ROLE OF AMINO ACIDS IN PROTECTING
PECTINOPHORA GOSSYPIELLA (SAUND.) AGAINST
STRONG PROTEIN-DENATURING ACTIVITY OF
PHENOLIC COMPOUNDS IN COTTON PLANTS

DINA A. AHMED 1; EL-SHARKAWY, MANAL A.A. 1;
RANIA, M. EL-SHENNAWY 1

1. Bollworm Dept., Plant Protection Research Institute, ARC, Dokki, Giza-Egypt.

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Abstract

To test if the generality of glycine secretion in the pink bollworm larvae related to the concentration of phenolic compounds (PCs), a feeding experiment with two concentrations of selected two PCs (pyrocatechol; Pyro & resorcinol; Res) was performed. Then, we determined and compared the lysine and glycine contents in the total body homogenate of treated and untreated 4th instar larvae. Results showed that the lysine and glycine contents recorded were 2.29 & 1.47 mg/100 mg of control larval body weight. Lysine content increased at low PCs concentration by 5.68 & 7.42% for Pyro and Res, respectively. On the other hand, its concentration decreased at high PCs concentration by 0.87 & 0.44%, respectively. On the other hand, glycine content generally decreased (except at low concentration of Pyro; 8.84%) by 4.76, 4.08 & 12.24%. Also, it could be noted that the generality of glycine secretion related to the concentration of PCs; whereas the less in glycine content was more notable in high concentration of PCs. And, the loss in glycine content was more than that for lysine.

Also, studies extend to other amino acids (AA) and the results indicated that it could be grouped AA in 3 groups:
1. A high AA concentration (more than 3 mg/100mg): GLU and ASP acid.
2. A moderate AA concentration (more than 2 mg/100mg): VAL, LEU, LYS, ARG, ALA and TYR.
3. A low AA concentration (less than 2 mg/100mg): THR, ILE, PHE, HIS, CYS, SER, GLY and PRO.

In the control PBW, glutamic acid and aspartic acid were present in high concentration (4.24 & 3.19 mg/100mg, respectively). As same manner with glycine; the losses in GLU and ASP contents were more than that for LYS. So, it could be used by PBW to overcome PCs toxicity. In addition, generally, at low PCs concentration, AA content increased except in the case of VAL and CYS. On contrast, at high PCs concentration, AA content decreased except in the case of MET, PHE, HIS and TYR. Commonly, it could be noted that Val, Cys, Gly and Ala had a higher percent of reduction. So, it means that other free amino acids may be used by some herbivores as counteractive agents against the denaturing activity of PCs.

INTRODUCTION

The pink bollworm (PBW), Pectinophora gossypiella (Saund.) supposed to be a major cotton pest. Plants have established several chemical defenses against insects. Cotton plants produce numerous secondary plant chemicals including phenolic compounds (PCs) (Perveen et al., 2001). Cotton PCs induce a reduction in viability of dietary protein in lepidopterous insects by making covalent bonds with the amino
residue of lysine side chain in proteins, so it reduces the amount of lysine in proteins (Appel, 1993). To overcome these compounds, insects have evolved various detoxification mechanisms. In 1996 Konno et al. started series of studies about the chemical methods which used by insects to resist compounds which causing protein denaturing like PCs. They observed a high concentration of free glycine in the digestive juice and midgut of the silkworm, Bombyx mori, and several other Lepidoptera larvae. In 1997 Konno et al. throw In vitro studies found that free glycine totally inhibits the denaturing activity of the privet leaves. Pierpoint (1969) mentioned that the amino residue of glycine and lysine reacts with PCs in the same manner. The glycine amino residue competes with the lysine amino residue, and hence inhibits the PCs denaturing activity (Konno et al., 2010). Also, according to their theory, not just free glycine but either other amino acids (GABA, β-alanine and alanine) are able to inhibit PCs denaturing activity, indicating that amino residues are responsible for the inhibition.

Previously in 2014 we found that the amount of free glycine in PBW appears to have a clear association with the PCs detoxifying activity. Also, we noticed unexpected presence of some other amino acids (AA) like: glutamic acid. In the current study, we tried to covered some remaining questions. Firstly, we tried to study if the glycine secretion generality related to the concentration of PCs. Secondly, we were concerned about figuring out if glycine is the only AA which has a role against the PCs defense. Thirdly, we asked what amino acids structure is important for the PCs activity inhibition.

MATERIALS AND METHODS

Insects: Pectinophora gossypiella were reared on a modified artificial diet according to Abd El-Hafez et al. (1982), at the laboratory of Bollworms Department, Plant Protection Research Institute, ARC, Dokki, Giza.

Tested phenolic compounds: Two PCs namely; pyrocatechol and resorcinol (Roth: Bestellen sie zum Nulltarif); were used in present study. Figure (1) illustrated the chemical structure of these compounds. According to the results of Ahmed (2007), pyrocatechol and resorcinol proved to be toxic to PBW. For this reason, these compounds were chosen for studying the role of AA as detoxifying agent against cotton PCs in PBW. In order to examine the generality of glycine secretion related to the concentration of PCs, two concentrations of PCs were used:

1. Twice the LC50 (1.718 & 1.46 g/100g diet, for pyrocatechol & resorcinol, respectively).
2. Half of LC50 (0.4295 & 0.365 g/100g diet, for pyrocatechol & resorcinol, respectively).

The data for LC50 was taken from Ahmed et al. (2014).
Amino acids analysis: This part of study was conducted in order to determine free amino acids content in the 4th instar larvae of PBW after treatment with tested PCs concentrations. Newly hatched larvae (≈100 larvae) of PBW were allowed to feed on artificial diet containing the tested concentrations of the two tested PCs, for 2 hours and then transferred to feed on control diet. Fourteen days after treatment, the survived 4th instar larvae were collected and refrigerated (at 5ºC) for few minutes and then dried in the oven overnight (at 60ºC) for biochemical analysis. The total larval bodies were homogenized to get dry powder. Homogenates were weighted and kept till the biochemical determinations. Quantitative determination of amino acids was carried out at Regional Center for Food and Feed, ARC, Dokki, Giza, by high perform Amino Acid analyzer (Biochrom 30) and EZ chrome manual (software for data collection and processing), according to AOAC (2012).

RESULTS AND DISCUSSION

Does glycine secretion relate to the concentration of PCs?

To test if the generality of glycine secretion related to the concentration of PCs, a feeding experiment with two concentrations of each PCs was performed. Then, we determined and compared the lysine and glycine contents in the total body homogenate of treated and untreated 4th instar larvae (Table 1).

Lysine and glycine contents recorded 2.29 & 1.47 mg/100mg of control larval body weight. Lysine content increased at low PCs concentration by 5.68 & 7.42% for Pyro and Res, respectively. On the other hand, its concentration decreased at high PCs concentration by 0.87 & 0.44% for Pyro and Res, respectively. On the other hand, glycine content generally decreased (except at low concentration of Pyro; 8.84%) by 4.76, 4.08 & 12.24%. Also, it could be noted that the generality of glycine secretion related to the concentration of PCs; whereas the less in glycine content was more notable in high concentration of PCs. And, the loss in glycine content was more than that for lysine. It could be due to that glycine is assumed to be lost by binding to PCs, but this loss is less detrimental for herbivores than the loss of lysine. The larvae could be expending glycine (nonessential AA), for saving lysine (essential and a more valuable AA) (konno et al., 2010). So, the amount of free glycine in PBW seems to have a clear
association with the detoxification activity of the PCs. These findings approve our early findings (Ahmed et al., 2014), and in agreement with (konno et al., 2009).

Whether glycine is the only amino acid that has a role against the PCs defense? And what amino acids structure is important for the PCs activity inhibition?

To study if not just glycine but also other AA which has amino residues has a role against the PCs defense, we extend the determination to other amino acids (Table 1). Results indicated that it could be grouped AA in 3 groups:

1. A high AA concentration (more than 3 mg/100mg): GLU and ASP acid.
2. A moderate AA concentration (more than 2 mg/100mg): VAL, LEU, LYS, ARG, ALA and TYR.
3. A low AA concentration (less than 2 mg/100mg): THR, ILE, PHE, HIS, CYS, SER, GLY and PRO.

Table 1. Concentration of free amino acids (mg/100mg) in the total body homogenate of P. gossypiiella 4th instar larvae.

| Amino acids       | Control | After treatment with Pyrocatechol | %Change after Treatment With* | Resorcinol |
|-------------------|---------|----------------------------------|------------------------------|-----------|
|                   |         | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 |
| Threonine (THR)   | 1.66    | 1.79 | 1.60 | 1.71 | 1.63 | 7.83 | -3.61 | 3.01 | -1.81 |
| Valine (VAL)      | 2.28    | 2.18 | 2.02 | 1.94 | 1.78 | -1.22 | -11.40 | -14.91 | -21.93 |
| Methionine (MET)  | 1.03    | 1.15 | 1.12 | 1.11 | 1.16 | 11.65 | 8.74 | 7.77 | 7.63 |
| Isoleucine (ILE)  | 1.58    | 1.68 | 1.5 | 1.6 | 1.44 | 6.33 | -5.06 | 1.27 | -8.86 |
| Leucine (LEU)     | 2.55    | 2.64 | 2.43 | 2.62 | 2.47 | 3.53 | -4.71 | 2.75 | -3.14 |
| Phenylalanine (PHE)| 1.82   | 1.98 | 1.86 | 1.99 | 1.88 | 8.79 | 2.20 | 9.34 | 3.30 |
| Lysine (LYS)      | 2.29    | 2.42 | 2.27 | 2.46 | 2.28 | 5.68 | -0.87 | 7.42 | -0.44 |
| Histidine (HIS)   | 1.16    | 1.25 | 1.21 | 1.28 | 1.24 | 7.76 | 4.74 | 10.34 | 6.90 |
| Arginine (ARG)    | 2.26    | 2.48 | 2.22 | 2.29 | 2.2 | 9.73 | -1.77 | 1.33 | -2.65 |
| Cystine (CYS)     | 0.55    | 0.51 | 0.46 | 0.48 | 0.48 | -7.27 | -16.36 | -12.73 | -12.73 |
| Aspartic acid (ASP)| 3.19   | 3.24 | 3 | 3.47 | 3.06 | 1.57 | -5.96 | 8.78 | -4.08 |
| Serine (SER)      | 1.37    | 1.47 | 1.28 | 1.41 | 1.33 | 7.30 | -6.57 | 2.92 | -2.92 |
| Glutamic acid (GLU)| 4.24   | 4.27 | 3.84 | 4.29 | 3.91 | 0.71 | -9.43 | 1.18 | -7.78 |
| Glycine (GLY)     | 1.47    | 1.6 | 1.4 | 1.41 | 1.29 | 8.84 | -4.76 | -4.08 | -12.24 |
| Alanine (ALA)     | 2.28    | 2.65 | 2.11 | 1.81 | 1.65 | 16.23 | -7.46 | -20.61 | -27.63 |
| Tyrosin (TYR)     | 2.53    | 2.87 | 2.77 | 2.65 | 2.77 | 13.44 | 9.49 | 4.74 | 9.49 |
| Proline (PRO)     | 1.82    | 1.95 | 1.66 | 1.88 | 1.63 | 7.14 | -8.79 | 3.30 | -10.44 |

* %Change = \frac{(Treatment - Control)}{Control} \times 100
In the control PBW, Glutamic acid and aspartic acid were present in high concentration (4.24 & 3.19 mg/100mg, respectively). As same manner with glycine; the losses in GLU and ASP contents were more than that for LYS. So, it could be used by PBW to overcome PCs toxicity. Amino acids used to overcome PCs toxicity must be non-essential amino acids (insects can synthesize by themselves), like glycine, or derivatives, such as GABA (γ-amino butyric acid) and β-alanine, where it could be simply formed from glutamate and aspartate, respectively (Konno et al., 2010). They found that GABA and β-alanine inhibited the lysine-decreasing activity as effectively as glycine.

Generally, at low PCs concentration, AA content increased except in the case of VAL and CYS (underlined data; Table 1). On contrast, at high PCs concentration, AA content decreased except in the case of MET, PHE, HIS and TYR (dotted underlined data). Commonly, it could be noted that Val, Cys, Gly and Ala had a higher percent of reduction. So, it means that other free amino acids may be used by some herbivores as counterractive agents against the denaturing activity of oxidized PCs as mentioned previously by konno et al. (2010). They also indicated that amino residues are responsible for the inhibition, and the inhibitory activity differed significantly among the amino acids. While glycine, GABA, and β-alanine inhibited lysine-declining activity approximately completely at lower concentrations, alanine did not do this even at 5 times higher concentrations. It could be attributed to the structure similarity between glycine, β-alanine and GABA, where they have amino residues at the molecules ends, versus alanine that does not. They also indicated that these AA appear to be the only AA that are at the same time economically and efficiently, and therefore the benefits of production overreached the costs.

In conclusion, PBW could develop molecular strategies based on the secretion of AA like glycine, glutamic acid, and aspartic acid as a counteracting, to feed on the cotton plants which defense by accumulating PCs.

REFERENCES
1. AOAC 2012. Official method of analysis AOAC international No. 994.12 chapter 4: 18-19. 19th Edition, revision 2012.
2. Abd El-Hafez, Alia, A.G. Metwally and M.R.A. Saleh. 1982. Rearing pink bollworm *Pectinophora gossypiella* (Saund.) on kidney bean diet in Egypt. Res. Bull., Fac. Agric., Zagazig Univ. 576: 1-10.
3. Ahmed, Dina A. 2007. Biochemical and toxicological studies on the effect of some plant extracts on pink bollworm, *Pectinophora gossypiella* (Saunders), in relation to their phenolic contents. *Ph.D. Thesis, Faculty of Agriculture, Cairo University.*
4. Ahmed, Dina A. 2014. Role of glycine in protecting Pectinophora gossypiella against strong protein-denaturing activity of phenolic compounds in cotton plants. *J. Plant Prot. and Path., Mansoura Univ.*, vol. 5 (12): 1053-1063.

5. Appel, H.M. 1993. Phenolics in ecological interactions: the importance of oxidation. *J. Chem. Ecol. 19*: 1521-1552.

6. Konno, K., C. Hirayama and H. Shinbo. 1996. Unusually high concentration of free glycine in the midgut content of the silkworm, *Bombyx mori* and other lepidopteran larvae. *Comp. Biochem. Physiol., 115A*: 229-235.

7. Konno, K., C. Hirayama and H. Shinbo. 1997. Glycine in digestive juice: a strategy of herbivorous insects against chemical defense of host plants. *J. Insect Physiol. 43*: 217-224.

8. Konno, K., C. Hirayama, H. Shinbo and M. Nakamura. 2009. Glycine addition improves feeding performance of non-specialist herbivores on the privet, *Ligustrum obtusifolium*: *In vivo* evidence for the physiological impacts of anti-nutritive plant defense with iridoid and insect adaptation with glycine. *Appl. Entomol. Zool. 44* (4): 595-601.

9. Konno, K. C. Hirayama, H. Yasui, S. Okada, M. Sugimura, F. Yukuhiro, Y. Tamura, M. Hattori, H. Shinbo and M. Nakamura. 2010. GABA, β-Alanine and glycine in the digestive juice of privet-specialist insects: convergent adaptive traits against plant iridoids. *J. Chem. Ecol. 36*: 983-991.

10. Perveen, S.S., T.M. Qaisrani, S. Bhutta, R. Perveen and S.H.M. Naqvi. 2001. HPLC analysis of cotton phenols and their contribution in bollworm resistance. *OnLine Journal of Biol. Sci. 1* (7): 587-590. http://www.pjbs.org/ansinet/ojbs/journal/2001/toc1(7).

11. Pierpoint, W.S. 1969. α-Quinones formed in plant extracts, their reactions with amino acids and peptides. *Biochem. J. 112*: 609-616.
دور الأحماض الأمينية في حماية دودة اللوز القرنفلي من التأثير
المثير للبروتين الحاد بفعل المركبات الفينولية المتواجدة في نبات القطن

تشتري نباتات القطن على مركبات فينولية والتي تُعد من مرسبات البروتين، وقد وجد أنها تحدث تأثيرًا من خلال الاستقلال مع الحمض الأميني الليسين (حمض أميني ضروري) مما يؤدي لانخفاض كمية الليسين في البروتين. وقد أثبتت الدراسات السابقة أن عدد من حمض الديستنينج تتميز بوجود تركيز عالية من الحمض الأميني الجليسين (حمض أميني غير ضروري) وأنها تستخدم هذا الحمض في مقاومة العمل المرسوب للبروتين الحاد بفعل المركبات الفينولية، وذلك لأن الجليسين يرتبط مع مرسبات البروتين بدلاً من الليسين كوسيلة للحفاظ على محتواها من الحمض الأميني الليسي. وقد وجد دراسات سابقة وجود علاقة بين وجود الجليسين في دودة اللوز القرنفلي ومقاومة البروتين الفينولية، بالإضافة إلى أنه لوحظ وجود تركيز مرتفع من أحماض أمينية أخرى كالكالجاوثاميك. وعلى هذا فقد اهتم البحث الحالي

1. هل هناك علاقة بين إفراز الجليسين وتركيز المركبات الفينولية؟
2. هل الجليسين هو الحمض الأميني الوحيد الذي له دور في مقاومة المركبات الفينولية؟
3. هل هناك تركيب محدد للأحماض الأمينية التي تساهم في مقاومة الأحماض الأمينية؟

من الدراسات السابقة تم اختيار تركيزين مستويين من المركبات الفينولية (البروكاتيكلوئ والريزوسيول) التي تثبت استثمارها لدودة اللوز القرنفلي، وتم قياس محتوى كل من الجليسين وليسي، فكانت الكمية المسجلة هي 2.29 و1.47% في حالة الحشرات غير المعالمة. ارتفعت نسبة الليسي مع استخدام التركيز المنخفض لمركب الفينولية بتحليق 5.68 و 7.42% للبروكاتيكلوئ والريزوسيول على التوالي. في حين حدث انخفاض في نسبة التركيز المرتفع بتحليق 0.87 و 0.44% على التوالي. وعلى الجانب الآخر فقد انخفض تركيز الجليسين بصفة عامة، كما أن الإنخفاض الحاد في تركيز الجليسين كان أعلى من ذلك الحاد في تركيز الليسي، مما يؤكّد على دور الجليسين في مقاومة فعل المركبات الفينولية مما كان تركيزها.

كانت دراسة وجود تركيزات مرتفعة من أحماض الجلوتاميك (4.24%) والإسبارتيك (3.19%)، وأن الفقد الحاد لمحتواه أكبر من الفقد الحاد لمحتجز الليسي. هذا بالإضافة إلى أن التركيزات المنخفضة من المركبات الفينولية أدت بصفة عامة إلى زيادة تركيز الأحماض الأمينية فيما جذل الأحماض الأمينية الفينيل النسيتيتات، بينما في التركيزات المرتفعة حدث انخفاض لمحتجز الأحماض الأمينية فيما عدا الميثيونين، الفينيل ألانين، الهستيدن، والترسيز، وكان أعلى معدل خفض لالأحماض الأمينية الفينيل النسيتيتات، الجليسين، والكالجاوثاميك، مما يشير إلى إمكانية مشاركة أحماض أخرى غير الجليسين في مقاومة فعل المركبات الفينولية.
ROLE OF AMINO ACIDS IN PROTECTING PECTINOPHORA GOSSYPIELLA (SAUND.) AGAINST STRONG PROTEIN-DENATURING ACTIVITY OF PHENOLIC COMPOUNDS IN COTTON PLANTS