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Resistance of some Soybean Genotypes to Bruchids: *Callosobruchus chinensis* L. as Influenced by Different Biochemical and Seed Quality Traits

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ABSTRACT

Bruchids have high fecundity that cause damage to stored soybean; which leads to significant losses in yield. This study was aimed to assess the susceptibility of four soybean genotypes(H6 L58, Misr 6, Giza 22, and PI 416937)to bruchid (*Callosobruchus chinensis* L.) infestation. Different seed quality and biochemical tests were done on the genotypes including; moisture content (MC%), electrical conductivity (EC), content of total oil, crude protein, phenolic(TPC), additionally, seed coat thickness and germination % to measure their effect on the insect resistance parameters as; percent seed weight loss and Dobie susceptibility index(DSI), to *C. chinensis.* Soybean genotypes yield and its components were evaluated in the field as well. Although Misr 6 gave the highest seeds yield/plot (2.91 kg) and it was moderately resistant to insect infestation with6.35 DSI. The genotype PI 416937 recorded the highest MC% (15%) and its coat thickness was 6.6 µm, which helped to resist the *C. chinensis* infestation. Moreover, PI 416937 and Giza 22 recorded low EC 68.6 and 47.6 $\mu Scm^{-1}g^{-1}$, which led to higher germination%(61.5% and 85%), respectively, than the others. Although there was no significant increase among the genotypes in TPC, it helped in resistance against the infestation. The TPC recorded a negative correlation with the F_1 progeny traits for *C. chinensis* on soybean. Furthermore, the coat thickness and MC% have a significant negative correlation with the F1progeny traits. Taken together, the biochemical traits of seed quality and their contribution to soybean resistance to *C. chinensis* may be crucial for breeders to consider.

Keywords: Callosobruchus chinensis, Phenolic, Moisture, Dobie Susceptibility

INTRODUCTION

Soybean (*Glycine max* L. Merril) performs as a major oil-seed grownup and consumed in the world, because of its various usages. Soybean seed is mostly used in food due to its high protein content, and is a vital component in the fight against malnutrition in rural areas (Chadare *et al*., 2018). Since soybean is a particularly important crop in Egypt and contains edible oil, maintaining a high seed oil content is essential to securing the seeds from environmental stressors (Morsy *et al*., 2016).

Seed quality is a crucial factor in the commercialization and production of the seed, and it can impact the end product and its value (Kandil *et al*., 2019). The seed quality can be clear via a group of characteristics, which could be genetic, physical, physiological, and sanitary (Groot *et al*., 2021). Insect infestation affects the quality of soybean seeds, including germination, oil content, and protein content, in addition to seed yield. Additionally, storage insect attacks can result in significant post-harvest losses for soybean seeds, with a predicted loss of 10% of produced soybeans (Chelladurai *et al*., 2016). Furthermore, the soybean grain quality rapidly deteriorates due to these storage insects, and the viability of germination is lost (Ulemu *et al*., 2016), and (Credland, 2000) reported that the cultivars and the extent of storage up to 6 months have an impact on the germination of soybean seeds.

Since bruchid species are the main pests of stored grain legumes, they cause significant damage to soybean during postharvest. When stored in certain conditions, three species—*Callosobruchus chinensis* (Linnaeus), *Callosobruchus maculatus* (Fabricius), and *Callosobruchus analis* (Fabricius)—cause significant losses (Tarver *et al*., 2007). They are significant because their infestation begins in the field and spreads throughout the value chain, causing direct and irreversible harm to the seed industry, which is the economic component (Acrey and Kananji, 2007). Even at low initial infestation rates, the high fertility and short generation times of beetles can cause substantial damage. Due to bruchids contain insect excrement, frass, and dead insects in and on the seed, they reduce the viability of the seed and change its nutritional quality. Particularly, *Callosobruchus chinensis* (L.) (Coleoptera: Chrysomelidae) is an economically significant insect pest and a serious interior feeder of seeds from multiple legume species (Tuda *et al* ., 2005). This weevil lowers the quality of the product by feeding directly into the seed and contaminating it with its webbing and excrement (Sanful and Darko, 2010). Additionally, bruchids trigger overall weight loss, thus about 18.6% of the weight of the pulses was lost due to *C. chinensis* (Rawat and Srivastava, 2011). Furthermore, (Neupane *et al* ., 2016) reported 55-60% loss in seed weight and 45.50 - 66.30% loss in protein content due to its destruction and pulse seed became inappropriate neither for human consumption nor for planting.

Pesticides are expensive, hazardous, and can cause pest resistance. Hence, genetically resistant varieties,

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including antixenosis and antibiosis, are cost-effective and environmentally beneficial for pest management (Keneni *et al*., 2011). Nevertheless, the processes of resistance involve morphological, physiological, and/or biochemical mechanisms. Regression analysis, according to (Msiska *et al* ., 2018), showed that while seed color could be used to determine genotype DSI with up to 74% coefficient of determination, adult bruchid emergence explained seed weight loss with a 62% coefficient of determination. Msiska *et al*., (2019), also reported that the flavonoids and phenolics were linked to increased susceptibility to *C. chinensis* in soybeans, whereas secondary metabolites and other biochemicals were linked to higher resistance.

Even though bruchids are known to attack a wide variety of legume species, research suggests that little is known about the harm that bruchids due to soybeans. The majority of prior research has focused on other legumes, including common beans (Acrey and Kananji, 2007), cowpea, and chickpea (Sharma and Thakur, 2014); however, there is a dearth of information on soybean, indicating that bruchid damage to soybean was previously deemed insignificant.

Consequently, the aim of this study is to assess the effect of soybean (*Glycine max* L.) physical and chemical seed characteristics in addition to different seed quality traits on their susceptibility to *Callosobruchus chinensis* (L.), and identify the resistance sources in four soybean genotypes.

MATERIALS AND METHODS

- **Description of the study location**

The field experiment was carried out in Sakha Research Farm, Field Crops Research Institute, Agricultural Research Center, Kafr-Elsheikh District, Egypt during summer seasons of 2020 and 2021. The experimental design was a randomized complete block design (RCBD) with three replications. The minimum and maximum temperatures of Sakha Research Farm is about maximum temperature 20.21°C and 36.43°C, respectively, and the relative humidity is 46.76% of the two seasons on average. All agronomic procedures were carried out according to the Agricultural Research Center and the Ministry of Agriculture and Soil Reclamation recommendations. The seeds were then stored inside craft bags in a storage room in Sakha Research Station, Kafr-ElSheikh Governorate for the following season.

- **The materials**

Four soybean genotypes were used in the current study (Misr 6, H6L58, PI416937 and Giza 22), these genotypes were obtained from Food Legumes Crops Research Department, Field Crops Research Institute (FCRI), Agriculture Research Center (ARC), Egypt.

After being stored, the infected seeds were taken out of the storeroom. Four replications in the laboratory experiments were separated to infested and not infested (control) groups that were conducted in Seed Technology Department, Field Crops Research Institute, Agricultural Research Center, Egypt.

Growth parameters of the soybean genotypes

Flowering time: Days to first flower opened for 50% of plants.

Days to maturity: numbers of days from sowing to 95% of pods were matured.

At harvest time, different parameters were observed: i. Plant height (cm), ii. Number of branches plant^{-1}, iii. Number of pods plant^{-1}, v. Seed yield plot^{-1} (kg) and vi. 100–Seed weight (g) were recorded on ten guarded plants from each plot.

Physical and viability parameters

Moisture content (MC) %

The moisture content of soybean seed was measured in each genotype using a DICKEY-John mini-Gac moisture analyzer (Dickey-John, Auburn, IL, USA). Four replicates were used to measure the moisture content in each of infested and non-infested seeds.

Seed Coat thickness (µm)

The M165C stereomicroscope (Leica) was used to measure the thickness of the soybean seed coat. Every sample for each genotype from each group according to the insect infestation consisted of a minimum of ten seeds, each of which had its seed coats removed and measured five times from various angles.

Electrical Conductivity of Seed Leachate (EC)(µScm^{-1}g^{-1})

Weight of fifty seeds in four replications of each genotype either infested or not groups were soaked in 250 ml distilled water inside a glass conical flask, and kept at 25 ± 1°C for 24 hr. The electrical conductivity of seed leachate was measured with portable EC meter (9 V-1 AmP, Thermo Electron Corporation, USA) and expressed in µScm^{-1}g^{-1}.

Germination %

Taken from both groups of infested and non-infested seeds, fifty seeds from each of the genotypes were placed in 10-cm-diameter Petri dishes on Whatman No.2 filter paper humidified with 10 ml of distilled water. Seeds were kept at room temperature (25°C) under normal light. The number of germinated seeds was recorded 7 days after planting as final germination percent (ISTA, 1999).

Seedling dry weight (DW) (g) and seedling Vigor index (SVI)

The weight of the germinated seedlings from each genotype after oven drying at 70 ºC for 72 hr was recorded in grams using a sensitive scale. The seedling Vigor was measured according to the equation of (Orchard, 1977).

Seedling vigor index (SVI) = [seedling length (cm)×germination percentage]

Seed chemical composition

Oil Percentage (%)

For each genotype in both insect infested and non-infected groups, Soxhelt apparatus was used for evaluating of ether extract percent, heating by electric heaters; cold water was used through the condenser. Petroleum ether (60-80 °C) was chosen for extractions which continued for not less than eight hours (rate of siphoning was 6-7 hr.) according to AOAC (1990), (Horwitz, 2010). Eventually, oil percentage was calculated as (Weight of oil/weight of seed) *100.

Crude Protein content %

Nitrogen content (N) was determined using the micro-kejeldahl apparatus of as following, identified weight of the finely powdered seeds (ca 0.1 g) was digested with (98%H_2SO_4) and (30% H_2O_2). Finally, the percentage of crude protein was calculated by using the following equation;

The Crude protein = N * 6.25 according to (Sanful and Darko, 2010).

Seed Total Phenolic Content

The content of phenolic (TPC) was measured according to the method of (Sanful *and* Darko, 2010). Briefly, one gram of ground soybean sample was mixed with 10 ml of 80% methanol, ultrasonically extracted for thirty minutes, and then centrifuged. After adding 2% Na_2CO_3 for two minutes, a 50 µl of Folin-Ciocalteaus reagent was added to a 100-mL aliquot of the resultant solution. After 30 minutes, the absorbance was measured at 750 nm. Gallic acid was the standard used in this investigation, and the concentration was expressed in milligrams of Gallic acid equivalents (GAE) per gram of samples.

- **Preparation of seeds for insect infestation:**

The total of the four soybean genotypes Seeds were heated for 6 h at 50 ºC to ensure that any eggs or adult insects from the field were killed (Amusa *et al*., 2014). The disinfected grains were left in the laboratory to acclimatize for 24 hr.

- **Callosobruchus chinensis rearing:**

This experiment was conducted in the laboratory of Department of Stored Product Pests, Plant Protection Research Institute Sakha Agriculture Research Station, Egypt under no choice condition. Adult bruchids used in this study were reared on natural soybean medium for two generations. In 1L glass jar which provided with 500 g soybean previously sterilized, 100 adults were transferred to the jar. The jar capped with muslin cloth to allow ventilation, but prevent insects from escaping and the culture was kept at 28 ± 2 °C and 65 ± 5% R.H, with light: dark photoperiod of 16:8h. the adult insects were removed after seven days of F1 progeny's. The newly emerging adults (0-24 hr) were collected by sieving the diets. Adult insects, used for all bioassays were of mixed sexes.

- **Screening of soybean genotypes for susceptibility to *Callosobruchus chinensis* infestation under (non-choice):**

Fifty healthy seeds containing 12-13% relative humidity of each genotype were weighed and placed in small plastic jars (11.5 cm height and 6 cm diameter) and artificially infested with 10 unsexed randomly selected adult bruchids of 0-24 hr, from the bruchid colony as described by (Somta *et al.*, 2008). The set-up was left for ten days under fluctuating laboratory conditions (26.4 – 30 ºC and 73.5 – 85% R.H) with light: dark photoperiod of 16:8 h for the adults to oviposit. After 10 days, all the adults were sieved out and the number of eggs laid on seeds of each genotype was recorded then the jars kept in the laboratory until the beginning of adults emerged (F_1 progeny), which were removed, counted daily, and recorded till the emergence of last adult (Acrey *and* Kananji, 2007). The study was arranged in a Completely Randomized Design which was repeated 4 times under non-choice conditions.

The observed variables were:

1- The number of bruchid *eggs*: Total number of deposited eggs**.**

2- Emerged adults: F1 progeny**.**

3- Damaged seeds% based on the emergence holes calculated as: $= \frac{\text{damaged seeds}}{\text{total}} \times 100$

4- Seed weight loss %, which is an economic loss indicator (Amusa et al ., 2014) calculated as:

$$\text{Weight loss \%} = \frac{\text{iwt} - \text{fwt}}{\text{iwt}} \text{ x } 100$$

Where,

iwt = Initial seed weight, fwt = Final seed weight for the sample.

5- Insect emergence (I.E.) % estimated as:

$$\frac{\text{number of emerged adult}}{\text{total number of deposited eggs}} \times 100$$

6- Median developmental period (MDP) estimated as:

The time from the period of middle of oviposition to the emergence of 50 % of F_1 progeny (Acrey *and* Kananji, 2007).

7- Growth Index (GI):

It is an indicator of genotype suitability for development of insects (Wijenayake *and* Karunaratne, 1999) was estimated as:

$$GI = \frac{\%IE}{MDP} X\ 100$$

8- Dobie Susceptibility Index

At the end of the experiment, the Dobie susceptibility index was used to determine the susceptibility level of each genotype of soybean. Susceptibility index was calculated by using the formula (Dobie *and* Kilminster, 1978) with the equation:

$$\text{Dobie Susceptibility index (DSI)} = \frac{\text{LogF1}}{\text{D}} \text{x100}$$

Remarks: F1 = Total number of emerged F1 adults, D = Median developmental period as mentioned before.

Susceptibility level was categorized based on the Dobie's susceptibility index value of each soybean genotypes. It has been modified as described in Table 1**.**

Table 1. Susceptibility categories

Susceptibility Indices (0-11scale)	Susceptibility's Categories
0 – < 4	Resistant
4 – < 8	Intermediate/moderate Resistant
8 – < 11	Susceptibility
≥ 11	Highly Susceptibility

- **Statistical Analysis**

Analysis of variance was used to compare the differences between four soybean genotypes at α < 0.05. Tukey HSD was adopted as Post Hoc test for the significant one, and the means of genotypes were compared using the Duncan's Multiple Range Test at $p < 0.05$ (Duncan, 1955). Simple correlation of physio-chemical traits of tested cultivars and parameters of *C. chinensis* were evaluated by Pearson correlation coefficient using MINITAB 21.0.

RESULTS AND DISCUSSION

Growth behavior of the different studied soybean genotypes

The genotype PI 416937 showed the latest flowering time (48.33 days) and maturity date (146.67 days) when compared to the other genotypes; in contrast, genotype Giza 22 recorded the earliest one (Table 2). The genotypes Misr 6 and Giza 22 did not significantly differ in plant height; however, the genotype H6 L58 had the highest plant, measuring 112.25 cm (Table 2). Despite Misr 6 genotype has the fewest number of branches per plant (2.55 branches/plant), it produced the greatest seed yield/plot (2.91 kg) when compared to other genotypes; this could be because it had a large number of pods per plant (146.67 pods/plant). Otherwise, PI 416937 genotype recorded the lowest seed yield 1.37 kg/plot which was around half of that belongs to the uppermost one (Misr 6), as shown in Table 2. Therefore, the yield parameters are correlated together that there was a positive correlation between the flowering date and maturity date (Figure 7), in addition, the seed yield correlated positively with the number of pods/plant. However, a negative correlation between the flowering and maturity dates togetherness, and the seed yield /plot. That is in harmony with the results of Fordoński *et al* ., (2023).

Table 2. Mean performance of some yield components traits for the studied soybean genotypes, the averages of data are combined for both seasons 2020 and 2021.

Soybean genotypes	Days to 50% Flowering	Days to 50% Maturity	Plant height (cm)	Number of branches/plant	Number of pods/plant	100 seed weight(g)	Seed yield/plot (kg)
Misr 6	39.67 ± 0.24$_{b}$	135.67 ± 0.24$_{ab}$	102.25 ± 0.85$_{b}$	2.55 ± 0.13$_{b}$	146.67 ± 2.74$_{a}$	16.67 ± 0.14$_{b}$	2.91 ± 0.08$_{a}$
H 6 L58	41.00 ± 0.35$_{b}$	130.33 ± 0.20$_{b}$	112.25 ± 0.96$_{a}$	4.63 ± 0.24$_{a}$	122.06 ± 1.71$_{b}$	19.30 ± 0.13$_{a}$	2.50 ± 0.01$_{a}$
PI 416937	48.33 ± 0.24$_{a}$	146.67 ± 0.85$_{a}$	70.38 ± 3.05$_{c}$	3.57 ± 0.21$_{ab}$	89.93 ± 5.06$_{d}$	16.60 ± 0.16$_{b}$	1.37 ± 0.03$_{c}$
Giza 22	36.33 ± 0.24$_{c}$	123.67 ± 0.62$_{c}$	102.88 ± 2.70$_{b}$	4.59 ± 0.15$_{a}$	106.33 ± 3.07$_{c}$	18.53 ± 0.27$_{a}$	2.34 ± 0.03$_{b}$

***The values in the same column are expressed as means ± SE followed by distinct lowercase letters indicate significantly different results (P <0.05).**

Insect infestations result in significant financial losses for seed producers, and chemical pesticides are frequently used to control them. However, research into alternate strategies, such as the creation of more resistant genotypes, has been sparked by the development of resistance in insects as well as issues with toxicity to humans and the environment brought on by the ongoing use of these chemicals (Ventury *et al*., 2022). Morphological, physiological, or biochemical properties of a host can result in antibiosis resistance, which directly affects the biology of the pest, or by antixenosis resistance, which is non-preference type of resistance to the pest (Smith, 1989). Hence, the plant resistance to insect pests may be attributed to physiological, morphological, or biochemical parameters. According to our research, the seed morphological or physicochemical characteristics (*i.e*., seed MC%, seed coat thickness, seeds EC, seed phenolic content) of the soybean genotypes that *C. chinensis* developed on; were responsible for changes in the variances' F_1 progeny life history, and population growth of the insect.

Seed physical and viability characteristics and their relation to insect infestation

Seeds moisture content is one of the most crucial elements influencing seed quality and, in turn, seed susceptibility to insect infestation in the store (De Alencar and Dantonino Faroni, 2011). It was obvious that the MC % of the genotypes 'seeds increased from 13.5 % at harvest time to 15 % for the genotype PI 416937 after insect infestation (Figure 1). When compared the infected seeds of the two genotypes Misr 6 and PI 416937, their MC % showed no significant difference between them 14.8 % and 15 %, respectively, however the infested seeds of H6 L58 recorded the lowest MC% (14.15 %), with no discernible change from its uninfected seeds (13.75 %) at P < 0.05, as shown in Figure 1.

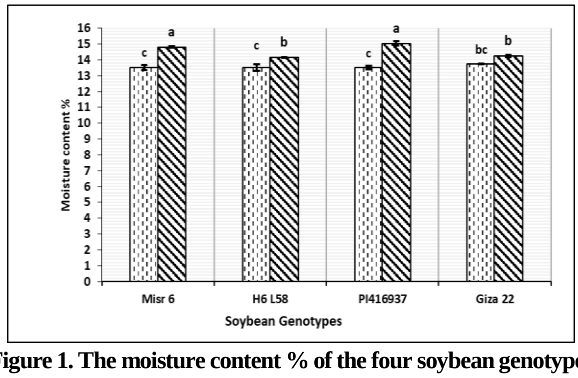

Figure 1. The moisture content % of the four soybean genotypes seeds in the insect infested seeds by (*Callosobruchus chinensis* L.) after storage that is represented as striped bar and the others are not infested seeds with dashed bars. Bars with different letters indicate significant differences (P ≤ 0.05), the represented data in 4 replicates as means ± SE.

The seed coat thickness of the host seed is one of the physical characteristics that can affect bruchids like *C. chinensis* oviposition behavior (Naseri *et al* ., 2022). The genotype Giza 22 retained the thickest coat (8.25 µm) for the infested seeds during the storage period, while the genotype Misr 6 demonstrated a significant decrease in its coat thickness, measuring half of what it had before insect infestation (Figure 2), that reflected on the laid eggs on their seeds since the genotypes H6 L158 and Misr 6 had the highest number of laid eggs 95.50 ± 1.04 and 89.00 ± 5.08 due to their thin and weak seed coat which ease the seed penetration by the insects to lay its eggs inside the grain of soybean , while Giza 22 and PI 416937 had the lowest number of deposited eggs with 42.00 ± 1.77 and 47.25 ± 4.53, respectively. The EC was also measured to determine the deterioration of cell wall of seeds that lead to leakage of the cell components because of the stresses. Hence, the EC values of the two genotypes, H6 L58 and Giza 22, did not significantly increase when compared to their non-infested seeds (Figure 3). However, the EC values of the other two genotypes, Misr 6 and PI 416937, increased considerably due to the insect infestation as a result of the storage conditions by approximately 8 $\mu Scm^{-1}g^{-1}$ and 9 $\mu Scm^{-1}g^{-1}$, respectively, when compared to the uninfected seeds. For soybeans stored with moisture contents of 11.2, 12.8, and 14.8 %, (Silva *et al*., 2008) employed electrical conductivity (EC) as a qualitative metric, the authors found that there was a general tendency toward higher electrical conductivity during storage, and that tendency became more noticeable as the moisture content rose. It is emphasized that electrical conductivity stayed nearly constant for the soybeans stored with a moisture content of 11.2 and 12.8 %, however the EC increased by the MC % rose in the studied genotype (Figures 2 and 3).

The emergence of the first discernible growth or root protrusion is known as germination, and it is influenced by number of variables such as insect attack, moisture content, and damage to the grains or seeds as EC increases (De Alencar and Dantonino Faroni, 2011). As anticipated, the infestation of insects had a detrimental effect on the percentage of germination of the four genotypes of seeds, which led to a significant negative result on the DW and SVI of the seedlings as well (Figure 4).

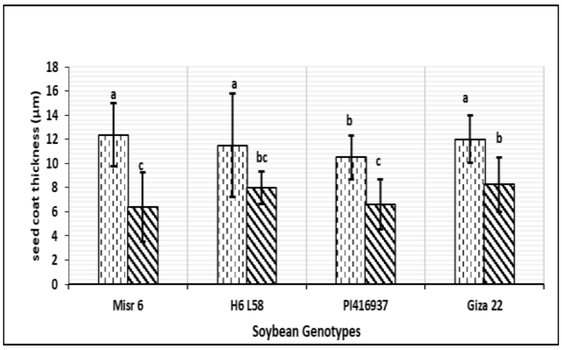

Figure 2. The seed coat thickness (µm) of the four soybean genotypes seeds in the insect infested seeds by (Callosobruchus chinensis L.) after storage that is represented as striped bar and the others are not infested seeds with dashed bars. Bars with different letters indicate significant difference (P ≤ 0.05), the represented data in 4 replicates as means ± SE.

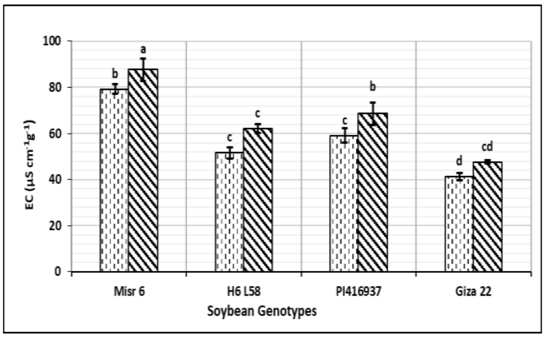

Figure 3. The Electric conductivity values of the four soybean genotypes seeds in the insect infested seeds by (Callosobruchus chinensis L.) after storage that is represented as striped bar and the others are not infested seeds with dashed bars. Bars with different letters indicate significant difference (P ≤ 0.05), the represented data in 4 replicates as means ± SE.

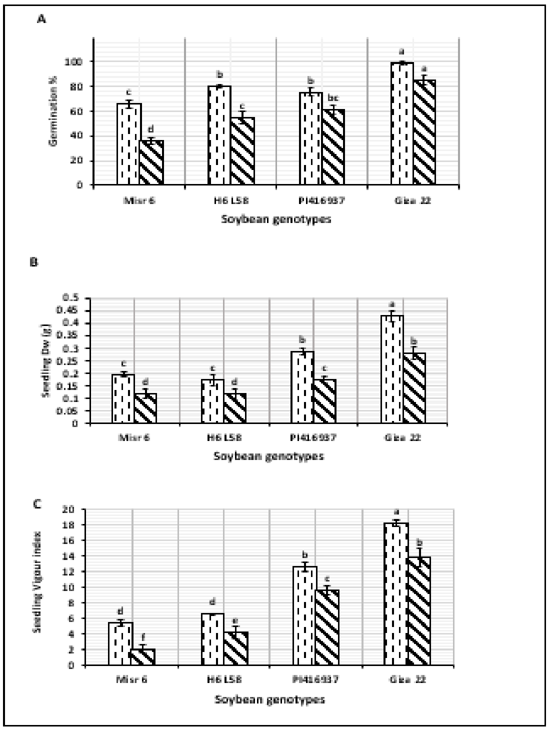

Figure 4. The germination % (A), seedling dry weight (B) and the seedling vigor index (C) of the four soybean genotypes seeds in the insect infested seeds by (Callosobruchus chinensis L.) after storage that is represented as striped bar and the others are not infested seeds with dashed bars. Bars with different letters indicate significant difference (P ≤ 0.05), the represented data in 4 replicates as means ± SE.

In this study, a direct correlation was found between increased infestation and decreased seed germination. According to (Verma *et al* ., 2018), chickpea seeds with the least amount of insect infestation had less seed mass loss and performed better during germination. Among the four genotypes, PI 416937 and Giza 22 were affected by the insect infestation less than the other two genotypes Misr 6 and H6 L58, as evidence both by the highest mean germination percentages 85 % and 61.5 % for Giza 22 and PI 416937, respectively, as shown in Figure 4A. Concerning seedling DW, Giza 22 genotype recorded the highest dry weight (0.28 g) for its infested seeds followed by the PI 416937 genotype (0.17 g), as illustrated in Figure 4B. However, for the other two genotypes there was no significant difference between them in the DW of the infested seed. In actual words, the SVI values markedly decreased after the insect infestation for all the genotypes (Figure 4C). The genotype Giza 22 showed the highest SVI (13.7), yet the genotype Misr 6 recorded the lowest SVI value (2.12), as presented in Figure 4C.

Seed biochemical characteristics effect on insect infestation

As seen in Figure 5A, the genotype Giza 22 had the highest oil content (21 %), while the genotype Misr 6 had the lowest oil content (16.5 %) in its clean seeds. The oil content of the infested Giza 22 seeds was significantly lower than that of the uninfected seeds, although the inset infested seeds for all genotypes under study showed no discernible variation in oil content. Oil content is essential to be maintained after storage as it is an important component in soybean seeds (Sugiyama, 2019).

When compared to seeds that were not insect infested, genotypes Misr 6 and PI 416937 both demonstrated a significant decline in seed crude protein following insect infestation; the other two genotypes, however, showed a slight decrease in crude protein content (Figure 5B). When it comes to insect-infested seeds protein content, H6 L58 and Giza 22 both have a mean of roughly 32.5 %; however, the genotype Misr 6 has the lowest protein content (29.4 %), as shown in Figure 5B.

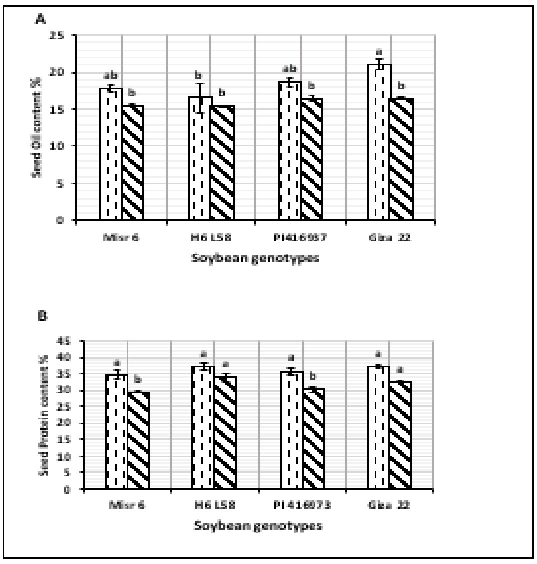

Figure 5. The oil content % (A) and the crude protein content (B) of the four soybean genotypes seeds in the insect infested seeds by (*Callosobruchus chinensis* L.) after storage that is represented as striped bar and the others are not infested seeds with dashed bars. Bars with different letters indicate significant difference (P ≤ 0.05), the represented data in 4 replicates as means ± SE.

Sharma and Thakur (2014) pointed out that protein content in soybean genotypes was not always a reliable indicator of their resistance to or susceptibility to *C. maculatus*. Therefore, in these genotypes, protein and oil contents of the four soybean genotypes did not affect the infestation of the *C. Chinensis.*

In the studied genotypes, the increased phenolic content and capacity to sustain higher phenolic levels may indicate a potential mechanism to support and maintain the structural integrity of the seed coat and cell wall against insect infection. Although there is no significant difference in the phenolic content in the four genotypes (Figure 6), their behavior under insect infestation is varied (Table 3).

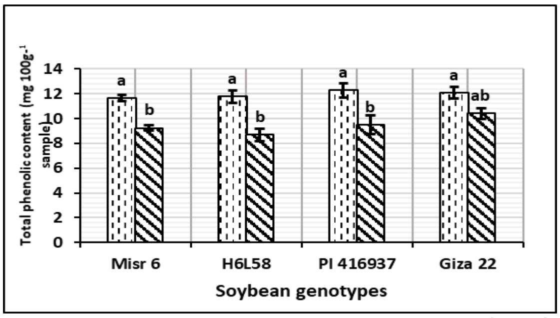

Figure 6. The total phenolic compounds content of the four soybean genotypes seeds in the insect infested seeds by (*Callosobruchus chinensis* L.) after storage that is represented as striped bars and the others are not infested seeds with dashed bars. Bars with different letters indicate significant difference (P ≤ 0.05), the represented data in 4 replicates as means ± SE.

The total phenolic content of the four genotypes' seeds decreased substantially after infestation when compared to the uninfected seeds, except Giza 22 genotype which recorded a slight decrease in the total phenolic content (1.6 mg/100 g sample), while the total phenolic compounds content of the infested seeds did not significantly differ among the four genotypes, as illustrated in Figure 6. This is in accordance with Elsawy *et al.* (2023) who demonstrated that, a phenol is one of the defensive compounds against stresses, including insects. On the contrary Msiska *et al.* (2019) demonstrated that proteins were not responsible for resistance to *C. chinensis* in soybean. Also, phenol in the susceptible and resistant genotypes may not be a good trait for determining resistance or susceptibility to bruchids in soybeans.

Susceptibility of soybean genotypes to bruchid infestation

Difference susceptibility of the tested soybean genotype to bruchid infestation was observed in this trial (Table 3). The cultivar had an effect on the biological parameters which including number of deposited eggs, emerged adults, % damaged seeds (magnitude of infestation), % Loss, insect emergence, median developmental period and growth index.

Table 3. Soybean genotypes and their susceptibility variables

Genotypes	Mean of numbers		Insect susceptibility variables				
	Eggs	Adults	%damaged seeds	%Loss	%IE	MDP	G. I.
Misr 6	89.00 ± 5.08$_a$	25.75 ± 1.65$_a$	30.50 ± 0.95$_{ab}$	0.59 ± 0.04$_{ab}$	29.19 ± 2.31$_a$	22.25 ± 0.94$_a$	1.31 ± 0.10$_{ab}$
H6 L58	95.50 ± 1.04$_a$	27.75 ± 1.70$_a$	37.50 ± 3.$_{04b}$	0.64 ± 0.07$_a$	29.01 ± 1.51$_a$	24.25 ± 0.85$_{ab}$	1.19 ± 0.09$_{ab}$
PI 416937	47.25 ± 4.53$_b$	11.25 ± 0.62$_b$	21.00 ± 0.57$_a$	0.40 ± 0.07$_{ab}$	24.42 ± 2.47$_a$	28.50 ± 0.64$_c$	0.85 ± 0.98$_b$
Giza 22	42.00 ± 1.77$_b$	12.50 ± 1.75$_b$	20.50 ± 0.95$_a$	0.36 ± 0.04$_b$	29.73 ± 0.57$_a$	26.00 ± 0.40$_{bc}$	1.14 ± 0.03$_{ab}$

% IE=Percent insect emergence, MDP=Median development period, %DSI= Dobie Susceptibility, GI =Growth index

Deposited eggs; *Callosobruchus chinensis* laid eggs with all studied genotype, H6L158 and Misr 6 had the highest number, while Giza 22 and P1 416937 had the lowest number of deposited eggs with 95.50±1.04, 89.00±5.08, 42.00±1.77 and 47.25±4.53, respectively. The ovipositional preference was not an indication of suitability for the larval development (Wijenayake and Karunaratne ,1999). Emerged adults. The genotypes H6L58 and Misr 6 were the most susceptible, producing two times approximately as f1 progeny in Giza 22 and PI416937 genotypes. % Damaged seeds. The percentage ranged from 20.50±0.95 to 37.50±3.04. Genotype H6L58 had the highest seed infestation with 37.50±3.04. % loss refer to magnitude of infestation, H6L58 had the highest seed weight loss with 0.64±0.07. % insect emergence (IE). There were not significantly different between the four genotypes, the emergence extended between 24.42±2.47 to 29.73 ± 0.57. Median development (MDP). The lowest MDP was observed on H6L198 genotype with 22.25± 0.94, which ranged from it to 28.50 days. Growth index (GI); P1 416937 genotype obtained the lowest one with 0.85±0.98 while the highest one was recorded on H6L198 genotype with 1.13±0.10.

The four genotypes were divided into two groups based on (DSI), as presented in Table 4. DSI is the most important factor to minimize the susceptibility level of each genotype of soybean. The genotypes with low values supported low values of progeny. The susceptibility index for P1 416937 soybean genotype was categorized as in resistant category to *Callosobruchs chinensis* infestation with 3.75. Meanwhile the three other genotypes were moderately resistant in category (DSI = 4-7) to infestation.

Soybean suffers damage from bruchid *Callosobruchus chinensis* during storage. This study found that soybean genotypes responded differently to *C. chinensis* infestation, which suggests a variation in genotype resistance (Allotey and Oyewo, 2004; Ulemu *et al*., 2016 and Msiska *et al.* 2018). The diversity in genotype resistance was mainly due to variations in biological parameters which including number of deposited eggs, emerged adults, % damaged seeds (magnitude of infestation), % Loss, insect emergence, median development period and growth index.

Table 4. Index of susceptibility and susceptibility's categories for four Soybean genotypes to *c. chinensis* Infestation based on Dobie susceptibility Index.

Soybean genotypes	Description	D.S.I.
Misr 6	Moderate resistant	6.35 ± 0.23$_a$
H6 L58	Moderate resistant	5.89 ± 0.34$_a$
PI 146937	Resistant	3.75 ± 0.13$_b$
Giza 22	Moderate resistant	4.21 ± 0.13$_b$

%DSI= Dobie Susceptibility IndexThe correlation between the studied seed

The results of this study showed that the F_1 progeny trait alone cannot differentiate the suitability of genotypes for *C. chinensis* development. Giza 22 genotype suffered considerably less weight loss compared to the others. The genotypes PI416937 and Giza 22 distinguished by delayed and low adult emergence; while the other genotypes, the adult emergence was relatively early, fast and in large numbers. The results on insect growth index which is an indicator of genotype suitability to bruchid development showed that the insect was able to infest and develop on all soybean genotypes tested but with significant differences. This is in accordance with Msiska *et al.* (2019). PI416937 had the lowest (GI) and presented the resistance genotype.

characteristics and insect infestation variables

The correlation coefficient between the studied traits of the four genotypes exhibited that, there is a positive correlation between the characteristics of each MC %, EC, Seed coat thickness and the germination % with its seedling vigor and DW and consequently the F_1 progeny traits of *C. Chinensis* insect (Figure 7). The seed coat thickness correlated oppositely with the ability of the bruchids to infest the soybean (Ventury *et al.,* 2022) However, there was a negative correlation between the total phenol content and each of weights of the eggs and adults of the insect. Furthermore, the total phenol content has a negative correlation to the damaged seeds %. There is a significant negative correlation between the flowering date, number of pods/plant and seed yield/plot and IE% (r = 0.90), (r = 0.84) and (r = 0.87), respectively. However, the correlation analysis revealed a positive and significant correlation between F_1 progeny and damaged seeds % (r = 0.64) and weight loss %

(r = 0.80), respectively Figure 7. Therefore, the physical and biochemical characteristics (MC %, seed coat thickness, oil content and phenolic content) affect negatively the insect infestation of *C. chinensis*. Likewise, a negative correlation between the F_1 progeny and seed viability parameters (seed germination %, SVI, seedling DW and seed EC), as illustrated in Figure 7. That leads to low viability of soybean seeds and resulted in decreasing the seed yield and production.

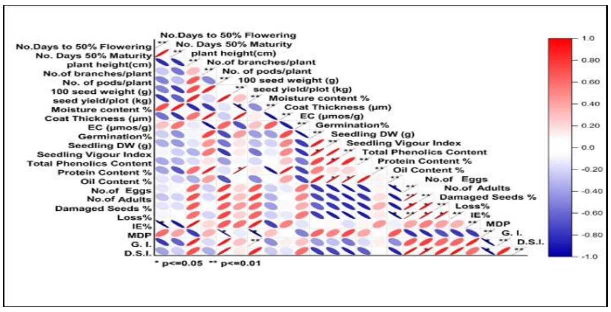

Figure 7. Pearson correlation coefficients (r) obtained between *C. Chinensis* parameters and seed properties of tested soybean genotypes. *and ** show significant correlations at P < 0.05 and P < 0.01, respectively.

CONCLUSION

The four studied genotypes of soybeans grown in Kafrl-Elsheikh, Egypt, differ in their basic physical and biochemical properties. In soybean breeding programs, the genotype PI 146937 was viable as a progenitor due to its resistance against *C. chinensis.* The resistance of the tested soybean genotypes to *C. chinensis* was significantly correlated negatively with seed coat thickness, seed moisture content, total oil content, and phenolic content. On contrary, there are positive effects of 100-seed weight, seed yield/plot, and seed protein content on soybean resistance to *C. chinensis*. Thus, the integration of those previous factors into a breeding program may enhance soybean seed resistance against this pest. Therefore, our study evaluated the susceptibility of soybean seeds of various genotypes grown in north Egypt to *C. chinensis* for the first time and identified the contribution of diffident physical and biochemical seed characters to their resistance.

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مقاومة بعض التراكيب الوراثية من فول الصويا للإصابة بحشرة خنفساء اللوبيا وذلك من خلال تأثرها بالصفات البيوكيماوية وصفات الجودة للبذور

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الملخص

تعتبر حشرة خنفساء اللوبيا من أهم الآفات التي تسبب ضررًا لبذور فول الصويا المخزنة وأيضا هي إحدى الآفات الرئيسية التي تسبب خسائر كبيرة أثناء التخزين للعديد من الحبوب الأخرى. لذلك كان الهدف من هذه الدراسة هو تقييم حساسية أربعة تراكيب وراثية من بذور فول الصويا وهي H6 L58، مصر 6، جيزة 22 و PI 416937. للإصابة بهذه الحشرة واختبار الأسباب المؤدية إلى تلك الإصابة حيث تم إجراء اختبارات مختلفة لجودة البذور والتحليل الكيميائي الحيوي على هذه التراكيب الوراثية لفول الصويا ومنها (محتوى الرطوبة، التوصيل الكهربائي لمنقوع البذور والمحتوى الكلي من (الزيت ,البروتين الخام والفينولات) بالإضافة إلى سمك قصره البذور ونسبة الإنبات لقياس تأثيرها على قدره البذور لمقاومة الإصابة بالحشرات من خلال قياس بعض مظاهر الإصابة مثل (النسبة المئوية لفقدان الوزن في البذور ,مؤشر حساسية دوبي. تم تقييم المحصول ومكوناته في تجربة حقلية لتلك التراكيب الوراثية وعلى الرغم من أن مصر 6 أعطى أعلى إنتاجية للبذور (2.91 كجم) إلا أنه كان متوسط المقاومة للإصابة الحشرية بمؤشر حساسية 6.35. وسجلت السلالة PI 416937 أعلى نسبة رطوبة (15%)وكان سمك القصرة لها 6.6 مايكرومتر، مما ساعدها على مقاومة وضع البيض للحشرة. وأيضا سجل PI 416937 وجيزة22 انخفاضًا في التوصيل الكهربائي بقيمه 68.6 و47.6 ميكروسيمنز / جم على التوالي مما أدى إلى زيادة نسبة الإنبات لتصل الى (61.5% و85%) على التوالي لهما مقارنة بالتراكيب الوراثية الأخرى. وعلى الرغم من عدم وجود زيادة معنوية في التراكيب الوراثية الأربعة في محتوى الفينولات الكلي إلا أن المحتوى الكلى من الفينولات ساعد في مقاومة الإصابة الحشرية. نستنتج من ذلك أن المحتوى الكلى من الفينولات، محتوى الرطوبة النسبي وسمك قصره البذور له علاقة عكسية مع صفات وضع البيض والتطور لحشرة خنفساء اللوبيا على هذه التراكيب الوراثية لبذور فول الصويا. ولذلك تعتبر هذه الصفات لجودة بذور فول الصويا هي أهم العوامل التي تؤثر على درجة حساسية بذور هذه التراكيب الوراثية للإصابة بحشره خنفساء اللوبيا أثناء التخزين، لذلك نوصى بأن تؤخذ هذه الصفات في الاعتبار خلال برامج التربية لإنتاج أصناف محصول فول الصويا المقاومة لحشرة خنفساء اللوبيا.

الكلمات الدالة : فول الصويا، الفينولات، الرطوبة، دليل الحساسية، خنفساء اللوبيا