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Morphological and Chemical Components of Resistance to Pod Borer, *Helicoverpa armigera* (Hübner) in Chickpea Germplasm

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ABSTRACT

Background: Chickpea pod borer (CPB), *Helicoverpa armigera* (Hübner) is a pest of great economic importance in chickpea and it is the major limiting factor in chickpea cultivation. In severe cases it causes about 75 to 90 per cent losses in seed yield, despite the application of insecticides. Therefore, development of a cost effective and an environmentally friendly approach like improvement of cultivars resistant to *H. armigera* is necessary for management of the pest in chickpea.

Methods: Morphological and chemical components of host plant resistance in chickpea germplasm was assessed under field and laboratory conditions against pod borer, *Helicoverpa armigera* at hotspot Pantnagar, during *rabi*, 2017-18 using standard protocols.

Result: Observations recorded revealed that germplasm ICC4484 recorded highest phenol (4.73 mg/g) and flavonoid (0.19 mg/g) content, whereas the maximum tannin, protein and trypsin content were recorded in ICC6263 (1.33 mg/g), ICC3137 (19.41 g/100 g of seeds) and ICC372351 (31.83 IU/g), respectively. Germplasm with higher phenol, tannin, flavonoid and trypsin inhibitor content recorded minimum per cent pod damage. Phenol, tannin, flavonoid, trypsin content showed negative correlation, while protein content showed positive correlation with the per cent pod damage by *H. armigera*.

Key words: Chickpea, Germplasm, Host plant resistance, Pod damage.

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is the second most important protein rich legume crop (Varshney *et al.,* 2013), containing 24% protein, 59.6% carbohydrates and 3.2% minerals (Bakr *et al*., 2004). The production and productivity of the crop is greatly hampered by Gram Pod borer (*Helicoverpa armigera* Hübner). This is a pest of great economic importance and it is the major restraining factor in chickpea cultivation. A full-grown larva feeds on grains by making a hole in the pod and thrusts its head inside pod, while its posterior part of the body remains outside. A single larva can consume 30-40 pods in its life time (Taggar *et al*., 2012). Gram pod borer (GPB) causes about 70 to 95 per cent losses in seed yield (Prakash *et al*., 2007), despite the application of insecticides. It is mainly due to its high reproductive rate, high voracity, high dispersal rate and resistance development against insecticides (Yang *et al.,* 2013). GPB causes economic and environmental problems that have been estimated to result in a loss of more than $2 billion annually worldwide (Tay *et al*., 2013). In addition to the huge direct economic losses, deleterious effects of pesticides remain in the environment. Therefore, development of a cost effective and an environmentally friendly approach like improvement of cultivars tolerant to *H. armigera* is necessary. The identification of crop cultivars with resistance/tolerance to insect pests has a great potential for integrated pest management, particularly under subsistence farming conditions in the developing countries (Sharma *et al.,* 1999). Many chickpea genotypes with low susceptibility to *H. armigera* or the genotypes that have high recovery potential from their damage have been identified in the past (Dua *et al.,* 2005). Host plant resistance denotes the presence of morphological or chemical plant factors that adversely alter insect behaviour resulting in poor establishment of the insects. Evaluation of crop germplasm for insect resistance has given a renewed incentive to the identification and use of HPR as an integral component of pest management worldwide. Many chickpea genotypes with low susceptibility to H. armigera or the genotypes that have high recovery potential from its damage have been identified in the past (Dua *et al.,* 2005). Therefore, the present study was carried out to know the importance of morphological and chemical factors in identification of tolerant lines against *H. armigera*.

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MATERIALS AND METHODS

Eleven germplasm along with resistant checks (GL25016 and ICCL86111), susceptible check (ICC3137) and local check (PG186) were screened at Norman E. Borlaug crop

research centre (NEB-CRC), G.B.P.U.A and T, Pantnagar, under natural field conditions during *Rabi* season of 2017-18. The germplasm were grown in plot of 2 m × 3 m with spacing of 10 × 30 cm, in randomized block design with three replications. The germplasm were assessed in per cent pod damage from five randomly selected plants of each replicated plot and compared with the biophysical and biochemical components of resistance.

Morphological components of resistance

Observations on the shape of fully-formed leaf from the upper canopy, plant type, days to 50 per cent flowering, seed weight, pod length, width and pod wall thickness were recorded on three uniformly developed pods of each test germplasm per replication using electronic Vernier caliper.

Chemical components of resistance

Ten gm of seed material was crushed with pestle mortar in the presence of liquid nitrogen to convert it into powder form. after grinding, a portion of 0.1 g powder was extracted in a 2 mL microfuge tube with 1 mL of 80% (v/v) methanol solvent. The mixture was shaken at 300 rpm at room temperature (25°C) for 3 h. Then the mixture was kept in the dark condition (12 to16 h) for additional extraction. The extracts were centrifuged by a microfuge at 12000 rpm for 15 min and the supernatants were removed into new tubes and these extracts were used for assessment of total phenols, flavonoids and tannins.

Estimation of total phenolic content

The total phenolic content of each extract was determined by using Folin-Ciocalteu's reagent (Chandrasekara and Shahidi, 2010).

Estimation of total flavonoids

The total flavonoids content was determined using a colorimetric method (Kim *et al*., 2003).

Estimation of total condensed tannins

Proanthocyanidins content was measured by using the vanillin/HCl assay (Sun *et al.,* 1998).

Estimation of total proteins

Powdered seeds (100 mg) were extracted with 25 ml of 0.1 N NaOH by keeping the tubes with water condensers on them overnight in the refrigerator. The contents were centrifuged at 5000 rpm for 15 min. Supernatant was used for the estimation of total proteins as per the method given by Lowry *et al*. (1951).

Extraction and estimation of trypsin inhibitor from the seeds of chickpea germplasm

The sample (100 mg) was homogenized with 1 ml of 0.01 M phosphate buffer (pH 7.5) containing 0.1 M NaCl and stirred for one hour at room temperature. The supernatant obtained after centrifugation at 10,000 rpm for 30 minutes was then kept in hot water bath at 80°C for 20 minutes. The homogenate was centrifuged again at 10,000 rpm for 30 minutes. Trypsin inhibitor potential was determined in the supernatant as per the method given by Hajela *et al*. (1999).

Statistical analysis

Tukey's HSD test was used to compare differences among treatment means ($P<0.05$) using statistical package for social sciences (SPSS) Software, version 16.

RESULTS AND DISCUSSION

Morphological basis of resistance in chickpea germplasm against *H. armigera*

Leaf shape

Leaves of promising chickpea germplasm were broadly grouped into narrow and broad on the basis of their shape (Table 1).

Plant type

On the basis of plant type characteristics, the chickpea germplasm were categorized into bushy, erect and bushy-spreading type under field conditions. Five germplasm (ICC4260, ICC2767, ICC244624, ICC3552 and ICC3404) were grouped under bushy type and four germplasm (ICC4484, ICC397375, ICC6263 and ICC372351) were categorized as erect type and remaining two germplasm (ICC3089 and ICC6938) as bushy-spreading type as compared to checks PG186 (bushy) and ICC3137 (bushy-spreading).

Days 50 per cent flowering

Days to 50 per cent flowering varied from 91 to 100 among the germplasm. The germplasm ICC6263 (91 days) was the earliest to 50 per cent flowering followed by ICC3404 (92 days), whereas the germplasm ICC2767 was late to 50 per cent flowering as compared to checks PG186 (99 days) and ICC3137 (98 days). The uniform flowering was observed in all the germplasm.

Seed weight

Maximum 100 seed weight was recorded on ICC3552 (32.15 g) followed by ICC2767 (18.7 g). Minimum 100 seed weight was recorded on ICC4484 (11.41 g) followed by ICC4260 (12.56 g), ICC6263 (12.68 g), ICC3404 (13.58 g), ICC3089 (13.80 g), ICC244624 (13.83 g) and ICC6938 (14.56 g) as compared to checks PG186 (18.31 g), ICCL86111 (20.87 g), GL25016 (13.57 g) and ICC3137 (27.92 g).

Pod wall thickness

Pod wall thickness of promising germplasm varied significantly. The lowest pod wall thickness was recorded on ICC6263 (0.231 mm) which was at par with ICC372351 (0.232 mm) and ICC4260 (0.24 mm). The highest pod wall thickness was recorded on ICC3404 (0.31 mm) which was at par with ICC3089 (0.29 mm) as compared to check varieties PG186 (0.29 mm), ICCL86111 (0.27 mm), GL25016 (0.28 mm) and ICC3137 (0.26 mm). Pod wall acts as a physical barrier for the pod boring insect. The increase in the pod wall thickness could results in the lowered level of

Table 1: Biophysical traits of promising chickpea germplasm.

Germplasm	Leaf shape	Plant type	Days 50% flowering	Test weight (100 seeds) grams	Pod wall thickness (mm)	Pod length (mm)	Pod width (mm)
ICC4484	Narrow	Erect	97	11.41	0.25^{bc}	14.43^{a}	7.37^{c}
ICC4260	Narrow	Bushy	96	12.56	0.24^{abc}	17.89^{e}	7.74^{e}
ICC2767	Broad	Bushy	100	18.7	0.27^{efg}	19.75^{h}	9.16^{j}
ICC397375	Narrow	Erect	93	16.15	0.27^{def}	15.8^{b}	7.92^{f}
ICC244624	Narrow	Bushy	99	13.83	0.26^{cd}	15.83^{b}	7.15^{b}
ICC3552	Broad	Bushy	99	32.15	0.27^{efg}	16.92^{c}	9.91^{k}
ICC6263	Narrow	Erect	91	12.68	0.23^{a}	16.84^{c}	7.59^{d}
ICC372351	Narrow	Erect	97	15.35	0.23^{ab}	15.72^{b}	6.73^{a}
ICC3404	Narrow	Bushy	92	13.58	0.31^{i}	17.01^{c}	7.58^{d}
ICC3089	Narrow	Bushy-spreading	95	13.80	0.29^{hi}	17.27^{d}	7.36^{c}
ICC6938	Narrow	Bushy-spreading	95	14.56	0.27^{efg}	18.29^{f}	8.85^{i}
ICC3137	Broad	Bushy-spreading	98	27.92	0.26^{cde}	17.78^{e}	7.92^{f}
GL25016	Narrow	Bushy	97	13.57	0.28^{fgh}	18.33^{f}	8.41^{h}
ICCL86111	Narrow	Bushy	95	20.87	0.27^{efg}	18.26^{f}	8.2^{g}
PG186	Narrow	Bushy	99	18.31	0.29^{gh}	18.87^{g}	7.69^{de}
SEm±					0.003	0.050	0.028
CD @ 5%					0.0096	0.145	0.081

*Means in a column followed by the same letter(s) do not differ significantly at the 5% level by Tukey's HSD test.

pod damage. The above results are in agreement with the findings of Brar and Singh (2017) who recorded average pod wall thickness varied from 0.27 mm to 0.32 mm.

Pod length and width

The pod length and width varied significantly among the chickpea germplasm. The pod length ranged from 14.43 mm to 19.75 mm as against 17.78 mm to 18.87 mm in check cultivars *viz*. ICC3137 and PG186, respectively. The lowest pod length was recorded in ICC4484 (14.43 mm), whereas the highest pod length was recorded from ICC2767 (19.75 mm). Similarly, pod width varied from 6.73 mm in ICC372351 to 9.91 mm in ICC3552 as compared to checks PG186 (7.69 mm), ICCL86111 (8.2 mm), GL25016 (8.41 mm) and ICC3137 (7.92 mm).

Effect of host chemical factors on *H. armigera* resistance in chickpea germplasm

The results of the studies on biochemical constituents of chickpea germplasm *viz*. protein, phenols, flavonoids, tannins and trypsin were presented in (Table 2 and Fig 1).

The protein content of the promising germplasm varied significantly. The minimum protein content was recorded from ICC4260 (10.33 g/100 g of seed), which was at par with ICC372351 (11.1 g/100 g of seed). The maximum protein content was recorded from ICC6263 (17.41 g/100 g of seed) followed by ICC2767 (17.25 g/100 g of seed) and ICC397375 (16.16 g/100g of seed) as compared to checks PG186 (10.58 g/100 g of seed), ICCL86111 (9.83 g/100 g of seed), GL25016 (15.5 g/100 g of seed) and ICC3137 (19.41 g/100 g of seed). The germplasm ICC6263, ICC2767 and ICC397375 with high protein content recorded higher pod damage by *Helicoverpa* (16.96, 15.99 and 10.18 per cent, respectively) indicating that these germplasm were more susceptible to *Helicoverpa*. It is mainly due to the sweetness which is responsible for higher pod borer infestation in chickpea. The hypotheses indicating that more pod damage would be there if the protein content increase and vice-versa. In the present study protein content of chickpea seeds had a non-significant positive correlation (0.496) with per cent pod damage (Table 3). Shaila (2017) reported the positive correlation between the protein content of chickpea seeds with damage rating by *Helicoverpa*. Results of the proximate composition are in agreement with Sharma *et al*. (2013) who recorded that, the crude protein content was varied from 18 to 31 per cent being higher in *kabuli* chickpea cultivars than *desi* chickpea. Bhatnagar *et al*. (2000) reported that susceptible chickpea genotypes had higher per cent protein content than tolerant genotypes.

The results obtained revealed that, the phenol content of the chickpea seeds varied from 1.34 mg/g to 4.73 mg/g. The minimum phenol content was recorded from ICC6263 (1.34 mg/g), which significantly differed from other germplasm. The maximum phenol content was observed in ICC4484 (4.73 mg/g), followed by ICC372351 (3.26 mg/g) as compared to checks PG186 (2.47 mg/g), ICCL86111 (2.90 mg/g), GL25016 (3.65 mg/g) and ICC3137 (3.21 mg/g). The germplasm ICC4484 and ICC372351 with higher phenolic content recorded low per cent pod damage (8.18 and 5.56 per cent, respectively) indicating that these germplasm were less preferred by *Helicoverpa*. The results of Sahoo and Patnaik (2003) are in close agreement with our findings, who reported that, the chickpea genotype BG256 with higher phenolic content recorded lower pod damage; on the other hand, genotype Annigeri and IICV2 with lower phenolic content recorded higher pod damage. In the present study

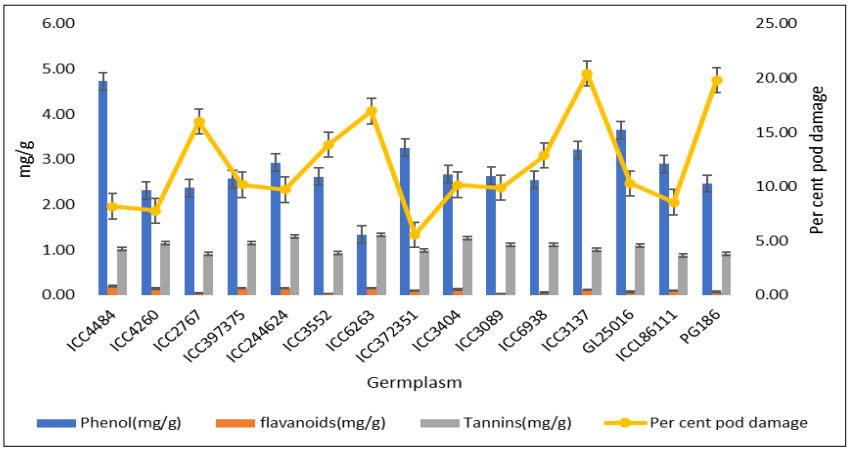

Fig 1: Effect of total phenol, flavonoid and tannin content on per cent pod damage by *H. armigera.*

Table 2: Biochemical composition of seeds of promising chickpea germplasm.

Germplasm	Phenol (mg/g)	Flavonoids (mg/g)	Tannin (mg/g)	Protein (g/100 g)	Trypsin inhibitor (IU/g)
ICC4484	4.73±0.10^{j}	0.19±0.019^{k}	1.02±0.031^{abc}	12.75±0.08^{c}	18.93±0.19^{g}
ICC4260	2.31±0.17^{b}	0.14±0.001^{ij}	1.15±0.017^{cde}	10.33±0.28^{ab}	30.19±0.26^{l}
ICC2767	2.37±0.15^{bc}	0.034±0.001^{ab}	0.91±0.039^{a}	17.25±0.24^{g}	7.51±0.10^{a}
ICC397375	2.57±0.25^{bcd}	0.15±0.010^{j}	1.15±0.061^{cde}	16.16±0.09^{f}	27.89±0.34^{k}
ICC244624	2.93±0.08^{efg}	0.15±0.004^{j}	1.3±0.055^{ef}	12.5±0.31^{c}	18.33±0.20^{f}
ICC3552	2.62±0.03^{bcde}	0.024±0.001^{a}	0.93±0.033^{a}	13.75±0.07^{d}	24.43±0.09^{j}
ICC6263	1.34±0.02^{a}	0.15±0.004^{j}	1.33±0.050^{g}	17.41±0.19^{g}	9.63±0.05^{c}
ICC372351	3.26±0.10^{g}	0.10±0.011^{fg}	0.98±0.053^{ab}	11.1±0.655^{b}	31.83±0.16^{m}
ICC3404	2.67±0.07^{cde}	0.12±0.002^{hi}	1.26±0.047^{def}	15.3±0.1^{e}	8.45±0.12^{b}
ICC3089	2.64±0.05^{bcde}	0.024±0.0001^{a}	1.12±0.096^{bcd}	15.83±0.14^{ef}	8.26±0.23^{b}
ICC6938	2.54±0.10^{bc}	0.050±0.003^{bc}	1.11±0.046^{bcd}	12.75±0.27^{c}	15.92±0.10^{e}
ICC3137	3.21±0.07^{fg}	0.11±0.008^{gh}	1±0.029^{abc}	19.41±0.08^{h}	14.20±0.15^{d}
GL25016	3.65±0.13^{h}	0.068±0.003^{cd}	1.09±0.096^{bc}	15.5±0.33^{ef}	22.33±0.16^{h}
ICCL86111	2.90±0.06^{def}	0.096±0.004^{ef}	0.89±0.007^{a}	9.83±0.075^{a}	23.83±0.24^{i}
PG186	2.47±0.06^{bc}	0.076±0.003^{de}	0.92±0.032^{a}	10.58±0.26^{ab}	8.70±0.05^{b}
SEm±	0.065	0.004039	0.030323	0.151431	0.1064
CD @ 5%	0.189	0.012	0.088	0.437	0.307

*Means in a column followed by the same letter(s) do not differ significantly at the 5% level by Tukey's HSD test.
Values are mean ± SD of triplicates.

the total phenol content recorded the negative correlation with phenolic content (-0.387). The results were in agreement with the findings of Bangar *et al.* (2018) who recorded that total phenol contents were negatively associated with egg count, larval incidence and pod damage percentage. Girija *et al.* (2008) also reported that phenolic content had negative correlation (-0.763) with per cent pod damage.

Total flavonoid content of the promising chickpea germplasm ranged from 0.024 mg/g to 0.19 mg/g. The flavonoid content of the chickpea germplasm varied significantly. The lowest flavonoid content was recorded from germplasm ICC3089 (0.024 mg/g) and ICC3552 (0.024 mg/g) which were at par with ICC2767 (0.034 mg/g). The highest flavonoid content was observed in ICC4484 (0.19 mg/g) as compared to checks PG186 (0.076 mg/g), ICCL86111 (0.096 mg/g), GL25016 (0.068 mg/g) and ICC3137 (0.11 mg/g). The germplasm ICC3089, ICC3552 and ICC2767 with lowest flavonoid content recorded maximum pod damage (9.88, 13.87 and 15.99 per cent, respectively) indicating these germplasm were susceptible to *Helicoverpa*. The activity of the flavonoids is mainly concentration-dependent and these compounds may be inhibitory or stimulatory, depending on the availability. The present result is in agreement with the findings of Sharma *et al.* (2013) who recorded the total flavonoid content in selected *desi* and *kabuli* chickpea cultivars ranged from 0.15 mg QE/ g of flour to 0.36 mg QE/ g of flour. The flavonoids had exhibited antifeedant and antibiotic activity towards the larvae of *H. armigera* (Simmonds and Stevenson, 2001).

Tannin content in the promising germplasm varied from 0.89 mg/g to 1.33 mg/g. Tannin content of the germplasm varied non-significantly and the lowest tannin content was recorded from ICC6938 (0.23 mg/g) which was at par with ICC2767 (0.91 mg/g), ICC3552 (0.93 mg/g), ICC372351

Table 3: Correlation between biochemical parameters of promising chickpea germplasm with per cent pod damage (%).

Variable	Phenol	Flavonoid	Tannin	Proteins	Trypsin
Pod damage	-0.387^{NS}	-0.233^{NS}	-0.156^{NS}	0.496^{NS}	-0.620*

** Significant at 1%, * Significant at 5%, NS = Non-significant.

(0.98 mg/g) and ICC4484 (1.02 mg/g). The maximum tannin content was recorded from ICC6263 (1.33 mg/g) followed by ICC3404 (1.26 mg/g) and ICC397375 (1.15 mg/g). In general, the germplasm with higher tannin content recorded low per cent pod damage and vice versa. Secondary substances of leguminous seeds suggest themselves to be the main defense mechanisms against insects and tannins acted by reducing the digestibility of tissues. Tannins are generally considered to be deleterious to herbivores. Tannins could affect the growth and development of insects in three main ways: they have an astringent taste, which affects palatability of the food, there by decreases the feed consumption, they form protein complexes and they act as enzyme inactivators. The most widespread secondary compounds in the Legumes are the tannins, lignin, lectins, alkaloids, enzyme inhibitors, polysaccharides, non-protein amino acids, toxic glycosides and miscellaneous toxins (Stamopoulos, 1987).

The results obtained revealed that the trypsin content of the promising chickpea germplasm varied from 7.51 IU/g to 31.83 IU/g as against 8.70 IU/g to 23.83 IU/g in checks. The trypsin content of the seeds varied significantly among the germplasm. The lowest trypsin content was recorded from ICC2767 (7.51 IU/g) which significantly differed from others. The maximum trypsin content was recorded from ICC372351 (31.83 IU/g) which possessed the lowest per cent pod damage. The germplasm ICC372351, ICC4260, ICC397375 and ICC3552 with higher trypsin content recorded minimum per cent pod damage (5.56, 7.77, 10.18 and 13.87 per cent, respectively) indicating these germplasm were resistant to *Helicoverpa*. The results were well supported by the findings of Patankar *et al*. (1999) who recorded the significant variation in the trypsin inhibitor and the *Helicoverpa armigera* gut proteinase inhibitor content in 8 chickpea cultivars. Highest TI (198 units/g) and HGPI (23 units/g) activities were shown by immature seeds of cultivar ICCV-2, whereas cultivar PG8505–7 (96.1 TI units/g) and Vijay (5 HGPI units/g) exhibited lower inhibitory activity. They also recorded more than 35 per cent inhibition from wild *Cicer*, suggesting that a large proportion of HGP was insensitive to PIs from *Cicer.* Nair *et al*. (2013) observed reduced larval weight and survival of final instar *Helicoverpa* larvae with the increased dose of trypsin inhibitor in the artificial diet. Similarly, Divija *et al.* (2020) recorded the lowest growth index for pulse beetle with increased level of protease inhibitors. PIs act as substrate mimics and hence they are able to bind stably with the proteinases, once ingested by the insects, these PIs bind to and inhibit the digestive serine proteinases in the insect (larval) gut, due to which protein digestion is blocked. PIs inhibition causes the depletion or assimilation of amino acids (Broadway, 1996), thus retards growth, development, fertility and fecundity of the adult moths (Telang *et al*., 2003). In the present study the germplasm ICC372351 with higher PIs content recorded the minimum mean egg and larval population (Divija and Agnihotri, 2020).

Conflict of interest

All authors declared that there is no conflict of interest.

CONCLUSION

The identification of crop cultivars with resistance/tolerance to insect pests has a great potential for integrated pest management, particularly under subsistence farming conditions in the developing countries. Evaluation of crop germplasm for insect resistance has given a renewed incentive to the identification and use of HPR as an integral component of pest management worldwide. The use of resistant genotypes is considered as simple, easy, cheap and ideal method of tackling pest problem, from farmer's point of view, this can be a most acceptable form of pest control technique. Among the biochemical parameters estimated higher phenol, flavonoid and tannins had negative correlation with the per cent pod damage by the *H. armigera*. The chickpea germplasm with low susceptibility to *H. armigera* or the germplasm that have high recovery potential from their damage can be exploited in breeding programme as a source of resistance to pod borer.

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