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Antifeeding Efficacy, Mechanisms and Molecular Response of Potato (*Solanum tuberosum* L.) Against Black Cutworm (*Agrotis ipsilon* Hufn.) Larvae

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Abstract

The Black cutworm (BCW), *Agrotis ipsilon* Hufn. (Lepidoptera: Noctuidae), one of the most destructive insect pests of potato (*Solanum tuberosum* L.), can destroy entire fields if not detected early. Therefore, a no-choice test was performed to evaluate the anti-feeding impact of ten potato cultivars on BCW larvae compared to castor (a preferred host - control) under laboratory conditions. BCW 2nd instar larvae were fed leaves of potato cultivars compared to castor and checked daily until adult emergence to record various biological and chemical parameters of the fed larvae. In comparison to castor leaves, feeding larvae on potato cultivars leaves reduced the average weight of both larvae and pupae. The protein, carbohydrate, and fat content of the fed larvae on either 'Lily' or 'Mondial' leaves were lower than control larvae. Feeding 'Lily' leaves to larvae had a negative influence on the amino acid content of the larvae. In comparison to castor leaves, larvae fed on the leaves of 'Carosso,' 'Hermmes,' 'Lily,' 'Mondial,' and 'Princces' reduced the activity of protease and glutathione S-transferase enzymes. According to the findings, 'Hermmes', 'Lily', and 'Princces' were low susceptibility to BCW larvae feeding, while 'Hana' and 'Mondial' were high susceptibility. Biochemical and molecular analyses were performed on two potato cultivars, high-sensitive 'Hana' and low-sensitive 'Lily', to define antifeeding mechanisms. The high antioxidative power, due to flavonoids and catalase, was determined in 'Lily'. Suggests a higher level of reactive oxygen species (potentially H_2O_2) management in larvae feeding resistance, in addition autotoxicity prevention in nearby intact cells. Phytohormone 28-Homobrassinolide was raised in 'Lily', while methyl jasmonate amounts were much higher in 'Hana' after larva feeding. Blocking brassinosteroid (BR) biosynthesis using brassinazol (BRZ) elevated the expression of jasmonates biosynthesis gene OPR (OPDA reductase) in both cultivars by almost 4-fold comparing to mock wounded plants. However, the magnitude of expression was much higher with low-sensitive 'Lily' than sensitive 'Hana' larval feeding wounding. Collectively, the induction of antioxidants in infested potato plants is greatly involved in antifeeding mechanisms against BCW larvae. Relative gene expression studies suggest that antagonistic interaction between BR and JA could debilitate the anti-herbivory traits of potato plants against attacking BCW larvae.

KEYWORD: Protease, Glutathione S-transferase, Brassinosteroids, Methyl Jasmonate, Catalase, Flavonoids

INTRODUCTION

Potato (*Solanum tuberosum* L.) is the world's largest crop after rice and wheat in terms of human consumption. Potato provides humans with an inexpensive source of carbohydrate, vitamins (B and C) and mineral (Kolasa 1993). Global annual production of potato exceeds 300 million metric tons (Visser et al. 2009). Egypt is Africa's largest potato producer and one of the top 20 global potato producers. Potatois the largest horticultural crop exported in Egypt, and it is the second largest vegetable crop after tomatoin terms of financial value and total tonnage produced (FAOSTAT, 2015).

Unfortunately, potato is significantly harmed by several destructive insects, causing yield losses of 16% of the global production (Oerke et al. 1994). If poorly managed, tuber yield and quality could be reduced by 30 to 70% (Mujica and Kroschel 2013, Kroschel and Schaub 2013). The black cutworm (BCW), *Agrotis ipsilon* Hufn. (Lepidoptera: Noctuidae), is a destructive insect of all vegetables and most crops, including potato. (Abdou and Abdel-Hakim 2017, Abd-El-Aziz et al. 2019). It is widespread in most regions of the world, except in some tropical and cold regions. BCW is not a climbing cutworm and feeds primarily at soil level. Newly hatched larvae attack the young plants at night and feed on the epidermis of the leaves, whilethe older larvae feed by cutting plant stem under or above the soil (Shakur et al. 2007). Potato is very sensitive to BCW larvae at the beginning of the plant life, with 4-6 pairs of leaves. A single larva can cutdown a lot of plants overnight. Thus, if it is not detected early, it may cause the destruction of all plants in the field (Kroschel et al. 2020). The situation is getting worse because in many agricultural ecosystems the damage has been unpredictable, so it not detected until a significant reduction in vegetative and yield has occurred (Amin and Abdin 1997).

Integrated pest management (IPM) within a sustainable agriculture system is based on four main aspects: host plant resistance, agricultural practices, biological control, and chemical control. Chemical pesticides are reasonably effective at reducing the harm caused by pests. However, it has a harmful impact on the environment and humanity. Pests can also develop resistance to pesticides. Therefore, it is urgent to prevent/reduce its use (Brozozowski and Mazourek, 2108). Agricultural practices (i.e., crop rotation, polyculture and manipulating planting date) and biological control are limited in effectiveness, and require additional capabilities that increase production costs (Vincelli, 2016). Resistant cultivars are the easiest, safest, most practical, and best environmentally friendly way to control pest damage and reduce its spread. There is limited evidence of potato. resistance/tolerance to BCW damage. In India, potato cultivars JN2626F_1 (Gulab et al. 2001), Kufri Alakhar, Kufri Jawahar (Kumar and Tiwary 2009) had minimal leaf damage, while 'Kufri Sutlej' had minimal tuber infestation. Also, cultivar Kurfi Anand had the highest no. of healthy tubers with higher yielding cultivars Atlantic, KufriJyoti, and Kufri Surya (Tudu et al. 2019). Salah et al. (2012) found significant differences between ten potato cultivars in BCW larval weight gains between the variousfeeding trials in the Sudanese fields and the laboratory. 'Alpha', 'Lesita' and 'Bright' showed higher levels of resistance, while 'Desiree' and 'Spunta' showed the highest level of susceptibility.

Understanding of plant defense mechanisms against herbivorous insects is key for to developing resistant cultivars through plant breeding programs or DNA recombinant techniques. In addition to the opportunity to promote resistance to susceptible cultivars to improve farmer profitability and keep the environment clean and healthy, improving metabolic efficiency, resulting in increased yield and enhanced crop quality (Abbas and Hussain,2020). However, the information available on antifeeding mechanisms in potato against BCW larvae is very limited. Only Salah et al. (2012) reported a negative correlation between the dry matter and solanine content of potato plant and the weight gain of BCW larvae.

Generally, the plant defense response is influenced by plant hormones and antioxidants. The main plant hormones involved in plant-pest interactions are salicylic acid (SA), jasmonic acid (JA), ethylene (ET), and brassinosteroid (BR). Brassinosteroids (BRs) are a group of steroidal plant hormones that have multiple roles in plant growth, development, and responses to biotic and abiotic stress (Nolan et al. 2019, Peres et al. 2019, Lozano-Durán and Zipfel 2015, De Bruyne et al. 2014). The functional and molecular mechanisms of BRs in plant development and abiotic stress tolerance have been analyzed and clearly

identified in numerous previous studies (Peres et al. 2019, Ahammed et al. 2020, Hafeez et al. 2021, Krishna et al. 2017). In addition, the relationship between BRs and pathogen resistance is being clarified (De Bruyne et al. 2014, Lozano-Durán and Zipfel 2015), but the effects of BRs on herbivorous insect feeding remain largely unclear (Miyaji et al. 2014, Pan et al. 2018). Jasmonic acid (JA) is an organic acid biosynthesized from linolenic acid by the octadecanoid pathway. JA and its derivative are also produced in response to pathogen infection, probably due to an increase in lipoxygenase and 1-amino-cyclopropane-1-carboxylic acid (ACC) oxidase activities, respectively (Gundlach et al. 1992, Hammond-Kosack et al. 1996, May et al. 1996, Penninckx et al. 1996, Thomma et al. 1998). JA, in addition to being a regulator of plant growth and development has been identified as a stress hormone that regulates plant responses to biotic (i.e., those elicited by herbivores and pathogens) (Wang and Wu 2013, Ye et al. 2021) and abiotic stress (i.e., wounding and ultraviolet radiation) (Farhangi-Abriz and Ghassemi-Golezani 2019). JA induces the production of antimicrobial compounds such as phytoalexins and pathogenesis-related proteins (PRP) (Wang and Wu 2013, Hazman et al. 2019, Ali 2021, Ye et al. 2021; Ali et al. 2018).

The production of reactive oxygen species (ROS) in plants, such as superoxide (O_2^-) and hydrogen peroxide (H_2O_2), occurs early in plant defense response to external stimuli (Mittler 2002, Neill et al. 2002, Overmyer et al. 2003). ROS are rapidly accumulated in response to biotic stresses like herbivores feeding and pathogens or abiotic stresses such as drought and cold stress (Gill and Tuteja 2010). High level of ROS is damaged the bodies of living organisms. Plants have evolved complex protective mechanisms for scavenging ROS. These include the enzymatic scavenging systems, such as catalase (CAT), peroxidase (POD), and superoxide dismutase (SOD) (Howe and Schilmiller 2002) and non-enzymatic scavenging systems, such as ascorbic acid, glutathione (GSH), phenols, tannins, and flavonoids (Kaur et al. 2017). Therefore, plant antioxidants are believed to play an important role in chemical defense against herbivores, but their specific physiological effects on insects are variable and poorly understood (Richardson 1985). They have been variously described as antifeedants (Wrubel and Bernays 1990), digestibility reducers (Feeny 1976), and toxins (Bernays 1981, Steinly and Berenbaum 1985).

Therefore, this study was conducted to evaluate the effect of antifeeding defenses of ten different potato cultivars compared to the castor (*Ricinus communis* L.) against BCW larvae under laboratory conditions, and to study the role of some phytohormones and antioxidants in antifeeding mechanisms.

MATERIALS AND METHODS

Plant materials and planting

Antifeeding effects of potato cultivars Caroso, Hana, Hermes, Charlotte, Inova, Lady Rossita, Lily, Marspeer, Mondial, and Princces against BCW were evaluated under laboratory conditions at the Entomology laboratory, Faculty of Agriculture, Cairo University, Giza, Egypt. Potato seeds (tubers) were obtained with the support of Daltex Company, Egypt. On November 1, 2018, seeds were planted in plastic pots (3l volume, tuber/pot) filled with a mixture of peatmoss and perlite (1:1 volume) at Faculty of Agriculture, Cairo University, Giza, Egypt (30°01’00.8’’N; 31°12’19.0’’E). Calcium carbonate (3kg/300 l peatmoss) and Rizolex T50% WP fungicide (50g/300 l peatmoss) were added to the mixture to adjust the pH and disinfection. The pots were distributed according to the randomized complete block design (RCBD) with three replicates under open field conditions. The experimental unit (EU) was consisted of five pots/cultivar. Potato plants were irrigatedwith half strength Hoagland’s nutrient solution without sprayed any insecticides. Castor was used in the experiment as the preferred host for feeding black

cutworm larvae (Amin and Abdin 1997). Castor leaves were obtained from naturally growing plants.

Insect rearing

The 2ed instar larvae of BCW and eggs loaded on a gauze cloth obtained from Cutworm and Mole Cricket Department, Plant Protection Research Institute, Agricultural Research Center, Egypt. The eggs were placed in a glass jar (1 l capacity) until hatching. The larvae were placed in plastic cages (1 l capacity) containing 5 g sawdust to reduce humidity and incubated in a laboratory at 25±2°C and 65±5% RH. The larvae were fed on castor leaves. The 4th instar larvae were reared individually in glass petri dishes, diameter 9 cm, until pupation to avoid cannibalistic behavior. The Adults were placed in chimney glass cages (three pairs/cage). Adults were fed on 10% bee honey solution and left for mating (female and male) and laying the females for eggs. The insect was reared for several generations to use in the laboratory and field experiments (Ahmed et al. 2013).

Laboratory evaluation

Antifeeding effect of potato cultivars against BCW was evaluated by feeding larvae on leaves of the evaluated cultivars compared to castor in vitro at 25±1°C and 65%RH with an estimate the insect life parameters, i.e., growth, duration of developmental stages, fecundity, and fertility. The 2nd instar larvae were placed in plastic cups (100ml; five larvae/cup) containing sawdust to reduce moisture content and covered with muslin and a rubber band. The larvae were fed on a leaf/cup/day until the 4th instar larvae, which separated into a larva/cup to avoid cannibalism. The 25 cups were randomly arranged on five replicates/cultivar (five cups/rep.). The 6th instar larvae were weighted and left to pupate. The newly emerged pupae were weighted and left until the adult emerged. The larvae and pupae checked daily until complete development to estimate the duration of larvae, prepupa, and pupae. Five pairs of newly emerged adults/cultivar were transferred into small glass vials (one pair/ vial) covered with muslin and a rubber band. Adults were fed a 10% honey solution and checked daily to estimate the longevity of adults (male and female) and no. of eggs/female. Newly healthy eggs (500 eggs/cultivar) were transferred into petri dishes at a rate of 100 eggs/dish over five replicates, and were checked daily to estimate the incubation period and hatchability percentages.

Attraction of 2nd instar larvae to potato cultivars

Fifty leaves of each cultivar were taken and divided into five replicates. The leaves were put on white paper as cycle shape in a no-choice test (ten leaves/paper). Fifty 2nd instar larvae / replicate were put in center of cycle. The total no. of larvae which attracted to each leaf was counted after 2, 4 and 6 h to determine the % attractive larvae / cultivar (Souza *et al*. 2012)

Chemical composition and enzymatic activity of BCW larvae

The quantity of amino acid, carbohydrates, lipids, and proteins in 4th instar larvae, as well as the activity of digestive enzymes (proteinase and glutathione S-transferase), were estimated to compare the metabolic efficiency of larvae fed on potato vs castor leaves. The described method by Amin (1998) was used to prepare the 4th instar larvae for chemical analysis. Larvae were homogenized in distilled water (50 mg /1 ml). The homogenates were centrifuged at 8000 rpm for 15 min at 2 ºC in a refrigerated centrifuge. The supernatants, referred as the enzyme extract, were stored at 5°C for least one week to estimate the activity of protease and glutathione S-transferase by methods described by Tatchell et al. (1972) and Habig et al. (1974), respectively. The deposits were re-homogenized and the supernatants referred to as the base material extract were used for the estimation of amino acid, carbohydrates, lipids, and proteins

by methods described by Lee and Takahashi (1966), Crompton and Birt (1967), Knight et al. (1972), and Bradford (1976), respectively.

Antifeeding mechanisms of potato against BCW larvae

According to results of feeding behavior and development of BCW larvae on the evaluated potato cultivars under laboratory conditions, high-sensitive cultivar Hana and low-sensitive cultivar Lily were selected to study antifeeding mechanisms in potato. On November 1, 2019, 10 pots/cultivar were planted with potato seeds (tuber/pot) as described in the section plant materials and planting. Pots were placed in greenhouse at Faculty of Agriculture, Cairo University, Giza covered with black fabric with narrow holes to prevent the entry and exit of insects. Five pots/cultivar were artificially infested with 2nd instar BCW larvae after three weeksfrom planting. Larvae were placed in small bags of perforated gauze and tied well on the plant branch. Leaves of infested and un-infested plants/cultivar were collected three days after infestation by surface disinfected clipper and immediately kept in liquid nitrogen (LN) for at least 10 min then stored in -80 °C for molecularand biochemical analysis.

Estimation of phytohormones and enzymatic and non-enzymatic antioxidants

Roles of phytohormones and non-enzymatic and enzymatic antioxidants in antifeeding defenses of potato against BCW larvae were studied by estimation hormones BR and JA and antioxidants CAT, glutathione reductase (GR), and flavonoids in the leaves of the infested and healthy plants for both cultivars.

Enzymatic antioxidant

The activity of antioxidative enzymes CAT and GR were measured in leaves stored in liquid nitrogen. The leaves were grinded and the fine leaf powder was homogenized in cold extraction buffer (50mM sodium phosphate buffer (pH 7.5), 1m Mpolyethyleneglycol, 1m Mphenylethylemethylesulfonyl fluoride, 8% (w/v) polyvinylpyrolydone and 0.01% (v/v) triton X-100). The mixture was centrifuged at 14,000 rpm/30 min at 0 °C. The supernatant was used to estimating CAT. CAT (EC1.11.1.6) activity was estimated spectrophotometrically by monitoring the disappearance of H_2O_2 at 240 nm (extinction coefficient 39.4 $mM^{-1}cm^{-1}$) of a reaction mixture containing 50 mM K-phosphate buffer (pH 7.0), 33 mM H_2O_2 and enzyme extract (Aebi 1984). GR (EC 1.6.4.2) activity was determined by measuring the oxidation of NADPH at 340 nm (extinction coefficient of 6.2 $mm^{-1}cm^{-1}$). 50µl of leaf extract were added to 1 ml of the reaction mixture. The mixture consisted of a solution of 0.2 mm Tris/HCl buffer (pH 7.8) containing 3 mm EDTA, 0.2 mm NADPH, and 0.5 mm oxidized glutathione (Hazman et al. 2015).

Flavonoids

Total flavonoids content was measured by the aluminum chloride colorimetric assay. An aliquot of methanolic extract of the samples (250ul) or the standard solution (different concentration of quercetin in ug/ml) was added to 10 ml of volumetric flask containing 4 ml dd H_2O to the flask was added 300 ul of 5% $NaNO_2$. After 5 min, 300ul of 10% $AlCl_3$ was added. At 6th min, 2 ml of 1N NaOH was added and total volume was made up to 10 ml with dd H_2O. the solution was mixed well and the absorbance was measured against prepared reagent blank at 510nm. Total flavonoids content of potato leaves methanolic extract was expressed at mg quercetin per gram fresh weight (Hazman et al. 2015).

Phytohormones

For hormone measurements, samples were grinded using mortars and pestles in LN till fine powder and weighted then sent to Regional Center for Food & Feed (RCFF), Agricultural Research Center (ARC), Giza, Egypt, for hormonal analysis. Three biological replications were collected in each treatment case and were

quantified simultaneously using a standardized ultraperformance liquid chromatography–tandem mass spectrometry (UPLC-MS/MS)-based method according to Balcke *et al.* (2012).

Effects of inhibiting BRs biosynthesis on BCW larvae antifeeding defenses

The phytohormones analysis revealed a decrease in JA biosynthesis and an increase in BR biosynthesis of low-sensitive cultivar 'Lily' compared to high-sensitive cultivar 'Hana'. Therefore, this experiment was performed to see if blocking the BR biosynthesis would enhance the JA biosynthesis, the main well-known plant defense hormone against damage caused by herbivores attacks. To achieve this purpose, brassinazole, a specific inhibitor of BR biosynthesis, was used in the form of a 1 µ M solution prepared in water containing 0.02% Tween 20 (vol/vol) as described by Deng et al. (2015). On November 1, 2020, potato seeds (tubers) of both cultivars were planted under controlled greenhouse condition at the Agricultural Genetic Engineering Research Institute (AGERI), Agricultural Research Center (ARC), Giza (30°01'26.8''N; 31°12'09.7''E). Tuber seeds were planted in rows 1.2 m wide, 2m long, and 50 cm apart between tubers. Plants subjected to the common agricultural practices without applying insecticides. Plants 60 days after sowing of each cultivar were divided into two groups: one was infested with BCW larvae as described previously, and the other was healthy. Each group was divided into two subgroups, one spraying with BRZ solution (BRZ-plants) and the other spraying with water containing 0.2% Tween-20 (Mocked plants). Spraying was carried out once, 12 h before larval infestation. A RCBD with three replicates was used to arrange the treatments. Each EU included one row. Gene expression level was measured using a real-time polymerase chain reaction (RT-PCR).

Total RNA extraction and RT-PCR

Total RNA was isolated from the shoots of healthy and infested plants using Trizol Reagent (Life technologies, USA) according to the manufacturer's instructions. The cDNA synthesis was performed with cDNA synthesis kit (Invitrogen, USA) using total RNA as a template. Real time (qPCR) was performed on 10 ng cDNA with a SYBR green dye protocol using Stratagene Mx3000P (Stratagene, USA) as follows: 95◦C for 3 min, and 40 cycles (95◦C for 10 min, annealing at 60 ◦C for 30 s and extension at 72◦C for 30 s).To compare the transcript levels between different treatments, the delta Ct method was used, the difference in the cycle threshold (Ct) values between the endogenous control genes actin and target gene was calculated (Livak and Schmittgen, 2001). The primer sequences for the target genes and reference genes are as follow: CYP450 942A-like (BR biosynthesis gene), forward: 5' CCGTTTGTGTATCCAGTGTTTC 3', reverse: 5'TGTCCTTTCCTCAATCCTCAC 3'. OPR (OPDA reductase, jasmonic acid biosynthesis gene), forward: 5' CCAAGTGCTTTGGGACTTTAC 3', reverse:5' GTATGGCCAACAACAGGATCTA 3'. Reference gene actine, forward: 5' CGCGTAGTTTCACCACCTATT 3', reverse: 5' GTTATGTTCTCATGTCTAATCCCAAATC 3'.

Data analysis

Statistical analysis of data performed with SPSS computer program version 14 (Snedecor& Cochran 1967). The data were analyzed by ANOVA and mean comparisons were performed according to Duncan's multiple range test (P <0.05).

RESULTS

Development of BCW larvae

In vitro feeding on leaves of the evaluated potato cultivars compared to castor as a control had higher significant effects on the duration of cutworm development stages, except for the pre-pupa stage as shown in Table 1. Potato cultivars and the control had insignificant effect on prepupa duration, which

ranged from 1.86 day with the control to 2.1 day with 'Caroso'. The lowest significant mean of incubation period of eggs observed with the control and 'Marspeer' (2.14 and 2.60 day, respectively), while the highest significant incubation period was with 'Princces', 'Lady Rossita', and 'Hermes' (4.20, 3.73, and 3.67 days, respectively). Castor and potato cultivars Mondial, Marspeer, Inova, Hermes, and Lily recorded the longest larval duration (26.67, 25.92, 25.84, 24.93, 24.87, and 24.67 days, respectively) without significant differences between them. On the other hand, potato cultivars Princces, Caroso, Hana, Lady Rossita, and Charlotte recorded the shortest significant larval duration (22.44, 23.79, 23.83, 24.07, and 24.16 days, respectively). The longest significant pupal duration observed with 'Caroso' and 'Lily' (12.37 day for both) without significant differences than the control (13.09 day). 'Inova', 'Lady Rossita', 'Hana', and 'Marspeer' had the shortest significant pupal duration (11.07, 11.37, 11.86, and 11.71 day, respectively). The control and all potato cultivars had the longest significant male adult duration, except 'Marspeer', 'Mondial', 'Charlotte', and 'Hana' which had the shortest significant duration. Also, all potato cultivars had the longest significant female adult duration, except 'Hana', 'Liliy', and 'Lady Rossita' which had the shortest significant duration with castor (Table 1).

Larval and pupal weight of BCW

In vitro feeding on leaves of the evaluated potato cultivars and castor significantly affected the average weight of 6th instar larvae and pupae of BCW (Table 2). In comparison to castor, which gave the highest significant weight for larvae and pupae (0.92 and 0.48 g, respectively), potato cultivars negatively affected weight of both. 'Charlotte' gave the lowest significant weight of larvae and pupae (0.38 and 0.31 g, respectively), followed by 'Inova' (0.15 and 0.11 g, respectively) and 'Liliy' (0.19 and 0.13 g, respectively) without significant differences between them.

Fecundity and fertility of BCW

Table 3 presents highly significant differences between potato cultivars and castor in their effect on no. of laid eggs/female of BCW and their hatchability. The control (castor) had the largest significant no. of laid eggs/female and the highest significant hatchability. The lowest no. of laid eggs/female observed with potato cultivars Charlotte, Liliy, Princces and Inova (0, 63.33, 119.0 and 134.50, respectively). 'Marspeer', 'Lady Rossita', 'Caroso', 'Hana' and 'Hermes' showed moderate no. of laid eggs without significant differences between them. The highest no. of laid eggs recorded with 'Mondial' without significant differences than control. The highest hatchability percentages were found with potato cultivars Hana, Princces, (81.92 and 78.78 %, respectively) without significant differences than control. The lowest hatchability observed with 'Inova', 'Liliy' and 'Lady Rossita' (7.33, 22.43 and 31.23 %, respectively).

Attractive of the 2nd instar BCW larvae

Data in Table 4 showed highly significant differences between the evaluated potato cultivars in the no. of BCW larvae attracted to their leaves after 2h of treatment, while there were non-significant differences between them after 4 and 6 h of treatment. After 2 h of treatment, potato cultivars Princces, Hana, and Inova had high attractiveness to BCW larvae (11.2, 9.2 and 8.4 larvae, respectively). Potato cultivars Marspeer, Charlotte, and Mondial were moderately attractive (5.6, 5.2, and 3.6 larvae, respectively), while Lady Rosetta, Hermes, and Caruso were less attractive (2.8, 2.0, and 1.6 larvae, respectively). In general, potato cultivars Hermes, Caroso, Liliy, and Charlotte obtained the lowest percentages of attracted larvae (21.6, 22.4, 26.6 and 28.0%, respectively). Potato cultivars Lady Rossita, Mondial and Marspeer had moderately percentages of attracted larvae (28.8, 30.4 and 32.8%,

respectively), while cultivars Princces, Inova and Hana recorded the highest percentages of attracted larvae (48.0, 45.6 and 40.8%, respectively).

The metabolic activity of the 4th instar BCW larvae

Table 5 shows that feeding the 4th instar larvae of BCW on the leaves of the evaluated potato cultivars and castor had a highly significant influence on its metabolic activity. 'Mondial' and 'Lily' had the lowest significant influence on larvae content of protein (11.7 and 12.6 mg/g.b.wt, respectively), carbohydrate (8.36 and 7.96 mg/g.b.wt, respectively), and lipid (3.73 and 2.31 mg/g.b.wt, respectively) compared to control, except carbohydrate content. 'Hermes' had the similar significant effect as control on larvae content of carbohydrate and protein, but less significant for lipid content. The protein and carbohydrate content of larvae was greatly improved by feeding on leaves 'Inova', 'Hana', 'Lady Rossita', and 'Charlotte'. Only, feeding on 'Hana' leaves gave the highest significant lipid content (5.2 mg/g.b.wt) compared to control.

Table 6 shows the highly significant effects of the evaluated potato cultivars compared to the castor on activity of protease (digestive enzyme) and GST (detoxifying enzyme) enzymes as well as amino acid content of the 4th instar BCW larvae. In comparison to control, protease activity (µg aalanine/min/g.b.wt) was low with 'Lily'(42.0), 'Hermes' (45.7), 'Princces' (52.0), and 'Caroso' (84.0), but high with 'Charlotte' (293.3), 'Hana' (194.7), and 'Marspeer' (186.7). GST activity (mmol sub. conjugated/min/g.b.wt) was low with 'Hana' (39.7), 'Mondial' (50.3), 'Charlotte' (62.7), 'Hermes' (76.3), and 'Caroso' (83.0), while was high with 'Inova' (185.3) and 'Marspeer' (153.0) in comparison to the control (96.7). 'Pricces' and 'Lily' had a detrimental effect on the amino acid content of larvae (196.3 and 167.7 µg alanine/g.b.wt, respectively), but 'Mondia', 'Lady Rosetta', 'Marspeer', and 'Caroso' had a positive effect.

Antifeeding Mechanism in potato against BCW larvae

Antioxidants plant content

The activity of CAT and Flav antioxidants was differed significantly between infested and healthy plants of low-sensitive 'Lily' and high-sensitive 'Hana', while GR activity was showed no significant differences between all treatments (Fig. 1). GR activity was not responsive either between cultivars or in response to infestation (Fig. 1C). The flavonoids content of did not differ significantly between healthy plants of both cultivars (Fig. 1a). The flavonoid content was increased by BCW larvae infestation, the increase was significantly larger in low-sensitive 'Lily' than high-sensitive 'Hana'. CAT activity was also comparable in healthy plants from both cultivars (Fig. 1B). Only low-sensitive 'Lily' was affected by BCW larvae infestation, which enhanced CAT activity.

content of endogenous Phytohormones

The leaf content of 28-homobrassinolide (HBL) and methyl jasmonate (MeJA) was significantly different ($P<0.05$) between infested and healthy plants of both cultivars (Fig. 2). The leaf content of HBL for the healthy plants of both cultivars was significantly similar (Fig. 1B). BCW larvae infestation increased HBL synthesis only in low-sensitive 'Lily', while it remained unchanged in high-sensitive 'Hana'.The leaf content of MeJA differed significantly between potato cultivars as healthy plants, and the content washigh in high-sensitive 'Hana'. BCW larvae infestation increased MeJA synthesis, with a high increase in 'Hana'.

BCW larvae attack induced brassniosteroids biosynthesis gene

The relative expression of several defense related genes was accomplished in either cultivar under healthy and infested conditions (Figure 3A). It is observed that the highest expressed gene was cytochrome P450-dependent fatty acid hydroxylase (CYP450 94A2-like) that is involved in biosynthesis of brassinosteroids,

and there was no significant difference between cultivars Lily and Hana. Jasmonic acid biosynthesis gene OPDAreductase (OPR) was also induced in both cultivars in response to infesting damage, however, the gene was more expressed in 'Lily' than 'Hana'.

Effect of BR biosynthesis blocking on activate Jasmonates biosynthesis gene

As shown in Fig. 3B, the OPR gene (OPDA reductase, jasmonic acid biosynthesis) was dramatically upregulated in BRZ-plants compared to mocked-plants by 4.25- and 3.6- fold of increase with 'Hana' and 'Lily', respectively. The relative expression level of the OPR gene in high-sensitive cultivar Hana was significantly lower than low- sensitive cultivar Lily by 41%.

DISCUSSION

Black cutworm infestation causes severe damage to many important economic crops, including potatoes.It can destroy entire fields, especially if not detected early. The situation is getting worse because damagein many agro-ecosystems is unpredictable, so it is not detected until there is a significant reduction in vegetative and yield (Kroschel et al. 2020). The use of resistant potato cultivars is the easiest, safest, most practical, and best environmentally friendly way to control pest damage and reduce its spread. However, there is a scarcity of knowledge on resistant potato cultivars to black cutworm larvae and their mechanisms. Therefore, this study was carried out to identify resistant potato cultivars and their possible defense mechanisms against feeding of black cutworm larvae. A no-choice test was performed to evaluate 10 different potato cultivars in terms of their anti-feeding impact on black cutworm larvae compared to castor (a preferred host - control) under laboratory conditions. In no-choice-test, only one host is available for feeding black cutworm larvae and larvae that cannot feed will be impeded in their growth or die. Biological and behavioral bioassays have been used to characterize black cutworm resistance. This approach of evaluating potato resistance to feeding of BCW larvae was followed by Salah et al. (2012) under field and laboratory conditions.

Clear significant differences were observed in the degree of interaction of potato cultivars with BCW larvae. This is consistent with the findings of Gulab et al. (2001) and Kumar and Tiwary (2009) who identified significantly differences between potato cultivars in leaf damages caused by BCW larvae feeding. The evaluated potato cultivars exhibited an antifeeding impact on BCW larvae. Feeding larvae on leaves of potato cultivars caused a decrease in average weight of both larvae and pupae compared to castor leaves (Table 2). This coincides with the short lifespan of the larvae and pupae, especially with 'Princces', 'Carosso', 'Hana', 'Charlotte', and 'Lady Rossita' (Table 1). Oviposition for BCW females and egg hatchability (%) were lower with all potato cultivars compared to control, except for 'Mondial' and egg hatchability with 'Hana' and Princces (Table 3). Different studies refer to the differences in the biological and biochemical performance of insects in different cultivars depending on their susceptible degree (Metspalu *et al*. 2000). Adults of Colorado potato beetle (*Leptinotarsa decemlineata* Say; Coleoptera: Chrysomelidae) limit egg numbers according to the capacity of potato cultivars to support larvae feeding (Pelletier 1995). Also, feeding of *Phthorimaea operculella* larvae on resistant potato cultivar decrease larval survival, weight and size of larvae and pupae, in addition to increasing larval development times (Horgan et al. 2010).

Most herbivorous insects have qualitatively similar nutritional requirements since the basic chemical composition of their tissues and their metabolic processes are generally similar. Proteins, amino acids, lipids, and carbohydrate contents are generally the essential biochemical components necessary for insect development, growth, synthesize chitin, and perform its vital activities (Sayle 1928). However, the chemical and enzymatic reactions associated with digestion, absorption and detoxification of food in the

insect gut are sensitive to physicochemical parameters of the feeding host. So, the content of amino acid, proteins, carbohydrate, and lipids, as well as the activity of digesting (protease) and detoxifying (GST) enzymes were estimated in the 5th instar larvae. The content of protein, carbohydrate, and lipids was lower in larvae fed either 'Lily' or 'Mondial' leaves than in control larvae (Table 5). Feeding 'Lily' leaves to larvae also had a detrimental impact on their amino acid content (Table 6). Larvae feeding on the leaves of 'Carosso', 'Hermmes', 'Lily', 'Mondial', and 'Princces' inhibited the activity of protease and GR enzymes compared with castor leaves (Table 6). This has a significantly impact on larval growth and development at these cultivars.

According to the estimated parameters, the evaluated potato cultivars can be divided into three groups: the low- sensitive cultivars were 'Hermmes', 'Lily', and 'Princces; the moderate effect cultivars were 'Caroso','Charlotte', 'Inova', 'Lady Rossita', and 'Marspper'; and the high- sensitive cultivars were 'Hana' and 'Mondial'. Salah et al. (2012) classified the evaluated potato cultivars as highly resistant ('Alpha', 'Lesita', and 'Bright') and highly sensitive ('Desiree' and 'Spunta') based on the findings of BCW larval weight gains fed on leaves of cultivars under laboratory and field conditions.

Plants have evolved various resistance mechanisms to overcome insect pest damage (Schoonhoven et al. 1998). Insect resistance mechanisms are either constitutive (the inherited ability of the host plant to defend itself against the insect pests) or induced (the plant response to insect attack). They can be grouped into three main categories: antixenosis or non-preference, tolerance and antibiosis. Antibiosis refers to a plant's adverse effects on insect's survival, development, or fecundity (Smith and Clement, 2012). The adverse effect of antibiosis may be mild or cause death, including larval mortality, disturbance of the life cycle and the reduction in fecundity of the insect. The no-choice test evaluates the antibiosis mechanisms (Van Driesche and Murray 2004). The morphological and biochemical of plants are responsible of defense strategies against herbivorous insects. The antifeeding mechanisms in potato against BCW larvae were little studied. Only, Salah et al. (2012) found a negative correlation between dry matter and solanine of potato plant and black cutworm larvae weight gain. Several studies have shownthat enzymatic and non-enzymatic antioxidants, as well as phytohormones, play a direct or indirect role in plant defenses against herbivore insects (Chen et al. 2009, Duffey and Felton 1991, Duffey and Stout 1996, Gill et al. 2010, Simmonds 2003, Treutter 2006, Usha Rani and Jyothsna 2010, War et al. 2011).

External stimuli like insect feeding induce the plant to enhance the production and accumulation of ROS as an early plant defense response. The high levels of ROS as H_2O_2 damages living organisms. Plant-produced H_2O_2 molecules have the potential to cause oxidative stress damage to insect midgut (Maffei et al. 2007). While plants have developed complex protective mechanisms for scavenging ROS (Howe and Gander 2008). These include enzymatic scavenging systems, such as CAT (Howe and Schilmiller 2002) and non-enzymatic scavenging systems, such as flavonoids (Kaur et al. 2017). This explains why BCW larvae feeding causes an increase in flavonoid synthesis, notably in low- sensitive cultivar Lily, as well as an increase in catalase enzyme activity solely in it. CAT activity was associated with increased plants defenses against some pests as aphid in tobacco (Zhao et al. 2016), *Helicoverpa armigera* in pigeonpea (*Cajanus cajan*L.) (Kaur et al. 2017). Flavonoids have also been linked to herbivorous insect resistance as cytotoxins affecting oviposition and feeding of insect, or as free-radical scavenging (Baur et al. 1998, Simmonds 2001&2003,Treutter 2006)

The most remarkable finding of the phytohormone analysis was that feeding BCW larvae increased 28-HBL synthesis and accumulation in the low- sensitive cultivar Lily (Fig. 2A). On the other hand, feeding BCW larvae increased MeJA synthesis and accumulation in the high- sensitive cultivar Hana (Fig. 2B).

HBL or homobrassinolide is one of the most bioactive forms of brassinoseroids (BRs) beside brassinolide and 24-epibrassinolide (Anwar et al. 2018). BRs is a class of steroid plant hormones that effectively regulate responses to biotic and abiotic stresses (De Bruyne et al. 2014, Lozano-Durán and Zipfel 2015, Nolan et al. 2019, Peres et al. 2019, Saini et al. 2015). The functional and molecular mechanisms of BRs in abiotic stress tolerance and pathogen resistance have been analyzed and clearly identified in numerous previous studies (Ahammed et al. 2020, De Bruyne et al. 2014, Hafeez et al. 2021, Krishna et al. 2017, Lozano-Durán and Zipfel 2015, Peres et al. 2019), but the effects of BRs on herbivorous insect feeding remain largely unclear (Miyaji et al. 2014; Pan et al. 2018). Generally, BRs signaling adaptive mechanismsin response to stress are associated with RBOHB-dependent oxidative burst (Miyaji et al., 2014 and Deng et al., 2016). Under the auspices of BRs, there is a trad-off between growth and adaptation under stressful conditions. The BRs transcription factor BES-1 promotes plant growth and inhibit flavonoid biosynthesis. Upon on the inhibition of BES-1 activity suppression (by UV-B for example), the signaling of BRs is redirected to activating the synthesis (H_2O_2) and elimination of reactive oxygen species by flavonoid biosynthesis genes (Liang et al., 2020). In this work, the accumulation of HBL in low- sensitive 'Lily' could berelated to the induced antioxidative power (Enzymatic and non-enzymatic).

MeJA is a physiologically active derivate of the phytohormone jasmonic acid (JA), collectively known as jasmonates (JAs). Jasmonates are well-known for responding quickly and effectively to herbivore attacks by induce the synthesis of secondary metabolites such as phytoalexins and PRP that are either deterring or harmful to infesting feeding insects (Wang and Wu, 2013, Ali et al. 2018, Hazman et al., 2019, Ye et al. 2021). Jasmonates burst in wounding spot to aid in immediate anti-herbivore response, but they also play a critical function in long-distance systemic signaling for alerting and priming intact nearby tissues of the same plant or even surrounding individual plants (for review see Okada et al. 2015). Jasmonates with a volatile nature are the most effective in such a defensive approach. MeJA may reach distant places in plants via the vapour phase or intercellularly through phloem, unlike free JA, which may not be able to enter cellular membranes due to its acidic nature (for review see Cheong and Choi 2003). In this study, MeJA concentration showed the opposite trend to BR at the timepoint when samples were collected after 72 h following infestation. MeJA was much lower in the low- sensitive cultivar Lily compared to the high-sensitive cultivar Hana (Fig. 2B). This finding might be linked to the hostile BR-JA relationship (Miyaji et al. 2014, Saini et al. 2015).

Gene expression profile revealed BR-JA antagonism interaction

The expression profile of several genes related to hormones biosynthesis and antioxidative defense was performed. The most noted observation is the higher expressed CYP450 94A2-like BRs biosynthesis gene comparing to jasmonates biosynthesis (OPR) and phenylalanine ammonium lyase (PAL) (Fig. 3A). Jasmonates synthesis was reported to be swiftly synthesized in wounded rice plants in minutes scale then catabolized or deactivated within few hours (Hazman et al. 2019). Thus, it is expected to find jasmonate biosynthesis gene OPR expression being attenuated comparing to BRs biosynthesis gene CYP450. For more insights about the interaction between BRs and JA, we have treated potato plants grown in pots with BRZ (brassinazol) as BRs biosynthesis blocking agent (He et al., 2017) and triggered a mechanical wounding manually using clean sterile forceps. The gene expression of OPR was greatly induced in either cultivar (Hana and Lily), yet the expression was higher in cultivar Lily than Hana (Fig. 3B). Campos et al. (2014) reported that jasmonate negatively interact with BR and reduced anti-herbivory resistance level in tomato.

Conclusion

We hypothesis that potato resistance to leaf chewers herbivores (here cutworm larvae) constitutes of several cooperative strategies including the production of ROS, mainly H_2O_2, at the site of wounding for offsetting damage derived from invading larvae or insects. Since H_2O_2 can diffuse quickly from wounding spot to other intact tissues causing sever cellular damaging, ROS accumulation should be combined with self-toxicity prevention enzymatic antioxidants as catalase. In parallel, non-enzymatic antioxidants are extremely useful as anti-herbivory compounds with potential toxicity to invading larvae. The accumulation of Methyl jasmonate is believed to involve in long-distance systematic signaling to prime adjacent intact tissue. The mechanical wounding stress could highlight the antagonistic interaction between BRs and JA that seemed to be involved in inhibiting anti-herbivory traits in potato susceptible cultivars. Finally, there is still much work needed to be accomplished to know more details about anti-herbivory molecular mechanisms in potato. Achieved and planned research work could help with improving host plants resistance against invading herbivores using sustainable agricultural practices.

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Table 1. Duration of developmental stages of *Agrotis ipsilon* Hufn. after feeding larvae on the leaves of ten potato cultivars compared to castor leaves in a no-choice test.

Cultivar	Egg	Larva	Pre-pupa	Pupa	Adult male	Adult female
Caroso	3.15±0.15^{bcd}	23.79±0.80^{bc}	2.10±0.10	12.37±0.14^{ab}	21.59±1.43^{ab}	12.40±0.65^{ab}
Hana	2.71±0.18^{d}	23.83±0.50^{bc}	1.96±0.04	11.86±0.25^{bcd}	17.29±1.88^{bcd}	9.0±1.28^{c}
Hermes	3.67±0.21^{ab}	24.87±0.65^{ab}	2.0±0.0	11.50±0.22^{cd}	22.75±0.92^{a}	13.62±0.69^{a}
Charlotte	ND	24.16±0.59^{bc}	2.0±0.0	12.17±0.35^{bc}	16.89±3.14^{bcd}	13.07±0.92^{ab}
Inova	3.0±0.0^{cd}	24.93±0.66^{ab}	2.13±0.09	11.07±0.28^{d}	22.46±0.76^{a}	13.53±0.57^{a}
Lady Rossita	3.73±0.14^{ab}	24.07±0.42^{bc}	2.0±0.0	11.37±0.34^{cd}	24.64±1.479^{a}	10.90±0.39^{bc}
Lily	3.33±0.33^{bc}	24.67±0.60^{abc}	2.0±0.0	12.37±0.31^{ab}	20.44±1.76^{ab}	9.37±1.33^{c}
Marspeer	2.60±0.24^{de}	25.84±1.02^{ab}	2.0±0.0	11.71±0.26^{bcd}	14.43±2.28^{d}	14.25±1.42^{a}
Mondial	3.0±0.05^{cd}	25.92±0.82^{ab}	2.04±0.08	11.94±0.18^{bc}	15.08±0.78^{cd}	13.04±0.78^{ab}
Princces	4.20±0.20^{a}	22.44±0.29^{c}	2.0±0.0	11.94±0.20^{bc}	23.28±1.36^{a}	14.21±0.49^{a}
Castor (control)	2.14±0.09^{e}	26.67±0.54^{a}	1.86±0.11	13.09±0.19^{a}	19.62±16.73^{abc}	9.81±0.33^{c}
F. value	**20.189**	**2.810**	**0.847^{ns}**	**4.229**	**5.069**	**5.277**
P. value	**0.000**	**0.003**	**0.584**	**0.000**	**0.000**	**0.000**

Mean value ± standard error (n=5). Means in each column with different letters are significantly different at *P* <0.05 according to Duncan's multiple range test. ^{ns}Non-significant.

ND: Not Detected (the females not laid eggs).

Table 2. Average weight of the 6thinstar larvae and pupae of *Agrotis ipsilon* Hufn. after feeding larvae on the leaves of ten potato cultivars compared to castor leaves in a no-choice test.

Cultivar	6thinstar Larvae	Pupae
Caroso	0.71±0.02^{cd}	0.39±0.01^{b}
Charlotte	0.38±0.02^{g}	0.31±0.03^{c}
Hana	0.61±0.03^{e}	0.38±0.01^{b}
Hermes	0.74±0.03^{bc}	0.40±0.01^{b}
Inova	0.15±0.01^{h}	0.11±0.02^{d}
Lady Rossita	0.82±0.03^{b}	0.43±0.02^{b}
Lily	0.19±0.02^{h}	0.13±0.02^{d}
Marspeer	0.49±0.02^{f}	0.41±0.01^{b}
Mondial	0.63±0.02^{de}	0.37±0.02^{b}
Princces	0.55±0.11^{ef}	0.40±0.04^{b}
Castor (control)	0.92±0.03^{a}	0.48±0.02^{a}
F. value	**49.929**	**39.347**
P. value	**0.000**	**0.000**

Mean value ± standard error (n=5). Means in each column with different letters are significantly different at *P* <0.05 according to Duncan's multiple range test.

Table 3. Fecundity and fertility of *Agrotis ipsilon* Hufn. after feeding larvae on the leaves of ten potato cultivars compared to castor leaves in a no-choice test.

Cultivar	No. of laid eggs/female[z]	Hatchability[z] (%)
Caroso	571.70±28.08^{b}	57.86±4.09^{c}
Charlotte	0.0±0.0^{d}	ND
Hana	574.33±74.28^{b}	81.92±3.16^{a}
Hermes	666.00±73.59^{b}	69.95±4.73^{bc}
Inova	134.50±97.24^{cd}	7.33±3.77^{e}
Lady Rossita	518.44±65.98^{b}	31.23±6.54^{d}
Lily	63.33±6.66^{cd}	22.43±7.46^{d}
Marspeer	357.25±78.09^{bc}	55.35±5.11^{c}
Mondial	1055.00±129.51^{a}	64.28±3.30^{c}
Princces	119.00±11.20^{cd}	78.78±4.77^{ab}
Castor (control)	1061.67±70.90^{a}	86.32±1.42^{a}
F. value	**18.080**	**37.030**
P. value	**0.000**	**0.000**

[z]Mean value ± standard error (n=5). Means in each column with different letters are significantly different at *P* <0.05 according to Duncan's multiple range test.

ND: Not Detected (the females not laid eggs).

Table 4. Attractiveness of the 2nd instar larvae of *Agrotis ipsilon* Hufn. to leaves of ten potato cultivars.

	No. of attracted larvae[z]			Percentage of attracted larvae (%)
Cultivar	**2h**	**4h**	**6h**	
Caroso	1.6±0.7^{c}	4.8±1.0^{ab}	4.4±1.3	21.6
Charlotte	5.2±1.7^{bc}	4.0±2.1^{ab}	4.8±1.3	28.0
Hana	9.2±1.9^{ab}	7.6±1.6^{ab}	3.6±0.7	40.8
Hermes	2.0±1.1^{c}	3.6±0.9^{ab}	5.6±1.9	22.4
Inova	8.4±0.9^{ab}	9.2±1.5^{a}	5.2±0.8	45.6
Lady Rossita	2.8±0.8^{c}	5.6±1.6^{ab}	6.0±2.5	28.8
Lily	3.2±1.8^{c}	7.2±2.1^{ab}	4.4±1.2	26.6
Marspeer	5.6±1.9^{bc}	6.8±2.3^{ab}	4.0±1.1	32.8
Mondial	3.6±0.9^{c}	3.2±1.0^{b}	8.4±1.7	30.4
Princces	11.2±2.1^{a}	9.2±1.9^{a}	3.6±1.7	48.0
F. value	**4.719**	**1.709^{ns}**	**0.884^{ns}**	
P. value	**0.000**	**0.119**	**0.548**	

Mean value ± standard error (n = 5). Means of no. of attracted larvae after 2h with different letters are significantly different at *P*< 0.05 according to Duncan's multiple range test. [ns]Non-significant.

Table 5. Total protein, carbohydrate, and lipids content in 4th instar larvae of *Agrotis ipsilon* Hufn. fed on the leaves of ten potato cultivars compared to castor leaves.

Cultivar	Total proteins[z] (mg/g.b.wt)	Total carbohydrates[z] (mg/g.b.wt)	Total lipids[z] (mg/g.b.wt)
Caroso	20.36±0.89^{c}	6.76±.41^{d}	3.60±0.15^{d}
Charlotte	25.80±1.50^{ab}	13.33±0.64^{bc}	4.28±0.14^{bc}
Hana	23.27±.0.69^{bc}	16.20±0.47^{a}	5.20±0.18^{a}
Hermes	16.0±0.61^{d}	7.30±0.40^{d}	1.41±0.15^{f}
Inova	26.60±0.46^{a}	13.40±0.38^{bc}	4.63±0. 18^{b}
Lady Rossita	22.80±1.21^{bc}	16.10±1.15^{a}	4.68±0.12^{b}
Lily	12.63±.0.68^{e}	7.96±0.46^{d}	2.31±0.12^{e}
Marspeer	21.43±1.34^{c}	14.47±0.33^{ab}	4.00±0.11^{cd}
Mondial	11.73±0.73^{e}	8.36±0.55^{e}	3.73±0.11^{d}
Princces	20.26±1.08^{c}	12.0±0.29^{cd}	3.93±0.12^{cd}
Castor (control)	16.43±0.94^{d}	11.46±0.66^{d}	4.23±0.18^{bc}
F. value	**26.196**	**37.314**	**56.041**
P. value	**0.000**	**0.000**	**0.000**

Mean value ± standard error. Means in each column with different letters are significantly different at *P* <0.05 according to Duncan's multiple range test.

Table 6. Activity of proteases and glutathione S-transferase (GST) enzymes and total free amino acids content of *Agrotis ipsilon* Hufn. larvae fed on leaves of ten potato cultivars compared to castor leaves in a no-choice test.

Cultivar	Proteases activity[z] (µg alanine/min/g.b.wt)	GST activity[z] (mmolsub.conjugated/min/g.b. wt)	Total free aminoacids[z] (ug alanine/g.b.wt)
Caroso	84.00±3.05^{e}	83.00±1.15^{d}	102.67±3.71^{d}
Charlotte	293.33±14.53^{a}	62.67±3.18^{e}	66.00±2.08^{e}
Hana	194.67±5.84^{b}	39.67±2.96^{f}	65.67±1.20^{e}
Hermes	45.67±2.96^{f}	76.33±3.18^{d}	71.33±5.20^{e}
Inova	126.33±4.37^{c}	185.33±9.33^{a}	92.33±3.84^{d}
Lady Rossita	112.67±3.93^{c}	105.67±2.33^{c}	167.67±6.23^{b}
Lily	42.00±1.73^{f}	106.00±2.52^{c}	42.33±1.45^{f}
Marspeer	186.67±6.69^{b}	153.00±5.57^{b}	125.33±7.42^{c}
Mondial	92.67±2.18^{de}	50.33±2.60^{ef}	196.33±13.69^{a}
Princces	52.00±4.04^{f}	96.00±1.5^{c}	47.33±3.18^{f}
Castor (control)	109.67±5.24^{cd}	96.67±5.239^{c}	73.33±4.33^{e}
F. value	**163.250**	**102.572**	**71.540**
P. value	**0.000**	**0.000**	**0.000**

Mean value ± standard error. Means in each column with different letters are significantly different at *P* <0.05 according to Duncan's multiple range test.

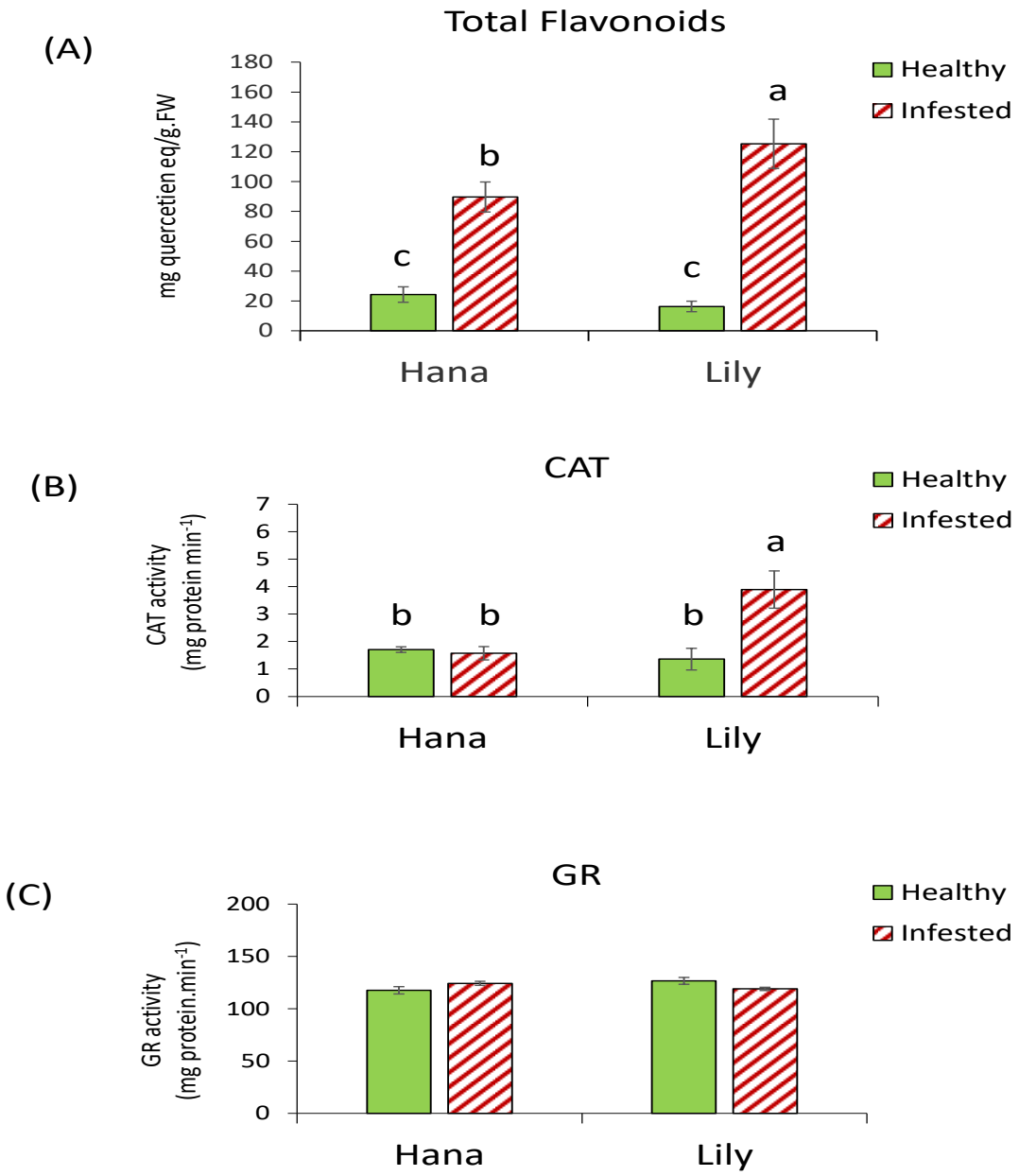

Fig. 1. Leaf content of flavonoids (A), and activity of catalase (CAT – B) and glutathione reductase (GR -C) enzymes of healthy and *Agrotis ipsilon* Hufn. infested plants of potato cultivars Hana (highly sensitive) and Lily (low sensitive). Columns in Fig. 1 A&B with the same letter represent means that are not significantly different according to Duncan's multiple range test (P <0.05). Vertical bars represent ± standard error of the mean (n = 3). Non-significant differences in GR activity.

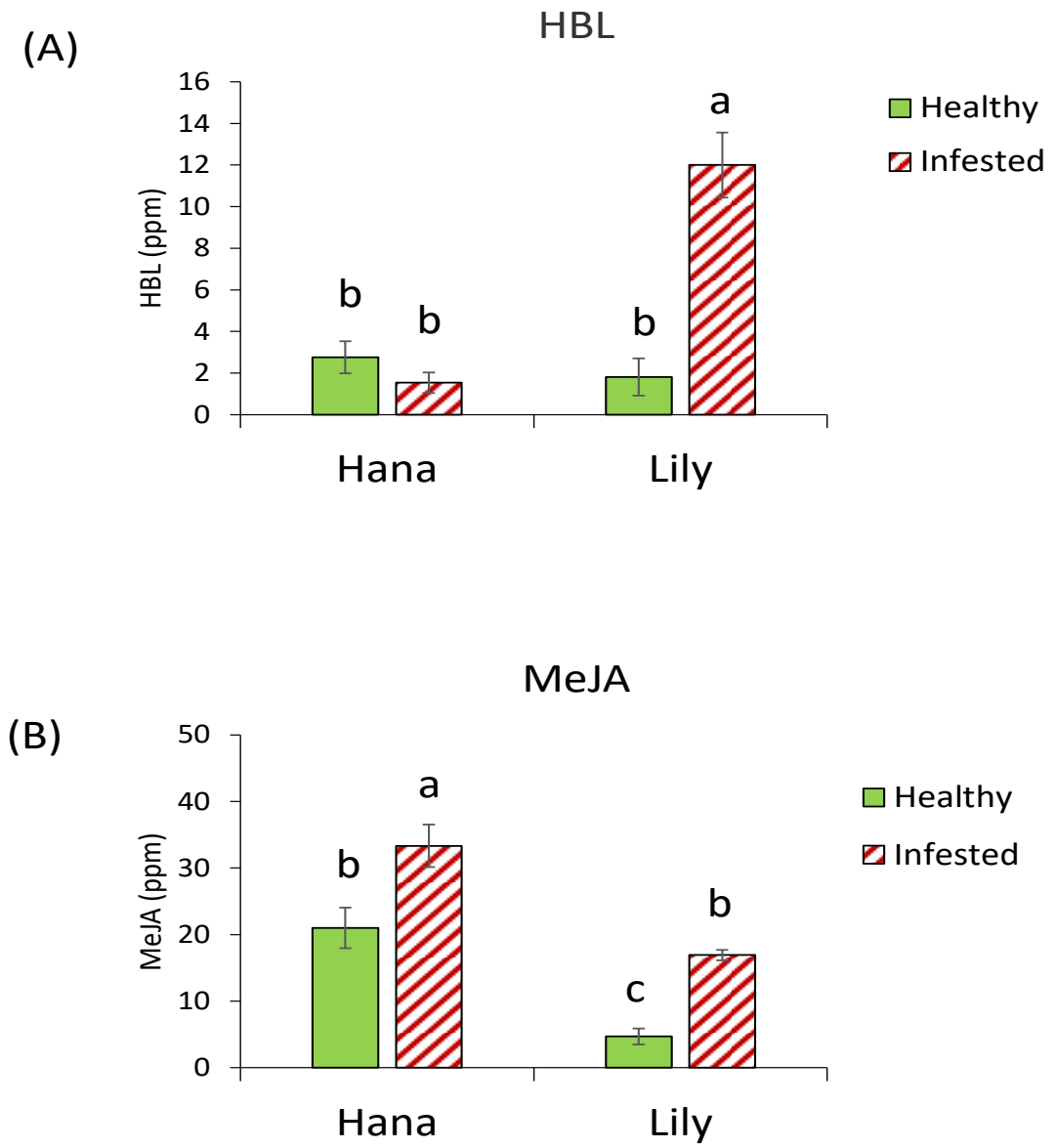

Fig. 2. Leaf content of phytohormones 28-homobrassinolide (HBL; A) and methyl jasmonate (MeJA; B) of healthy and *Agrotis ipsilon* Hufn. infested plants of potato cultivars Hana (high-sensitive) and Lily (low-sensitive).Columns with the same letter represent means that are not significantly different according to Duncan's multiple range test (P <0.05). Vertical bars represent ± standard error of the mean (n = 3).

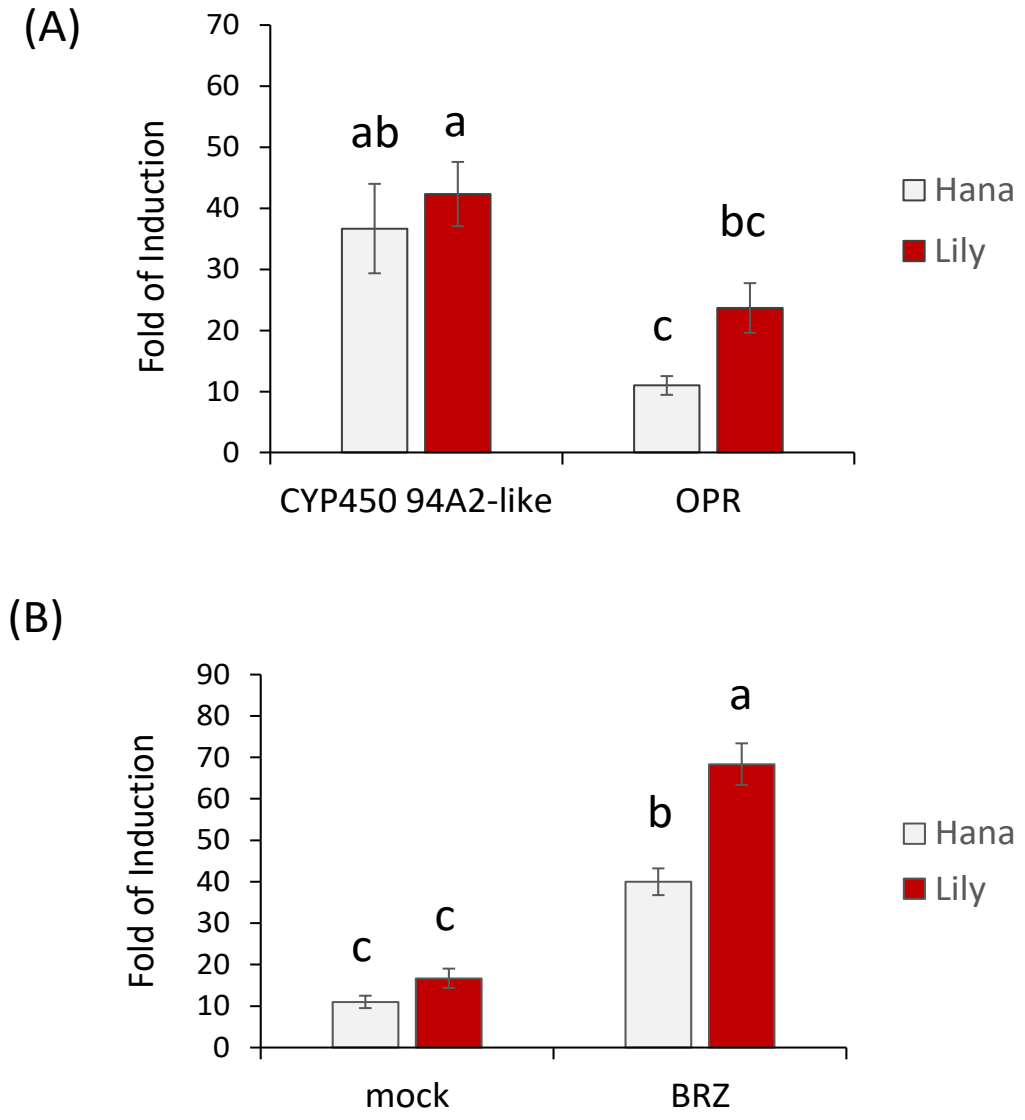

Fig. 3. Alterations in transcript accumulations of genes involved in phytohormone biosynthesis in the leaves of potato cultivars Lily (low-sensitive to *Agrotis ipsilon* Hufn. larvae feeding) and Hana (high-sensitive to *A. ipsilon* larvae feeding). A: CYP450 94A2-like (brassinosteroid biosynthesis) and OPR (OPDA reductase, jasmonic acid biosynthesis) genes. B: Effects of brassinazol (BRZ, brassinosteroids biosynthesis inhibitor) on OPR transcript accumulations in healthy and *A. ipsilon*-infested plants of both potato cultivars Columns with the same letter represent means that are not significantly different according to Duncan's multiple range test (P <0.05). Vertical bars represent ± standard error of the mean (n = 3).