Research Paper

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Partial purification of Glutathione-S-transferases in Plutella xylostella (L.) show imparting resistance against Flubendiamide

Agriculture

KEYWORDS : Glutathion-s-transferase, Partial Purification, Detoxifying enzyme, Flubendiamide.

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ABSTRACT

Present investigation was undertaken to study Partial purification of Glutathione-S-transferases in imparting resistance in Plutella xylostella (L.) against Flubendiamide. To know the molecular mechanism of flubendiamide resistance in DBM, It was selected against flubendiamide. The LC50 of flubendiamide was 0.38 ppm at F5 generation which increased to 6.35 ppm in F10 selected generation resulting 16.62 fold resistance increased as compared to F5 generation. In partial purification by ammonium salt precipitation susceptible strain showed GST activity 2.94 fold pure and In resistance strain 6.96 fold pure by ammonium salt precipitation. 60-100 percent ammonium sulphate precipitated fraction 1 showed high specific activity as compared to fraction 2. Resistance strain showed 52 fold purity and susceptible strain showed 9 fold purity. From this concluded that fraction 1 showed high moleculer weight containing high specific activity.

INTRODUCTION

The diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: plutellidae) sometimes called as cabbage moth, dominant in South East Asia (Robert and Wright, 1996). It is one of the most important pests of cruciferous crops in the world and usually only feed on plants that produce glucosinolates. Its Control used to be through the use of chemical insecticides but in the 1980s resistance to pyrethroids developed, and soon afterwards, virtually all insecticides became ineffective. (Sarfraz and Keddie, 2005). Flubendiamide is new to crop protection, developed by Nihon Nohyaku Co. Ltd., (Tokyo, Japan). It belongs to a chemical family of benzenedicarboxamides or phthalic acid diamides. It showed extramly high activity against a broad spectrum of lepidopterious pest. Insecticidal activity of Flubendiamide through the activation of the ryanodine-sensitive intracellular calcium release channels, leading to the cessation of feeding immediately after ingestion of the compound, (Settele et al. 2008).

The study of resistance enzyme, its behaviour with available synergist for enhancing the toxicity of insecticide and increases susceptibility in insect for control pest population. Glutathione-S-transferases can cause resistance to insecticides by conjugating reduced glutathione to the insecticide or its metabolites. Mechanism of resistance involving target sites insensitivity which could be modifications of the nervous system (Rajurkar et al. 2003). In India there is no information available regarding or associated with mechanism of resistance to flubendiamide molecule in *P. xylostella*. Hence this study was undertaken

MATERIAL AND METHODS

Rearing of *P. xylostella*

Population of *Plutella xylostella* collected from different geographical locations of Akola district (M. S. India). Mass rearing was done in Entomology Department, Dr. PDKV, Akola. Further studies were done at Biotechnology Centre, Dr. PDKV, Akola during the session 2010-11. Rearing procedure was followed according to Lu and Sun (1984) to maintained the culture of *P. xylostella*. Rearing done under controlled conditions of temperature 25 ± 2°C, relative humidity 75 ± 5 per cent and photoperiod of 13 hrs light : 11 hrs dark. Rearing done for upto F_4 generations for establishing homogeneous laboratory population. Mustard seedlings were used for rearing purpose.

Development resistance to fluben dia -mide

Leaf dip method of bioassay for assessing the resistance levels. According to Tabashnik et al*., (*1987) 5 cm diameter Cabbage leaves were first washed with distilled water and then dried for about 1 hrs to remove water then dipped in an insecticide solution for 10 seconds and drain to remove excess solution at room temperature. The bioassay were conducted at room temperature. Ten third instar larvae were released on each disc in an individual petriplate where in blotting paper was placed at the bottom. Similarly ten larvae were released on cabbage leaf disc dipped in water only, which was treated as control. The median lethal concentration LC_{50} value determined the log dose probit (ldp) assay of insecticide flubendiamide. The resistance ratio (RR), which was calculated by the formula (Regupthy and Dhamu, 1990). LC_{50} of resistant strain (RS) divided by LC_{50} of susceptible strain (SS)

Enzyme Preparation

The third instar larvae (3.0 - 4.0 mg) were starved for 7-8 hours to remove all digested food particles and chilled in refrigerator. Whole larvae were homogenized using mortar and pestle in 100 mM sodium phosphate buffer (PB) (pH 6.5), containing 0.1 mM of EDTA, PTU and PMSF each. The homogenate thus obtained was centrifuged at 10,000 rpm for 20 minutes at 4°C in high speed refrigerated centrifuge, solid debris and cellular material was discarded. The resultant supernatant obtained was stored at -20°C and used as enzyme source. The protein was estimated by Bradford (1976).

Purification of Glutathione-S-transferase by Ammonium sulphate fractionation

Ammonium sulphate fractionation was performed as a first purification step of the enzyme. The crude enzyme extract was brought to 30 percent saturation with gradual addition of solid ammonium sulphate. Ammonium sulphate was slowly added to aliquot with constant stirring for 1h at 4°C. The resulting precipitate was collected by centrifugation at 6000 rpm for 30 min (Roe, 2001). Supernatant was removed and pellet was drained. Pellet was resuspended in buffer containing sodium phosphate buffer (pH 6.5) centrifuged at 10,000 rpm for 1h (Wang et al., 2007). The above step was repeated twice to obtain 60 percent and 100 percent ammonium sulphate saturation. Protein concentration was determined by the method of Bradford (1976) using bovine serum albumin (BSA) as standard.

Molecular exclusion chromatography

The supernatant obtained in the Ammonium sulphate fractionation loaded on to a Bio-Sil SEC-125 column (80×7.8cm) equilibrated with the buffer containing 10 mM Tris-HCl (pH 8.0). The fractions eluted from this column containing protein peak were collected. Protein concentration was determined by the method of Bradford (1976) using bovine serum albumin (BSA) as standard.

RESULTS AND DISCUSSION

Development of Resistance to Flubendiamide

Development of Resistance to Flubendiamide, the LC_{50} value of F_{10} selected population of *P. xylostella* against flubendiamide was found to be 6.35 ppm. It was 16.62 fold greater as compared to unselected F_5 population which was 0.38 ppm. Nirmal and Singh (2001) reported the development of resistance in DBM to the extent of 198-615 fold resistance to cypermethrin and 590 - 4576 fold resistance to fenvalerate.

Purification of Glutathion-s-transferase from *Plutella xylostella*

Glutathion-s-transferase purified from the crude enzyme obtained from third instar learvae of DBM, according to the purification procedure given in material and methods. Crude enzyme obtained 0.023 mg of protein and 0.085 µM mg protein^{-1} min^{-} specific GST activity in susceptible strain and 0.0246 mg of protein and 0.055 µM mg protein^{-1} min^{-1} specific GST activity in resistant strain. The enzyme source obtained from third instar larvae of DBM, first partially purified and fractionated by ammonium salt precipitation (ASP). Due to gradual addition of ammonium sulphate responsible for protein precipitated. In 0-30 percent saturation fractions, GST activity for susceptible strain was 0.109 µM mg protein^{-1} min^{-1} and for resistance strain was 0.160 µM mg protein^{-1} min^{-}. In 30-60 percent saturation fractions, GST activity for susceptible strain was 0.098 µM mg protein^{-1} min^{-1} and for resistance strain was 0.148 U/mg. In 60-100 percent saturation fractions showed high GST activity for susceptible strain was 0.250 µM mg protein^{-1} min^{-1} and for resistance strain was 0.383 µM mg protein^{-1} min^{-1} (Indicated in Table 1, Fig. 1). Purification of GST by ammonium salt fractionation In this step, detoxifying enzymes specific activity and fold purity were increased same as Rajurkar et al. (2003) obtained highest GST activity in 65-70% and ammonium salt saturation fraction of *H. zea* and *H. armigera*, respectively while studing levels of GST, its isolation and purification.

Purification of GST by Size exclusion chromatography

In 60-100 percent saturation fractions showed high GST activity hence it further purify by size exclusion chromatography. The specific GST activity of fraction 1 was 0.009 µM mg protein^{-1} min^{-1} in susceptible strain and 0.052 µM mg protein^{-1} min^{-1} in resistance strain. Resistance strain showed 52 fold pure and susceptible strain 9 fold pure. In GST 60-100 per cent ammonium sulphate precipitated fraction 1 showed high specific activity as compared to fraction 2. From this concluded that fraction 1 showed high moleculer weight containing high specific activity (Shown in table 2 and Fig 2). This correlated with Singh et al. (2010) reported that GST purified 8 fold by size exclusion chromatography from insect cells.

Diamondback moth has long history of eventually becoming resistant to every insecticide used extensively against it, in many countries. Thus, this pest was chosen in the present work to study the newer molecule which would further assist in elucidating the toxicity level.

Table 1: Partial Purification of Glutathion-s-transferase from *Plutella xylostella* by ammonium salt precipitation method

Sr. No.	Ammonium sulphate precipitated fraction (%)		Protein (ug/ul)	GST specific activity (µM mg protein^{-1} min^{-1})	Fold purity
1	0-30	Susceptible	0.012	0.109	1.28
		Resistance	0.014	0.160	2.90
2	30-60	Susceptible	0.020	0.098	1.15
		Resistance	0.019	0.148	2.69
3	60-100	Susceptible	0.007	0.250	2.94
		Resistance	0.010	0.383	6.96

Fig. 1: Partial Purification of Glutathion-s-transferase from *Plutella xylostella* by ammonium salt precipitation method

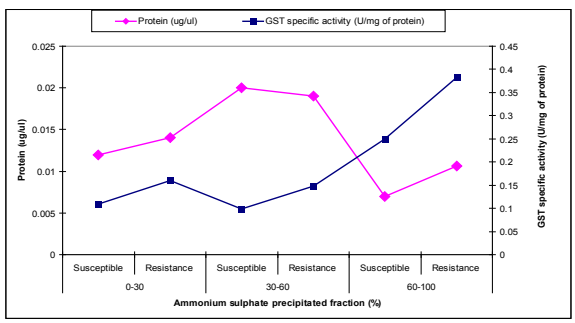
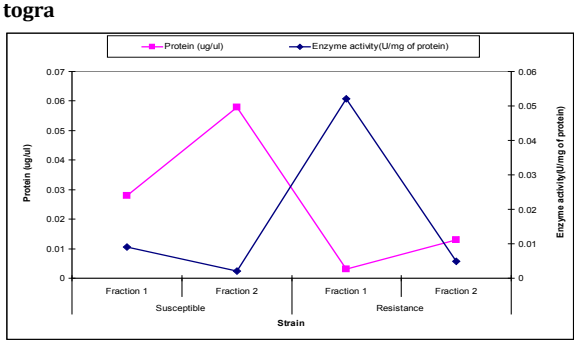


Table 2. Purification of GST Activity by Size exclusion chromatography

Strain	Fraction	Protein (ug/ul)	Enzyme activity(µM mg protein^{-1} min^{-1})	Fold purity
Susceptible	Fraction 1	0.028	0.009	9
	Fraction 2	0.058	0.002	2
Resistance	Fraction 1	0.003	0.052	52
	Fraction 2	0.013	0.005	5

Fig 2. Purification of GST Activity by Size exclusion chromatogra



REFERENCE

Bradford, M. (1976). A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein dye binding Anal. Biochem. 72 : 248-258. | Chopade, N. (2006). Studies on some morphological, biochemical and enzymological aspect of the Papilio demoleus Larvae. Ph.D. Thesis (Unpub.) Amaravati:110-147. | Lu, M. & Sun, C. (1984). Rearing Diamondback moth on rape seedling by modification of Koshihara and Yamada method, J.Econ.Entomol. 75:153-155 | Mohan, M. & G. Gujar. (2003). Local variation in susceptibility of the diamondback moth. Plutella xylostella (Linneaus) to insecticides and role of detoxification enzymes. Crop Prot. 22: 495-504. | Nirmal, B & Singh, T. (2001). Development of resistance by diamondback moth to synthetic pyrethroids in Andhra Pradesh. Pestic. Res. J. 13(1): 14-19. | Regupathy, A. & Dhamu, K. (1990). Statistic work book for Insecticide Toxicology, Coimbatore, Softteck Computers, 179. | Rajurkar, R., Khan Z. & Gujar G. (2003). Studies on levels of GST, its isolation and purification from Helicoverpa armigera. Currot Sci. 85(9): 1355-1360. | Robert, H. & Wright, D. (1996). Multitrophic interactions and management of the Diamondback moth : A review. Bull. Ent. Res. 86 : 205-216 | Roe, S. (2001). Protein Purification Technique. Second edition, Bath press, Avon:135-140 | Sarfraz, M. & Keddie, B. (2005). Conserving the efficacy of insecticides against Plutella xylostella (L.) (Lepidoptera: Plutellidae) J. Appl.Entomol. 129: 149-157. | Schroder, P., Lamoureux, G., Rusness, D. & Rennenberg, H. (1990). Glutathion-S-Transferase activity in spruce needles, pestic. Biochem. and Physiol. 37 (3):211-218. | Settele, B., Cordova, D. & Cheek, R. (2008). Insect Rynodine Receptors Molecules targets for novel pest control chemical. J. Invert Neurosci. 8:107-119. | Singh, P., Chan, P., Hibbs, M., Vazquez, M., Sequra, D., Thomas, D. & Theobald, A. (2010). High-yield production and characterization of biologically active GST-tagged human topoisomerase IIα protein in insect cells for the development of a high-throughput assay, Protein Expr Purif, 76(2):165-72. | Tabashnik, B., Cushing, N. & Finson, N. (1987). Leaf residue Vs. tropical bioassay for assessing resistance in the Diamondback moth (Lipidoptera : Plutellidae). FAO, Pl. Prot. Bull. 35 : 11-14. | Wang, L. & Yu, Y. (2007). Cross-resistance and biochemical mechanisms of abamectin resistance in the B-type Bemisia tabaci. J. Appl. Ento., Vol: 131(2):98-103. | Yu, S. & Naguyen, S. (1992). Detection and biochemical characterization of insecticide resistance in the diamondback moth. Pestic Biochem. Physiol. 44(1) : 74-81.