



BIOCHEMICAL RESPONSE OF CHICKPEA GENOTYPES AS INFLUENCED BY POD BORER *HELICOVERPA ARMIGERA* (HUBNER)

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ABSTRACT

This study evaluated the biochemical changes in 15 chickpea genotypes artificially infested with pod borer *Helicoverpa armigera* (Hubner). Infested samples revealed significantly more phenols, malic acid and protein, and less reducing sugar contents. Significant differences among the genotypes for all the biochemicals were observed in the infested samples. These differences were influenced by genotype and pod damage both individually and together. Correlation analysis indicated significant associations of parameters with pod damage.

Key words: *Helicoverpa armigera*, chickpea, genotypes, infestation, phenols, proteins, malic acid, reducing sugars, pod damage, correlation coefficients, relationships

Chickpea (*Cicer arietinum* L.) is the third most important legume crop globally after dry beans and peas (Golla et al., 2018), with its production (75%) and consumption majorly centered in India (Das et al., 2017). Gram pod borer *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae) is a major pest of chickpea causing significant annual losses up to 25-30% (Golla et al., 2018; Das et al., 2017). High polyphagy, fecundity, diapause and migratory behaviour ensure its survival in wide range of ecosystems. Management strategies are often compromised by varying levels of infestation, and resistance to major groups of insecticides (Kranthi et al., 2002). Further, insecticide resistance in *H. armigera* varies with space and time (Singh et al., 1994). The immediate urge to search for alternate viable control strategies drives our approach towards host plant resistance. Screening the germplasm against the pest has been done previously, however studies on biochemical responses in response to infestation are inadequate. It is important to understand the biochemical responses induced as a result of pod borer feeding to use these parameters as selection criteria in developing cultivars with resistance to the pest. Hence, the present study evaluating the biochemical responses in chickpea genotypes in relation to infestation.

MATERIALS AND METHODS

Fifteen chickpea genotypes viz., BGD (133, 1501, 1536, 103, 111-01), JAKI 9218, JG 11, A-1, DBGV (204, 209, 206, 215, 213, 212) and KAK-2

were sown in randomized block design replicated thrice with Annigeri-1 (A-1) as a susceptible check during rabi, 2017 and rabi-summer, 2017-18 at the College of Agriculture, Vijayapura, Karnataka, India. The experiments were conducted as a part of routine breeders' evaluation trial and hence a resistant check was not included. The crop was raised as per the package of practices, recommended by the University of Agricultural Sciences, Dharwad except for the insecticidal application. As the natural infestation was way below the ETL, artificially infesting plants was done. At flowering stage, two 4th instar *H. armigera* larvae were introduced on the terminal leaves of five random plants/ treatment early in the morning for feeding. Larval movement was restricted by enclosing a muslin bag and insect was allowed to feed for 24 hr. One *H. armigera* larva/ m row is above economic threshold level for chickpea crop. Uninfested and infested leaf samples were collected from upper half of the plant during both seasons at flowering stage which were later shade dried and used for estimation of phenols, proteins and reducing sugars. Leaf samples collected before artificial infestation were considered as uninfested while samples collected after infestation were considered as infested. Leaf samples were collected separately for malic acid estimation. Standard protocols were employed to estimate the total phenol content (Sharma et al., 2016), reducing sugars (Somogyi, 1952), total protein (Sharma et al., 2016) and malic acid content (Koundal and Sinha, 1983).

At harvest, number of damaged pods was recorded on ten random plants/ treatment. Data on pod damage was subjected to arc sine transformation. The data on all the parameters from both seasons were pooled and subjected to one way ANOVA followed by Tukey HSD test ($p = 0.05$). Further, the individual and combined effect of genotype and pod damage on various biochemical parameters was analyzed. Paired t-test was conducted to test the significant differences between uninfested and infested samples. Correlation coefficients between pod damage and biochemical parameters were conducted for individual genotypes to know the effect of infestation on damage responses. The data analysis was done in R software (4.0).

RESULTS AND DISCUSSION

The data presented in Fig. 1 represents the pooled mean of both the seasons. Significant differences can be observed in all the biochemical components between uninfested and infested samples. Feeding by *H. armigera* resulted in significantly less reducing sugars ($t = 15.38$; $df = 89$; $p < 0.05$) with enhanced phenols ($t = -11.5$; $df = 89$; $p < 0.05$), malic acid ($t = -9.9$; $df = 89$; $p < 0.05$) and protein contents ($t = -7.57$; $df = 89$; $p < 0.05$) compared to uninfested samples. No significant differences were observed among genotypes in uninfested samples. Induced biochemical defense is

the most active and dynamic form of defense ensuring sufficient protection to the host against herbivory. Among the fifteen genotypes, evaluated for induced resistance against *H. armigera*, revealed that maximum phenol production was triggered in BGD 111-01 followed by DBGV 215 which were significantly more. These were significantly negatively correlated with pod damage ($r = -0.85$ in BGD 111-01 and $r = -0.83$ in DBGV 215). The least response to damage was noticed in BGD-103, BGD-133, BGD-1501 and KAK-2. Phenols constitute one of the most common, important and extensively studied groups of compounds with a major role in providing resistance (Sharma et al., 2009; War et al., 2013; War et al., 2015). They act as first line of defense by reduction of reactive oxygen species (ROS) produced as a result of insect herbivory ultimately leading to the activation of defensive enzymes (Maffei et al., 2007; Meitei et al., 2018).

Irrespective of the genotype, infested plants revealed maximum phenol contents than the corresponding uninfested plants. This suggested their role in providing protection against ROS-induced damage due to pest infestation (Sharma et al., 2016). Further, oxidation of phenols results in quinone production which inhibits protein digestion in insects (Kaur et al., 2015; Sharma et al., 2016; War et al., 2012). Accumulation of phenols (Sharma et al., 2009), qualitative and quantitative

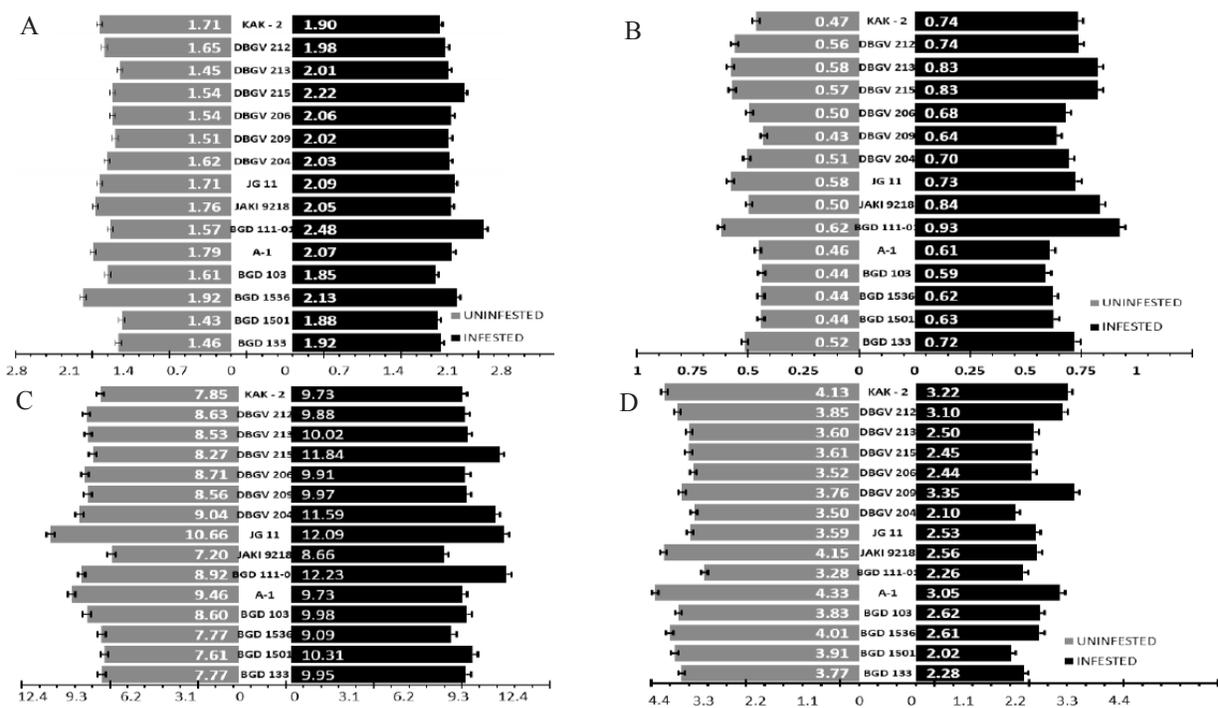


Fig. 1. Biochemical changes in leaves of uninfested and *H. armigera* infested chickpea genotypes. A) Phenols (mg GAE/ g); B) Malic acid (%); C) Proteins (mg/ g) and D) Reducing sugars (mg/ g); Error bars represent SEM.

changes in its contents in response to infestation is well known (Maffei et al., 2007). An increased production of phenols in infested leaves is in agreement with earlier reports in chickpea (War et al., 2011; Sharma et al., 2016; Kaur et al., 2017; Kaur et al., 2017b) pigeon pea (Green et al., 2003; Sharma et al., 2009; Sahoo and Patnaik, 2003) and many other plant systems (Rani and Jyothsna, 2010; Senguttuvan and Sujatha, 2000).

Similarly, malic acid content was significantly high in BGD 111-01 and exhibited significant negative correlation with pod damage ($r = -0.84$). Moderate to low response was noticed in JAKI-9218, DBGV-213, 215. Lowest production of malic acid in response to damage by *H. armigera* was noticed in BGD-103 which was below than susceptible check (A -1). Malic acid and oxalic acid constitute the major components in the exudates of chickpea leaves. Significantly higher amount of malic acid in infested leaves indicate its role in host defense. Malic acid might have a detrimental effect on the pest mediated through ovipositional antixenosis and antibiosis. The present results are in accordance with previous studies indicating malic acid as a source of resistance (Sharma et al., 2016; Cowgill and Lateef, 1996; Simmonds and Stevenson, 2001; Devi et al., 2013). On the contrary, some genotypes with considerable amounts of malic acid also suffered from relatively more pod damage suggesting that malic acid alone might not be the source of resistance (Bhagwat et al., 1995).

Significantly high protein content was observed in BGD-111-01, JG-11 and DBGV-215 genotypes showing significant negative correlation with pod damage ($r = -0.81$, -0.84 and -0.81 respectively). Least response was observed with JAKI-9218 ($r = -0.84$) and other genotypes responded moderately to infestation. A protein mediated defense is one of the most important and widely studied defense mechanism in plants against insects (Chen et al., 2009). Increased protein content in the infested samples could be explained by the plant requirement in synthesizing defensive enzymes and other non-enzymatic proteins in large quantities in response to infestation (Chen et al., 2009; War et al., 2012; War et al., 2015). These proteins might show antifeedant property against insects. Significantly higher amount of protein in insect infested plants confer stronger resistance against the pest. The present data on accumulation of proteins and their role in resistance confirms the earlier results (War et al., 2011; Kumar, 2017) and other crops (Chen et al., 2011; War et al., 2012; Prasad, 2015).

In response to pod borer feeding, DBGV-209 revealed maximum reducing sugar content followed by KAK-2 which were significantly positively correlated with pod damage ($r = 0.94$ in DBGV-209 and $r = 0.86$ in KAK-2) while BGD-1501 revealed the least reducing sugar content. Lesser reducing sugars in infested plants can be attributed to more foliar damage due to herbivory ultimately reducing the sugar contents. Similar results were also reported by Savitri, (2016) and Sharma et al. (2016) in chickpea; and Sahoo and Patnaik (2003) and Sharma et al. (2009) in pigeonpea. All the biochemical parameters were significantly influenced by genotype and pod damage both individually and when considered together (Phenols: F-value: Genotype = 8.64^{***} , Pod damage = 76.56^{***} , Genotype x Pod damage = 4.12^{***} ; Malic acid: F-value: G = 3.54^{***} , PD = 44.82^{***} , G x PD = 2.86^{**} ; Proteins: F-value: G = 6.2^{***} , PD = 10.94^* , G x PD = 8.77^{***} ; Reducing sugar: F-value: G = 9.3^{***} , PD = 6.0^* , G x PD = 10.53^{***}). Significant differences were observed in biochemical composition among various genotypes which indicate the ability of the genotypes to induce better damage responses as a result of pod borer feeding. It is possibly due to differences in innate nature of genotypes.

Among the genotypes, maximum pod damage was observed in BGD-103 followed by A-1 while BGD-111-01 was with the least pod damage, and significantly different from others. BGD-111-01 was superior in response to infestation followed by DBGV-215 with significantly more phenols, malic acid and proteins. BGD-103 and KAK-2 showed poor response to damage along with susceptible check (A-1) with lower phenols, malic acid and proteins and with higher reducing sugar content. Thus, the present study reports significant differences in biochemical parameters in various chickpea genotypes. Genotypes BGD-111-01 and DBGV-215 responded well to pest infestation by production of anti-nutrition factors and were found superior compared to other genotypes and hence can be used for developing resistant cultivars or as a source of resistance.

ACKNOWLEDGEMENTS

The authors thank Dr Vinod S Kukanur (ICRISAT, Hyderabad) for his help and guidance in the preparation of manuscript. Thanks to Dr M K Berwal (ICAR-CIAH, Bikaner) for help in statistical analysis.

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(Manuscript Received: February, 2021; Revised: March, 2021;

Accepted: July, 2021; Online Published: October, 2021)

Online published (Preview) in www.entosocindia.org Ref. No. e21032