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Impact of Olfactory Genes in Management of Lepidopteran Pest *Spodoptera Litura* by dsRNA Mediated RNAi

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Abstract

RNAi mediated by dsRNA is widely used for management of agriculturally important insect's pests. But, the screening of candidate gene of interest to be targeted in RNAi study is crucial for efficient RNAi. The aim of our study is to know the impact of olfactory genes, as potential target for dsRNA mediated RNAi, for management of Lepidopteran pest, *Spodoptera litura* F. (Noctuidae). We have targeted three genes in our present investigation, GOBP1 encoding General odorant binding proteins, OR1 and OR83b encoding olfactory receptors and olfactory co-receptor respectively. These genes belongs to insect chemoreceptor super family, involved in transmission of external environmental signals (chemicals) by transporters designated as binding proteins, and conversion of chemical signals into electrical signals that are perceived by insect nervous system, interprets and decides insect behavior, communication and interaction with host species. Inhibition of such interactions is targeted through dsRNA for designing effective management strategies. The results suggested, substantial decrease in larval feeding, inturn resulted in larval body weight ranges from 46 % to 100% and fecal pellet weight ranges from 50 % to 80% in targeted genes as compared to the control. OR1 had shown higher reduction in both larval weight (82%) and pellet weight (80%). Thus, indicated the alteration in feeding behavior in *S. litura* larvae by silencing olfactory receptors. Hence, the targeted gene (OR1) can be used as a good candidate gene for dsRNA mediated RNAi based insect pest management.

Keywords: RNAi, dsRNA, GOBP1, OR1, OR83b

Introduction

The tobacco cutworm, *Spodoptera litura*, is one of the major insect pests of cultivable species. It is polyphagous in nature and affects nearly 150 species [Huang et al., 2007]. About 26-100% yield losses were reported by Ahmad et al., [2008] and Dhir et al., [1992]. Its control mainly depends on synthetic pesticides, but extensive use of insecticides led to development of resistance in *S. litura* [Dong et al., 2013, Zhai and Robinson, 1992]. Ahmad et al., [2008] witnessed its evolved resistance against conventional insecticides such as organophosphates, carbamates and synthetic pyrethroids. Other new generation novel insecticides such as spinosad, indoxacarb, and some chitin synthesis inhibitors, such as lufenuron and novaluron were developed for management of insect pests but are quite expensive than conventional insecticides [Ahmad et al., 2008]. Therefore, new approaches like RNA interference (RNAi), is highly demanding in designing pest management strategies.

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It is possible to manage crop pests by employing RNAi approach by silencing some of the important genes that play a crucial role in insect-host plant interaction, growth and development, reproduction, communication with other insect species etc, through introducing dsRNA either as feeding or through transgenic plant. However, screening of potential target and mode of administration are the essential considerations before commencing RNAi experiments.

Based on the preliminary studies, we found insect sensory systems involved in communication or interaction with host plants, interesting area to work upon. Sensory systems involving chemoreceptors allow organisms to perceive the surrounding world to interact with the environment and survive. The central nervous system collects (receptor binding proteins) stimuli and translates them into an inner representation, which is used by the organism to respond with the most appropriate behavior. Chemoreceptors or chemosensory receptors of insects are mainly formed by the olfactory receptors (ORs) and gustatory receptors (GRs), which are located in the dendritic membrane of neurons.

We had selected three genes encoding olfactory receptors and binding proteins in our current study, General odorant binding protein (GOBP1), Odorant receptor (OR1) and Odorant co-receptor (OR83b). GOBPs located in the sensilla basiconica are thought to interact with general odorants (e.g., plant volatiles) that plays an important role in the detection of general odorants and has a conserved sequence across different species [Deng et al., 2012, Yu et al., 2009, Zhang et al., 2009]. The odorant receptors (ORs) belong to the large superfamily of G protein-coupled receptors (GPCRs), which detect chemicals in their environment [Clyne et al., 1999]. Odorants are thought to be translocated from the air to the chemoreceptors by a variety of protein mainly existing in the sensillar lymph, including odorant-binding proteins (OBPs) and chemosensory proteins (CSPs) [Steinbrecht, 1998, Vogt and Riddiford, 1981]. Gene encoding odorant receptor (OR83b) is co-receptor for most of the odour recognition in insects and is extremely well conserved in distant insect species [Jones et al., 2005]. Thus, Orco is a synonym of OR83b [Leal, 2013]. Based on this background, we had tried to study the olfactory genes as candidate targets for RNAi based management of *S. litura*.

Materials and Methods

RNA isolation and synthesis of dsRNA of GOBP1, OR1, OR83b gene : The *S. litura* larvae of different stages were collected from TNAU research fields and reared on castor leaves. Total RNA was extracted by homogenizing antennae of *S. litura* moths by employing Trizol method. The RNA isolated was converted into cDNA using cDNA Synthesis Kit (Fermentas, Cat# K1622). A cDNA fragment containing a gene specific partial sequence was ampliûed with T7 RNA polymerase binding site using forward and reverse primers incorporated with T7 promoter sequence (TAATACGACTCA CTATAGGGAGA) at the 5'ends (Table 1). The PCR products were puriûed by column purification and were then used for *in vitro* transcription with MEGAscript® RNAi kit (Ambion, Cat# AM1626) as per the manufacturer's instructions. The dsRNAs were annealed by incubating at 37ºC for 3.5 h, followed by slow cooling to room temperature. The annealed dsRNAs were treated with DNase I and RNase at 37ºC for one hour and then puriûed. The puriûed dsRNAs were analyzed on 1% agarose gel before being stored at -20ºC.

Bioassay study with dsGOBP1, dsOR1, dsOR83b : For the assay, two groups were maintained individually, first on artificial diet and other on 5:1 of rice bran and jaggery. Artificial diet containing chickpea seeds 100g, agar agar 12.8g, yeast 30g, methyl para hydroxyl benzoate 2g, sorbic acid 1g, ascorbic acid 3.2g, wesson's salt mix 7.2g, streptomycin sulphate 0.04g, vitamin supplement 2ml, formaldehyde (40%) 1ml and carbendazim 0.5g in 1lt distilled water was prepared, following protocol described by Chenchaiah and Bhattacharya [2005]. dsGOBP1, dsOR1, dsOR83b with final concentration of 300ng/10µl was overlaid on artificial diet and mixed in feed (5:1 ratio of rice bran and jaggery) under sterile conditions. Third instar larvae were allowed to feed the diet maintained in 80mm sterile cups with whereas diet treated with DEPC water was kept as control (Figure 1). The feeding alteration in larvae was measured in terms of weight of fecal pellets and larval body weight everyday up to 5 days after feeding. There were three biological replicates in each group and the observations were carried out accordingly. Data on larval body weight and pellet weight were analyzed by one-way analysis of variance (ANOVA).

Results and Discussion

In the present study, we had deployed third instar *S. litura* larvae and 300 ng/10il dsRNA concentrations for bioassay. The rationale for using third instar larvae is that, late instar larvae have well developed antennae for communication and host recognition, ascertain to detect feeding alterations. Larval body weight was measured for monitoring larval growth and development. In the first group, larvae fed on

artificial diet, larval body weight reduction observed was GOBP1 (100%), OR1 (82%) and OR83b (46%) (Figure 2a) while in second group, larvae fed on rice bran and jaggery, it was GOBP1 (25%) and OR83b (50%) at 48h after feeding (Figure 2b). No difference in body weight was found in OR1 treated larvae. Consistence effects on larval growth and development were observed in *S. exigua* with bacterially expressed dsRNA of chitin synthase [Tian et al., 2009]. However, we could not find any phenotypic variation in treated larvae, as our genes of study have different mechanism of action. Other reason could be the degree of knockdown may not reach the level required for lethal phenotypes. Therefore, it depends on dosage requirement for the target gene and sensitivity of the bioassay. In addition, second group had shown more gain in larval weight compared to first group. One possible reason could be the use of jaggery had enhanced the palatably in larvae as sugar solution mixed with dsRNA were reported to increase the RNAi efficiency [Bautista et al., 2009, Walshe et al., 2009]. Furthermore, statistical analysis ($P<0.05$, ANOVA) had shown significant differences within the treatments, as well with control in first group. By contrast, on the other hand, no significant difference was found within the treatments in second group; however, treatments were differing significantly from control. These results showed, though the larval body weight increased but it was substantially differ from control, so it was likely that reduction in larval body weight caused by dsRNA treatment.

Likewise, in order to know the feeding alterations, pellet weight were recorded in each group. Pellet weight was reduced in OR1 (80%), OR83b (53.33%) and GOBP1 (60%) as compared to control in first group (Figure 3a). In second group, it was reduced to 69, 84, and 46% in GOBP1, OR1 and OR83b, respectively (Figure 3b). Significant differences were found among the treatments as compared to control. This showed the potential of dsRNA to downregulate the three genes expressed in insect antennae. Notably, the expression of these genes varies with developmental stages and higher in adult antennae [Yin et al., 2012], yet we found differences in feeding behavior at the larval stage. Many studies on *Aphis gossypii* [Rebijith et al., 2016], *Manduca sexta* [Howlett et al., 2012], *Rhodnius prolixus* [Franco et al., 2016], *Tribolium castaneum* [Bai et al., 2011] had reported the chemoreceptor's as promising targets for RNAi studies.

The inconsistencies in RNAi were observed in earlier studies and it depends on various factors [Li et al., 2013, Yang and Han, 2014]. We also noticed the results were not consistence for three genes under study. However, all target genes had shown higher variability from control, suggesting them potent target for RNAi studies. To be more precise, among the target genes, OR1 had shown almost invariable results, compared to other target genes. Thus, OR1 could be used for carrying out further RNAi experiments. Furthermore, mode of administration of dsRNA, we could not find larval body weight difference at 48h after feeding in OR1, suggesting artificial diet mediated dsRNA may be more efficient compared to 5:1 rice bran and jaggery feed. Other drawback of 5:1 rice bran and jaggery feed, it cannot maintain moisture level making it unfit for feeding. Moreover, stability of dsRNA in feed is still an unanswered question. Further, expression analysis can be done to validate the results.

In summary, three olfactory genes were selected to study the effect on *S. litura* larval feeding, growth and development by dsRNA mediated RNAi. All targeted genes were downregulated in comparison to control. However, OR1 gene had shown more promising results, suggesting that olfactory genes may be considered as potential target for further RNAi experiments.

Table 1. Primers used for the amplification of target genes

Primer Name	Sequence (5'-3')	Amplicon Size (bp)
SL GOBP1	F-taatacgactcactatagggGTTCATCATGGAGGCTGAGG	
	R-taatacgactcactatagggATCGGCTGCCGTATTCTTAG	228
SL OR1	F-taatacgactcactatagggGTCTTTTGCTCCTGGAGTGC	
	R-taatacgactcactatagggCCACTGTTCTCAGCATCACG	347
SL OR83b	F-taatacgactcactatagggGCCAACACTATCACCGTCCT	
	R-taatacgactcactatagggCAGTCGAGGGGCTACTTCTG	332

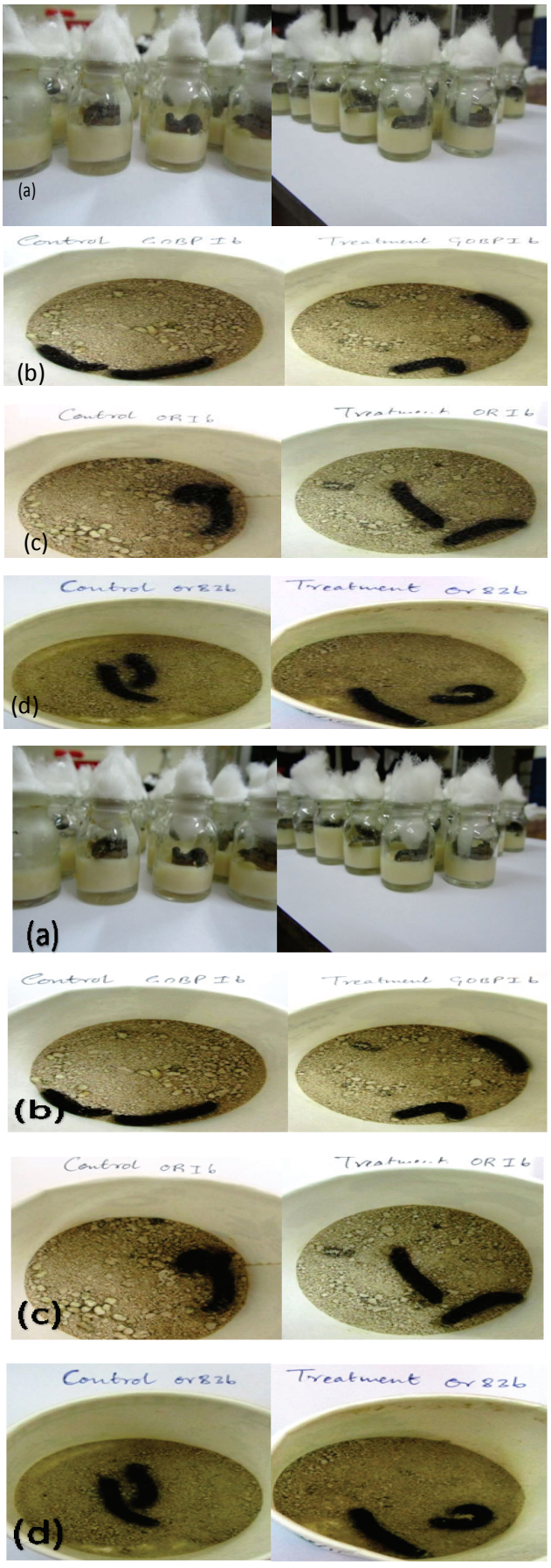

Figure 1. Bioassay study with double-stranded RNA (dsGOBP1, dsOR1, and dsOR83b) in the *S. litura* larvae. (a) Artificial diet containing dsRNA (b) 5:1 rice bran: jaggery feed mixed with dsGOBP1 (c) 5:1 rice bran: jaggery feed mixed with dsOR1 (d) 5:1 rice bran: jaggery feed mixed with dsOR83b.

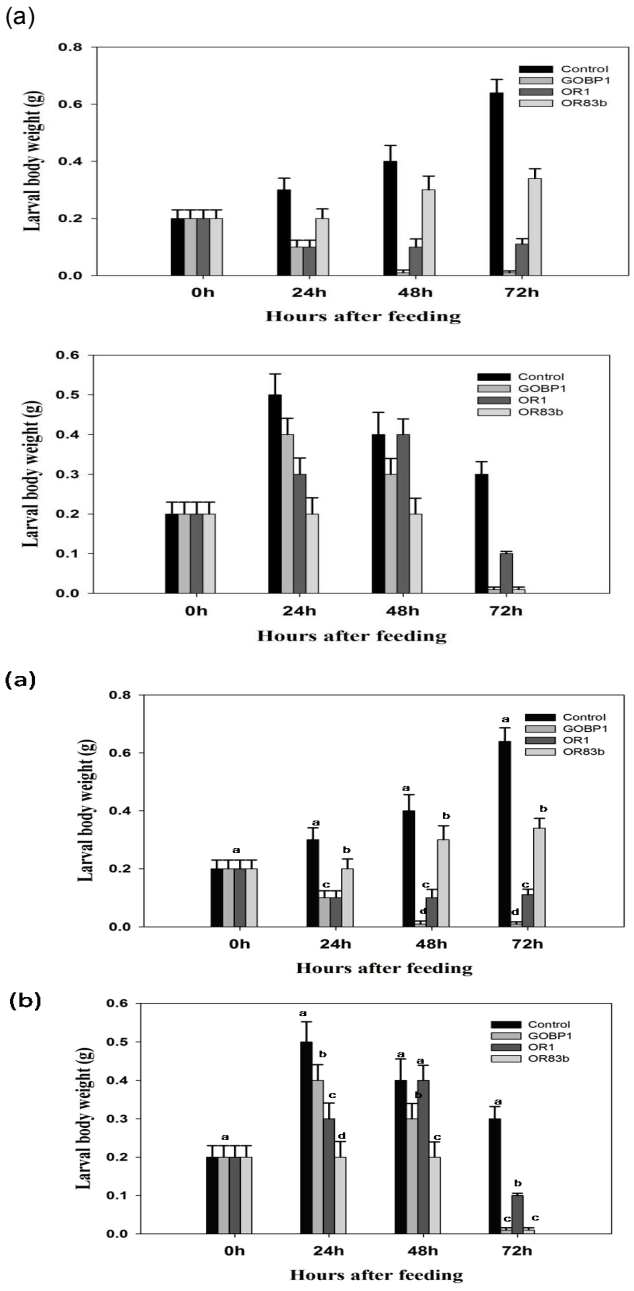

Figure 3. Effects of ingested dsGOBP1, dsOR1 and dsOR83b on *S. litura* fecal pellet weight. (a) Larvae fed on artificial diet containing dsRNA (b) Larvae fed on 5:1 rice bran: jaggery feed mixed with dsRNA. Error bars indicate standard error of mean. Statistical significance of difference were analyzed with ANOVA ($P<0.05$). Bars labelled with the same letter are not significantly different.

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