Biochem., **269**(2): 337-341.
- Pundir R.P.S., Mengesha M.H. and Reddy K.N. 1990. Leaf types and their genetics in chickpea (*Cicer arietinum* L.). Euphytica, **45**(3): 197-200.
- Pundir R.P.S., Rao N.K. and Van den Maesen L.J.G. 1985. Distribution of qualitative traits in the world germplasm of chickpea (*Cicer arietinum* L.). Euphytica, **34**(3): 697-703.
- Rahman M. M. 1989. Control measures for important insect pests of major pulses. In: Advances in Pulses Research in Bangladesh. Proceedings of the Second National Workshop on Pulses (pp. 6-8).
- Saeed A., Hovsepyan H., Darvishzadeh R., Imtiaz M., Panguluri S.K. and Nazaryan R. 2011. Genetic diversity of Iranian accessions,

improved lines of chickpea (*Cicer arietinum* L.) and their wild relatives by using simple sequence repeats. Pl. Mol. Biol. Reporter, **29**(4): 848-858.

- Sharma H. C., Stevenson P. C. and Gowda C. L. L. (2005). Heliothis/ Helicoverpa management: Emerging trends and prospects for future research. *Heliothis/Helicoverpa* Management Emerging Trends and Strategies for Future Research; Oxford & IBH Publishing Co. Pvt. Ltd.: New Delhi, India, **453-461**.
- Sharma H.C., Pampapathy G., Dhillon M.K. and Ridsdill-Smith J.T. 2005. Detached leaf assay to screen for host plant resistance to *Helicoverpa armigera*. J. Econ. Entomol., **98**(2): 568-576.
- Sharma H.C., Sujana G. and Rao D.M. 2009. Morphological and chemical components of resistance to pod borer, *Helicoverpa armigera* in wild relatives of pigeonpea. Arthropod-Plant Interact.*,* **3**(3): 151-161
- Singh A. K. 2020. Early History of Crop Presence/Introduction into India: VI. African and West and Central Asian Leguminous Crops. Asian Agri-History, **24**(1).
- Singh F. and Diwakar B. 1995. Chickpea Botany and Production Practices. Skill Development Series, 16, 8-9.Singh, D. (1981). The relative importance of characters affecting genetic divergence. Indian J. Genet. Pl. Breed., **41**: 237-245.
- Talebi S.M., Mahdiyeh M., Nohooji M.G. and Akhani M. 2018. Analysis of trichome morphology and density in *Salvia nemorosa* L.(Lamiaceae) of Iran. Botanica, **24**(1): 49-58.
- Temesgen A., Mandefro N. and Habtamu Z. 2015. Genetic divergence study among Kabuli chickpea (*Cicer arietinum* L.) genotypes. Scholarly J. Agric. Sci., **5**(5): 183-188.
- Upadhyaya H.D., Gowda C.L.L., Buhariwalla H.K. and Crouch J.H. 2006. Efficient use of crop germplasm resources: identifying

useful germplasm for crop improvement through core and mini-core collections and molecular marker approaches. Pl. Genet. Res., **4**(1): 25-35.

- Vanambathina P., Rachaputi R.C., Sultanbawa Y., Phan A.D.T., Henry R.J. and Brier H. 2021. Biochemical basis of resistance to pod borer (*Helicoverpa armigera*) in Australian wild relatives of pigeonpea. Legume Sci., **3**(4): e101.
- Von Wettberg E. J., Chang P. L., Başdemir F., Carrasquila-Garcia N., Korbu L. B., Moenga S. M. and Cordeiro M. A. 2018. Ecology and genomics of an important crop wild relative as a prelude to agricultural innovation. Nature Commun., **9**(1): 1-13.
- War A.R., Paulraj M.G., Hussain B., Buhroo A.A., Ignacimuthu S. and Sharma H.C. 2013. Effect of plant secondary metabolites on legume pod borer, *Helicoverpa armigera*. J. Pest Sci., **86**(3): 399-408
- Warschefsky E., Penmetsa R. V., Cook D. R. and Von Wettberg E. J. 2014. Back to the wilds: tapping evolutionary adaptations for resilient crops through systematic hybridization with crop wild relatives. American J. Botany, **101**(10): 1791-1800.
- Whitehead S. R., Turcotte M. M. and Poveda K. 2017. Domestication impacts on plant–herbivore interactions: a meta-analysis. Philosophical Transactions of the Royal Society B: Biol. Sci.*,* **372**(1712): 20160034.
- Wilk's S.S. 1946. Sample criteria for testing equality of means, equality of variances, and equality of covariances in a normal multivariate distribution. The Annals of Math. Stat., **17**(3): 257-281.
- Yaqoob M., Bakhsh A. and Zahid M.A. 2010. Studies on heritability and genetic advance in chickpea (*Cicer arietinum* L.). Science Technol. Develop., **29**(3): 1-9.

Supplementary Table S1. List of the genotypes with their parents used for evaluating pod borer

S.No	Genotype Name	Code used	Domestic Parents	Wild Parent	Wild Species	Genotype Group
1	2010	G ₁	ICCV 96029	Karab_81	Cicer echinospermum	Α
2	2018	G ₂	ICCV 96029	Karab_81	Cicer echinospermum	Α
3	2019	G ₃	ICCV 96029	Karab_81	Cicer echinospermum	Α
4	2020	G4	ICCV 96029	Karab_81	Cicer echinospermum	Α
5	2021	G5	ICCV 96029	Karab_81	Cicer echinospermum	Α
6	2026	G6	ICCV 96029	Karab 81	Cicer echinospermum	$\overline{\mathcal{A}}$
7	2027	G7	ICCV 96029	Karab_81	Cicer echinospermum	\overline{A}
8	2043	G8	ICCV 96029	Karab_81	Cicer echinospermum	Α
9	2045	G ₉	ICCV 96029	Karab_81	Cicer echinospermum	Α
10	2048	G10	ICCV 96029	Karab_81	Cicer echinospermum	\overline{A}
11	2049	G11	ICCV 96029	Karab_81	Cicer echinospermum	Α
12	2050	G12	ICCV 96029	Karab_81	Cicer echinospermum	Α
13	2056	G13	ICCV 96029	Karab_81	Cicer echinospermum	\overline{A}
14	2057	G14	ICCV 96029	Karab_81	Cicer echinospermum	Α
15	9626	G15	ICCV 96029	Karab_81	Cicer echinospermum	Α
16	9628	G16	ICCV 96029	Karab_81	Cicer echinospermum	\overline{A}
17	10360	G17	ICCV 96029	Karab_81	Cicer echinospermum	Α
18	8349	G18	ICCV 96029	Karab_92	Cicer echinospermum	D
19	8380	G19	ICCV 96029	Karab_92	Cicer echinospermum	D
20	8382	G20	ICCV 96029	Karab_92	Cicer echinospermum	D
21	8348	G ₂₁	ICCV 96029	Karab_92	Cicer echinospermum	D
22	8351	G ₂₂	ICCV 96029	Karab_92	Cicer echinospermum	D
23	8352	G ₂₃	ICCV 96029	Karab_92	Cicer echinospermum	D
24	8355	G ₂₄	ICCV 96029	Karab_92	Cicer echinospermum	D
25	8356	G ₂₅	ICCV 96029	Karab_92	Cicer echinospermum	D
26	8357	G ₂₆	ICCV 96029	Karab_92	Cicer echinospermum	D
27	8367	G ₂₇	ICCV 96029	Karab_92	Cicer echinospermum	D
28	8370	G28	ICCV 96029	Karab_92	Cicer echinospermum	D
29	8372	G29	ICCV 96029	Karab_92	Cicer echinospermum	D
30	8373	G30	ICCV 96029	Karab_92	Cicer echinospermum	D
31	8383	G31	ICCV 96029	Karab_92	Cicer echinospermum	D
32	8387	G ₃₂	ICCV 96029	Karab_92	Cicer echinospermum	D
33	8397	G33	ICCV 96029	Karab 92	Cicer echinospermum	D
34	8404	G34	ICCV 96029	Karab_92	Cicer echinospermum	D
35	8405	G35	ICCV 96029	Karab_92	Cicer echinospermum	D
36	8413	G36	ICCV 96029	Karab_92	Cicer echinospermum	D
37	8414	G37	ICCV 96029	Karab_92	Cicer echinospermum	D
38	8415	G38	ICCV 96029	Karab_92	Cicer echinospermum	D
39	8416	G39	ICCV 96029	Karab_92	Cicer echinospermum	D
40	8418	G40	ICCV 96029	Karab_92	Cicer echinospermum	D

Supplementary Table S2: List of checks (Ck) used for evaluating the test genotypes for pod borer resistance