



Pyrosequencing Uncovers a Shift in Bacterial Communities Across Life Stages of *Octodonta nipae* (Coleoptera: Chrysomelidae)

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Bacterial symbionts of insects affect a wide array of host traits including fitness and immunity. *Octodonta nipae* (Maulik), commonly known as hispid leaf beetle is a destructive palm pest around the world. Understanding the dynamics of microbiota is essential to unravel the complex interplay between *O. nipae* and its bacterial symbionts. In this study, bacterial 16S rRNA V3-V4 region was targeted to decipher the diversity and dynamics of bacterial symbionts across different life stages [eggs, larvae, pupae, and adult (male and female)] and reproductive organs (ovaries and testis) of *O. nipae*. Clustering analysis at $\geq 97\%$ similarity threshold produced 3,959 operational taxonomic units (OTUs) that belonged to nine different phyla. Proteobacteria, Actinobacteria, and Firmicutes represented the bulk of taxa that underwent notable changes during metamorphosis. Enterobacteriaceae and Dermabacteraceae were the most abundant families in immature stages (eggs, larvae, and pupae), while Anaplasmataceae family was dominated in adults (male and female) and reproductive organs (ovaries and testis). The genus *Serratia* and *Lactococcus* were most abundant in eggs, whereas *Pantoea* and *Brachybacterium* represented the bulk of larvae and pupae microbiota. Interestingly the genus *Wolbachia* found positive to all tested samples and was recorded extremely high ($>64\%$) in the adults and reproductive organs. The bacteria varied across the developmental stages and responsible for various metabolic activities. Selection choice exerted by the insect host as a result of its age or developmental stage could be the main reason to ascertain the shift in the bacteria populations. Maternally inherited *Wolbachia* was found to be an obligate endosymbiont infecting all tested life stages, body parts, and tissues. These outcomes foster our understanding of the intricate associations between bacteria and *O. nipae* and will incorporate in devising novel pest control strategies against this palm pest.

Keywords: *Octodonta nipae*, microbiota, 16S rRNA gene pyrosequencing, *Wolbachia*, life stages, reproductive organs



Exploring the Role of Relish on Antimicrobial Peptide Expressions (AMPs) Upon Nematode-Bacteria Complex Challenge in the Nipa Palm Hispid Beetle, *Octodonta nipae* Maulik (Coleoptera: Chrysomelidae)

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The humoral immune responses of the nipa palm hispid beetle *Octodonta nipae* involves the inducible expression of the genes coding for antimicrobial peptides (AMPs) which are mediated by immune deficiency signaling pathways. In insects, the nuclear factor- κ B (NF- κ B) transcription factor, Relish, has been shown to regulate AMP gene expressions upon microbial infections. Here, we dissect the expression patterns of some AMPs in *O. nipae* during infections by entomopathogenic nematodes (EPNs) and their symbionts, before and after Relish knock down. Our results indicate that, prior to gene silencing, the AMPs attacin C1, attacin C2, and defensin 2B were especially expressed to great extents in the insects challenged with the nematodes *Steinernema carpocapsae* and *Heterorhabditis bacteriophora* as well as with their respective symbionts *Xenorhabdus nematophila* and *Photorhabdus luminescens*. The study also established the partial sequence of OnRelish/NF- κ B p110 subunit in *O. nipae*, with an open reading frame coding for a protein with 102 amino acid residues. A typical Death domain-containing protein was detected (as seen in *Drosophila*) at the C-terminus of the protein. Phylogenetic analysis revealed that in *O. nipae*, Relish is clustered with registered Relish/NF- κ B p110 proteins from other species of insect especially *Leptinotarsa decemlineata* from the same order Coleoptera. Injection of OnRelish dsRNA remarkably brought down the expression of OnRelish and also reduced the magnitude of transcription of attacin C1 and defensin 2B upon *S. carpocapsae* and *H. bacteriophora* and their symbionts infections. Altogether, our data unveil the expression pattern of OnRelish as well as that of some AMP genes it influences during immune responses of *O. nipae* against EPNs and their symbionts.

Keywords: insect immunity, Relish, antimicrobial peptide, *Steinernema carpocapsae*, *Heterorhabditis bacteriophora*, symbiotic bacteria



Genetic diversity of the families Aeshnidae, Gomphidae and Libellulidae through COI gene from South China

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ABSTRACT

Adult dragonflies (Anisoptera) were collected from different localities of South China covering eight provinces. Representative sequences were sixty-one, including 16 species, 11 genera and three families (Aeshnidae, Gomphidae and Libellulidae), under cytochrome oxidase subunit I (COI) gene. After alignment of sequences by BioEdit v6, genetic interaction and divergence were computed by MEGA 7 whereas all the indices of genetic diversity were calculated by DnaSP v5 software. Phylogenetic trees were constructed through Neighbor-Joining method under Jukes-Cantor model, and all species of respective families were assembled with each other into individual groups. Maximum divergence was observed by *Tritemis* genus (18.69%), followed by *Orthetrum* genus (18.16%), whereas a minimum value of divergence was noted for *Pantala* genus (0.31%). On the other hand, maximum genetic diversity was recorded for *Orthetrum* genus up to 142 mutations, followed by *Tritemis* genus (126 mutations), while the minimum value (two mutations) was observed for *Pantala* genus. Genetic diversity for overall and Libellulidae family sequences was much higher, up to 404 mutations and 344 mutations, respectively. Current results suggest a high diversity of odonates in the South China region and results are valuable in gaining a total obligation of the diversity of Asian odonates and conservation measures of this insect group.

1. Introduction

Odonata (Anisoptera), a diversified group of insect, widely distributed in different ecological zones, consists of more than 6000 species globally (Dijkstra et al., 2013). Order Odonata can be divided into two main sub-order Zygoptera (damselflies) and Epiprocta, respectively (Lohmann, 1996). Epiprocta is further divided into two sister group Anisoptera (dragonflies) and Anisozygoptera (Handlirsch, 1906) (damselfly dragonflies). However, Anisoptera can be further divided into 11 families and five super-families. The most dominated family is Libellulidae, that is divided into 11–13 subfamilies (Fraser and Tillyard, 1957; Bridges, 1994; Steinmann, 1997), whereas Aeshnidae and Gomphidae consist of 55 and 100 genera, respectively (Schorr and Paulson, 2018).

Majority of insect species have annual and long-distance migration ability, while the census sizes seem to be maximum in many insect species. Insect species from different orders and families show long-distance and multigenerational migrations as their essential part of the

life cycle. Till now, many insect's population genetics, migration pattern and inter-population connectivity stay indefinable (May, 2013; Pfeiler and Markow, 2017). The integrity of odonates is remarkable because of rich phenotypic and ecological diversity in a single insect order and widely used as a model species in various areas of ecology and evolution, such as the evolution of flight, sexual selection and life history (Córdoba-Aguilar, 2008). Odonata represents the unique insect model to integrate evolutionary genomics with ecology to study the insect evolution. These insects have many key features including extensive phenotypic and ecological diversity, historic phylogenetic position, exceptional evolutionary innovations, easy to study in usefulness and wild as bio-indicators of freshwater ecosystems worldwide (Bybee et al., 2016).

Dragonflies belonging to Aeshnidae family are worldwide distributed and diverse insect group (Von Ellenrieder, 2002) and known as “darner” (Kohli et al., 2014). The males are large with blue or yellow-spotted body, whereas females are having more frequent yellow-spotted and a single distinctive golden yellow vein at the edge of wings rarely

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Genetic interaction and diversity of the families Libellulidae and Gomphidae through COI gene from China and Pakistan

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ABSTRACT

A total of 300 dragonflies (Odonata) were collected from six different localities of China and Pakistan. Sixty seven representative samples were selected to sequence their mitochondrial cytochrome oxidase subunit I (COI). An examination of the resultant sequences identified 21 different dragonfly species, belonging to 15 distinct genera, two families, Libellulidae and Gomphidae. Sequence alignment was executed using Clustal-W in BioEdit v6. The phylogenetic tree was constructed through Neighbor-joining method by using Jukes-Cantor model, and genetic divergence was calculated via Kimura 2-parameter using MEGA7, while Genetic diversity was calculated by DnaSP v5. The maximum genetic divergence was observed for *Crocodylus servilis*, at 20.49%, followed by Libellulidae sp. with 22.30% while minimum divergence (0.82%) was observed for *Meligomphus ardens*. Likewise, a significant genetic diversity was observed for all species. However, *Crocodylus servilis* species presented maximum value (176 mutations) followed by Libellulidae spp. (150 mutations), whereas minimum value (3 mutations) was observed by *Orthetrum testaceum*. Interestingly, the diversity of *C. servilis*, all of which are collected from a single location of China, is much higher than those from Pakistan, which were collected from 5 different places with a spatial distance exceeding 500 Kms. Our results are useful in gaining a full appreciation of the global diversity of dragonflies and the development of conservation measures of this insect.

1. Introduction

Order Odonata, consisting of more than 6000 species, are widely distributed all over the world and commonly named as dragonflies or damselflies (Dijkstra et al., 2013). The order Odonata was further divided into two suborders, namely Zygoptera (Selys-Longchamps, 1854) (damselflies) and Epiprocta, respectively (Lohmann, 1996). Epiprocta comprises two sister groups named Anisoptera (Selys-Longchamps, 1854) (dragonflies) and Anisozygoptera (Handlirsch, 1906), respectively. Anisoptera has 11 families which were grouped into five super-families (Dijkstra et al., 2013). According to different authors (Bridges, 1994; Carle et al., 2013; Fraser and Tillyard, 1957; Steinmann, 1997), the family Libellulidae can be divided into 11–13 subfamilies. Likewise, Gomphidae is divided into 100 genera (Schoor and Paulson, 2018).

Members of the family Gomphidae are medium to giant dragonflies, males of which usually have expanded apical abdominal segments that give the appearance of a club. Owing to this, they are commonly known as club-tails. Previous work has suggested that relatively high diversity

of the Gomphidae was brought about by parallel shifts in behavior and niche space: the nymphs acquired the habit of hiding within or sprawling on aquatic substrates (Carle et al., 2015).

Similarly, Libellulidae family is distributed throughout the world and reported from all aquatic habitats. However, this family is more abundant within Anisoptera having more than 900 described species. For comprehensive classification of Libellulids, between 1909 and 1919, Ris (1912) was the first scientist who made a new revision and designs the foundation for it. While in 1917, the first comprehensive classification of Libellulidae was established by Tillyard (1917), including creation and definition of subfamilies. However, some authors did a minor modification, though the classification is in use. Mostly characters are variable within subfamily or found in other subfamilies as well as due to lack of diagnostic, unique character states, but usually, subfamilies are not well defined.

Because of the large size, colorful body and high visibility, it is more popular insects in public, and for this reason, Odonates are more focused in conservation efforts (Nigam et al., 2017). All Odonate nymphs

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Genomic evaluations of *Wolbachia* and *mtDNA* in the population of coconut hispine beetle, *Brontispa longissima* (Coleoptera: Chrysomelidae)

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ABSTRACT

Wolbachia pipentis is a diverse, ubiquitous and most prevalent intracellular bacterial group of alpha-Proteobacteria that is concerned with many biological processes in arthropods. The coconut hispine beetle (CHB), *Brontispa longissima* (Gestro) is an economically important pest of palm cultivation worldwide. In the present study, we comprehensively surveyed the *Wolbachia*-infection prevalence and mitochondrial DNA (*mtDNA*) polymorphism in CHB from five different geographical locations, including China's Mainland and Taiwan, Vietnam, Thailand, Malaysia and Indonesia. A total of 540 sequences were screened in this study through three different genes, i.e., cytochrome oxidase subunit I (COI), *Wolbachia* outer surface protein (*wsp*) and multilocus sequencing type (MLST) genes. The COI genetic divergence ranges from 0.08% to 0.67%, and likewise, a significant genetic diversity ($\pi = 0.00082$; $P = 0.049$) was noted within and between all analyzed samples. In the meantime, ten different haplotypes (H) were characterized (haplotype diversity = 0.4379) from 21 different locations, and among them, H6 (46 individuals) have shown a maximum number of population clusters than others. Subsequently, *Wolbachia*-prevalence results indicated that all tested specimens of CHB were found positive (100%), which suggested that CHB was naturally infected with *Wolbachia*. *Wolbachia* sequence results (*wsp* gene) revealed a high level of nucleotide diversity ($\pi = 0.00047$) under Tajima's *D* test ($P = 0.049$). Meanwhile, the same trend of nucleotide diversity ($\pi = 0.00041$) was observed in *Wolbachia* concatenated MLST locus. Furthermore, phylogenetic analysis (*wsp* and concatenated MLST genes) revealed that all collected samples of CHB attributed to same *Wolbachia* B-supergroup. Our results strongly suggest that *Wolbachia* bacteria and *mtDNA* were highly concordant with each other and *Wolbachia* can affect the genetic structure and diversity within the CHB populations.

1. Introduction

The coconut hispine beetle (CHB), *Brontispa longissima* (Gestro) (Coleoptera: Chrysomelidae), is a devastating insect pest of palm plants (Nakamura et al., 2006), and its larvae and adult damage delicate parts of the plant. After maturity of old fronds, the larvae and adults shift to other young fronds. Severe infestation of beetles leads toward the restricted development of palm cultivations due to browning and curling of leaves. The massive leaf damage retards the growth, production losses and eventually causes the death of palm tree (Nakamura et al., 2006). The CHB has been supposed to be local to Papua New Guinea and Indonesia, and after that, it has spread worldwide (Nakamura et al.,

2006; Zhang et al., 2015). During 2002, CHB was first time reported from Hainan province (China), and it caused substantial destructions to palm trees, exclusively the coconut plants (Hou et al., 2011; Wan et al., 2015). Later on, the palm plantations from other provinces of China (Fujian, Yunnan, Guangxi, and Guangdong) were damaged by CHB (Lu et al., 2004; Hou et al., 2011; Chen et al., 2015). Recently, the invasion of CHB is alarming, due to which it is listed in the Global Invasive Species Database (2010).

Wolbachia pipentis is a gram-negative alpha-Proteobacteria (Rickettsiaceae; Rickettsiales), and considered to be the most ubiquitous obligate bacterial genus among other endosymbionts (Cardaux et al., 2001; Rowley et al., 2004; Werren and Windsor, 2000).

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Infection Density Dynamics and Phylogeny of *Wolbachia* Associated with Coconut Hispine Beetle, *Brontispa longissima* (Gestro) (Coleoptera: Chrysomelidae), by Multilocus Sequence Type (MLST) Genotyping^S

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The intracellular bacterium *Wolbachia pipientis* is widespread in arthropods. Recently, possibilities of novel *Wolbachia*-mediated hosts, their distribution, and natural rate have been anticipated, and the coconut leaf beetle *Brontispa longissima* (Gestro) (Coleoptera: Chrysomelidae), which has garnered attention as a serious pest of palms, was subjected to this interrogation. By adopting *Wolbachia* surface protein (*wsp*) and multilocus sequence type (MLST) genotypic systems, we determined the *Wolbachia* infection density within host developmental stages, body parts, and tissues, and the results revealed that all the tested samples of *B. longissima* were infected with the same *Wolbachia* strain (*wLog*), suggesting complete vertical transmission. The MLST profile elucidated two new alleles (*ftsZ*-234 and *coxA*-266) that define a new sequence type (ST-483), which indicates the particular genotypic association of *B. longissima* and *Wolbachia*. The quantitative real-time PCR analysis revealed a higher infection density in the eggs and adult stage, followed by the abdomen and reproductive tissues, respectively. However, no significant differences were observed in the infection density between sexes. Moreover, the *wsp* and concatenated MLST alignment analysis of this study with other known *Wolbachia*-mediated arthropods revealed similar clustering with distinct monophyletic supergroup B. This is the first comprehensive report on the prevalence, infection dynamics, and phylogeny of the *Wolbachia* endosymbiont in *B. longissima*, which demonstrated that *Wolbachia* is ubiquitous across all developmental stages and distributed in the entire body of *B. longissima*. Understanding the *Wolbachia* infection dynamics would provide useful insight to build a framework for future investigations, understand its impacts on host physiology, and exploit it as a potential biocontrol agent.

Keywords: *Wolbachia*, *Brontispa longissima*, quantitative PCR, MLST, biocontrol agent, symbiont-host interactions

Introduction

Members of the family Chrysomelidae (Insecta: Coleoptera), also known as leaf beetles, are one of the most plentiful, diverse, and successful phytophagous Coleopteran insects, encompassing more than 35,000 species reported worldwide [1]. Owing to its devastating impacts on commercial and

ornamental palm cultivation, the pest has garnered a lot of attention [2]. Among the Chrysomelidae family, the genus *Brontispa* has 22 described species, out of which 17 species have been reported causing considerable damage to various palm species [3]. *Brontispa longissima* (Gestro) (Coleoptera: Chrysomelidae), commonly known as coconut hispine beetle, is a notorious pest and key defoliator of the



Original article

Insect pollinators diversity and abundance in *Eruca sativa* Mill. (Arugula) and *Brassica rapa* L. (Field mustard) crops

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ABSTRACT

Studies on the insect pollinators diversity and their relative abundance in *Eruca sativa* Mill. (Arugula) and *Brassica rapa* L. (field mustard) was carried out during spring season from February to April consecutively during all the three years of 2016–18. Insect pollinators observed belonged to four orders i.e. Hymenoptera, Diptera, Lepidoptera, and Coleoptera. A total of 20 major species of insect pollinators were recorded. The highest abundance of pollinator species belonged to Hymenoptera. The most prominent insect pollinator species were *Apis mellifera* followed by other three honey bee species of *A. cerana*, *A. florea*, and *A. dorsata* respectively. Some species of solitary bees were also recorded. From Diptera, four species of syrphid fly and one species from Muscidae family were also recorded. Insect pollinators recorded from order Lepidoptera were *Pieris brassicae*, *Vanessa cardui*, and *Papilio demoleus*. Lady bird beetle *Coccinella septempunctata* was recorded from Coleoptera order as occasional visitor. It was noticed that *E. sativa* attracted more insect pollinators than *B. rapa* which may be attributed to different amount and chemical properties of nectar, with number of pollen grains, and flower canopy of both crops. Further studies are needed to confirm the reasons for higher pollinator visitation to *E. sativa* than *B. rapa* through chemical analysis of nectar, amount of pollens, flower physiology and phenology of both crops. © 2018 Production and hosting by Elsevier B.V. on behalf of King Saud University. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

1. Introduction

Brassica rapa (Field mustard) belongs to family Brassicaceae. This crop is economically important because of seed oil contents

and some other plant parts like leaves which are edible and can be used as fodder crop. The oil extracted from their seeds is consumed by humans from centuries in the Asian continent. Seeds after oil extraction are changed to the shape of cake which is very nutritious and used to feed animals (Ramachandran et al., 2007). *Eruca sativa* (Arugula) also belongs to family Brassicaceae. Local name of *E. sativa* is Taramira. This crop has medicinal and economic value and can be consumed as salad and vegetable by humans. It can also be used as green fodder for feeding animals (Ghazali et al., 2014).

Insects help in pollination of these crops. Crops belonging to family Brassicaceae are predominantly dependent on insect pollination (Entomophilous). Increase in seed quality and quantity is

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Article

Development of Single Nucleotide Polymorphism (SNP) Markers for Analysis of Population Structure and Invasion Pathway in the Coconut Leaf Beetle *Brontispa longissima* (Gestro) Using Restriction Site-Associated DNA (RAD) Genotyping in Southern China

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Abstract: To determine population genomic structure through high-throughput sequencing techniques has revolutionized research on non-model organisms. The coconut leaf beetle, *Brontispa longissima* (Gestro), is a widely distributed pest in Southern China. Here, we used restriction site-associated DNA (RAD) genotyping to assess the invasion pathway by detecting and estimating the degree of genetic differentiation among 51 *B. longissima* accessions collected from Southern China. A total of 10,127 SNPs were obtained, the screened single nucleotide polymorphism (SNP) information was used to construct the phylogenetic tree, *F_{ST}* analysis, principal component analysis, and population structure analysis. Genetic structure analysis was used to infer the population structure; the result showed that all accessions were divided into Hainan population and non-Hainan population. The Hainan population remained stable, only the Sansha population differentiated, and the non-Hainan populations have gradually differentiated into smaller sub-populations. We concluded that there are two sources of invasion of *B. longissima* into mainland China: Taiwan and Hainan. With the increase of the invasion time, the Hainan population was relatively stable, and the Taiwan population was differentiated into three sub-populations. Based on the unrooted phylogenetic tree, we infer that Taiwan and Hainan are the two invasive base points. The Taiwan population invaded Fujian, Guangdong, and Guangxi, while the Hainan population invaded Yunnan and Sansha. Our results provide strong evidence for the utility of RAD sequencing (RAD-seq) in population genetics studies, and our generated SNP resource could provide a valuable tool for population genomics studies of *B. longissima* in the future.

Keywords: *Brontispa longissima*; restriction site-associated DNA sequencing; SNPs; population structure



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Full length article

In-vitro assessment of food consumption, utilization indices and losses promises of leafworm, *Spodoptera litura* (Fab.), on okra crop

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ABSTRACT

The lepidopteran insect pests have significant importance in vegetable production. The present study was performed to investigate the baseline studies about the assessment of feeding and consumption potential, utilization indices and losses promises of leafworm, *Spodoptera litura* (Fab.) on Okra. The data regarding feeding potential, food utilization and consumption indices as well as losses of different larval instars were recorded and subjected to appropriate statistical analysis. The results showed that, in the beginning, the approximate digestibility of various instars was increase, e.g. third instar (51.36%–64.03%), fourth instar (63.42%–69.45%) and fifth instar (70.25%–76.10%). However, after a certain period, the digestibility was decreased and efficiency to convert the ingested food into biomass varied significantly. The consumption index values increased with an increase in time but the consumption and growth rate was declined of fourth instar larvae. The ingestion and digestion increased of third (10.01–13.06, 8.32–11.91 mg), fourth (11.27–17.28, 10.96–14.03 mg) and fifth (12.60–19.40, 11.93–15.28 mg) larval instars. The corrected weight of consumed leaves increased with a gain in body weight. However, in the third instar, a decline was observed on the last day of feeding. Maximum leaf area was consumed by fifth instar larvae (44.66 cm²) followed by fourth (35.41 cm²) and third (27.98 cm²) instars. In conclusion, all the dependent parameters, including food utilization potential, consumption indices and losses were higher for fifth instar larvae than others. These results emphasized the re-establishment of fundamental (economic threshold level: ETL, economic injury level: EIL) integrated pest management concepts.

Introduction

Okra is native to Africa, North Australia to the Pacific and South-East Asia (Kaleri et al., 2011) and commercially grown in many countries of the world such as Turkey, Bangladesh, Malaysia, Southern United States, Afghanistan, Pakistan, Brazil, Thailand, India, Ethiopia, Iran, Western Africa, Yugoslavia, Japan, Myanmar and Cyprus

(Purseglove, 1987; Benjawan et al., 2007; Qureshi, 2007). In the world, its production is about six million tons per year (Bencharki, 2012). In 2009–2010, the area under okra crop was 0.43 million hectares, and the production stood at 4.54 million tons (Bencharki, 2012). India was the first country in the world, with 3.5 million tons of okra production from over 0.35 million hectares of land (FAOSTAT, 2008). India is the most important country in the world for okra production and shares a part of

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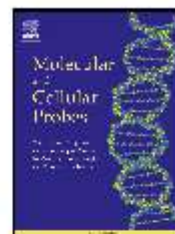
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Population genomics of honey bees reveals a selection signature indispensable for royal jelly production

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Potential of Moringa (*Moringa oleifera*: Moringaceae) as plant growth regulator and bio-Pesticide against wheat aphids on wheat crop (*Triticum aestivum*; Poaceae)

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ABSTRACT

Moringa oleifera commonly known as Moringa is a multipurpose plant. Field trials were conducted to assess the potency of Moringa leaf (MLE) and root extracts (MRE) as plant growth regulator (PGR) and a Bio-pesticide on wheat crop. At distinct crop growth stages (tillering, booting, and heading) with different concentrations (5, 10, 12.5, and 25% v/v or w/v or w/w) of MLE and MRE were applied. Results showed statistically significant increase in crop growth traits and reduction in aphid infestation (booting, milk, and heading stage). As plant growth regulator maximum leaf area duration (LAD), leaf area index (LAI), and total dry matter accumulation (TDM) were recorded at all growth stages for MLE 25%. Highest crop growth rate (CGR) ($24.91 \text{ gm}^2\text{day}^{-1}$) at tillering was achieved for MLE 5%, $13.04 \text{ gm}^2\text{day}^{-1}$ for MRE 10% at booting stage and $8.76 \text{ gm}^2\text{day}^{-1}$ for MLE 10% at heading stage. Maximum thousand grains weight (57.33g), highest number of spikes per plant (9.67plant^{-1}) and maximum grain yield (4446Kg hac^{-1}) were determined for MLE 25%. The heading stage of the crop was heavily infested with aphids and MLE 5% significantly reduced pest infestation compared to other concentrations of MLE and MRE and control group. Overall, MLE and MRE proved very effective as plant growth regulator and a Bio-pesticide against wheat aphid on wheat crop.

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INTRODUCTION

It is crucial to explore the potential of botanical and bio-pesticides in agriculture pest management (Jacobson and Crosby, 1971; Chakraborty and Basu, 1997; Habib *et al.*, 2015). Green pesticides provide materials for natural and beneficial pest control and increased production per unit area (Isman and Machial, 2006). Broad spectrum synthetic insecticides have consequences which badly affect human health, environmental cause pollution, destroy the food chain and kill beneficial insects (Sexena *et al.*, 1974; Chauhan *et al.*, 1987).

Botanical insecticides like neem (*Azadirachta indica* A. Juss) has been proved to be useful as bio-pesticide, efficiently reported (more than

400 species) against insects (Martinez, 2002) but there is an increasing need to discover some new bio-pesticides and their toxicity against insects, birds, fish and mammals as they are environment-friendly, economical, and pose no hazardous threat to human health and other non-targeted organisms (Tang *et al.*, 2002). Different plant species belonging to family Meliaceae such as *Tillia americana*, *A. indica*, *Melia volkensii*, and *Azadirachta exelsa* are effective against noctuid caterpillars, *Trichoplusia ni* and *Pseudaletia unipuncta* (Akhtar *et al.*, 2008). Without experimental clarification these bio-pesticides cannot be registered.

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Role of primary metabolites in plant defense against pathogens

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ABSTRACT

Plants under natural environment facing various pathogens, tend to produce defense to maintain their fitness and minimize pathogenic damage. Plant-pathogens interaction is gaining more importance by researchers as, their means of the fight are primary metabolites. The ultimate result of either means of defense is pathogenesis or resistance. Plant defense mechanisms can be grouped either into inducible and constitutive defense or chemical, structural and morphological defense. Majority of defense mechanisms have a passive role, i.e. only defensive against pathogens, but a few are very active. Plant primary metabolites are catching interest in their immunity role. Deep information of molecular mechanisms involved during the plant-pathogen system is need of the day for future disease control. This review will highlight the role of primary metabolites and their mechanism of action in plant defense.

1. Introduction

Plants respond to various biotic stresses and harmful environmental conditions through various biochemical, morphological and molecular mechanisms [1]. Specific signaling and recognition systems are present that make it possible to detect pathogen access and start an efficient defense response in plants [2]. Plants protect themselves by the production of chemical compounds against pathogenic infection [3].

Plant metabolism and pathogenic infection are associated with each other [4]. Nutrition is the basic requirement of a pathogen from the host [5] and plant metabolism affect this nutrition availability [6]. Energy supply is the key factor required for defense of plant against pathogens and this requirement fulfilled by primary metabolic activities [7]. Primary metabolites perform their function as molecules signaling to trigger defense response by signal transduction and pathogen recognition processes [8]. Production of primary metabolites occurs during the active growth phase (trophophase) and begins in the presence of required nutrients in a growth medium. Primary metabolism is important for growth, development and reproduction of cells. The primary metabolites participate in the primary response by the

regulation of carbohydrates, protein and lipids to infection by pathogens [6]. Plant growth and yield is affected by primary and secondary metabolism during pathogen infection [9]. Energy supply is compulsory for the defense response [10]. Pathogens easily manipulate plants metabolism in a limited supply of nutrients, which increase the nutrient demand for assimilation [11]. Metabolism sinks because after the infection of pathogen, photosynthesis decreases necrotic and chlorotic tissue development which change the accumulation of sugar [12].

Role of primary metabolic pathways in the regulation of plant defense responses for development and growth is very little known. The present review focuses on the role of primary metabolism in regulating plant defence against pathogen derived elicitor or potential pathogens.

1.1. Primary metabolites against plant virus

Several primary metabolites produced by plants like carbohydrates, proteins, and lipids but antiviral characteristics are present only in proteins and polysaccharides [13]. Defense responses produced by the proteins against specific viral, fungal and bacterial pathogens [14]. There are 17 families belongs to defence related proteins (DRPs) [15].

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Spatio-temporal distribution of the peach fruit fly, *Bactrocera zonata* (Diptera: Tephritidae) infesting citrus orchards at Sargodha, Pakistan

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Abstract: [Aim] This study was conducted to monitor the population changes of most dominant species of the peach fruit fly, *Bactrocera zonata* distributed at seven localities (Sargodha-I, Sargodha-II, Bhagtanwala, Sakessar, Chak# 75-SB, Chak# 46-SB and Chak# 104-NB) of Tehsil Sargodha from 2009 to 2011. [Methods] Population occurrence of *B. zonata* was recorded on weekly basis using methyl eugenol pheromone traps charged at fortnight intervals. [Results] The results revealed that the highest population abundance of *B. zonata* was recorded at Sargodha-I (53.67, 45.82 and 45.47 flies/trap) followed by Sakessar (41.13, 33.87 and 35.75 flies/trap) whereas Chak# 75-SB had the lowest population occurrence (15.78, 19.18 and 19.15 flies/trap) during all the three years (2009–2011), respectively. The highest peaks were observed during April (76.08, 71.94 and 61.51 flies/trap, respectively) followed by May (60.74, 52.63 and 64.00 flies/trap, respectively) and the lowest during February and October each year. Moreover, there were strong positive relationships between the maximum and minimum temperature and *B. zonata* population abundance where negative association was observed for the relative humidity and rainfall. Similarly regression coefficient demonstrated that the maximum temperature was the major contributing factor influencing *B. zonata* population occurrence and rainfall contributed the lowest share. [Conclusion] Regular inspection of *B. zonata* population should be carried out throughout the year, especially when temperature started to rise in April–May as weather factors greatly influence population counts of *B. zonata*.

Key words: Tephritidae; *Bactrocera zonata*; population dynamics; weather factors; citrus; pheromone trap

1 INTRODUCTION

Pakistan having 2.1 million tons of production is the sixth largest producer of mandarin and oranges in the world. Among all countries, Pakistan shares 0.9% and 3.6% value and volume of mandarin and oranges, respectively. With respect to very famous variety of citrus, *Citrus reticulata*, locally known as “Kinnow” Pakistan stands the first for its production in the world. Approximately 95% kinnow is produced in Pakistan, of which District Sargodha contributes to about 70% of total kinnow production and average yield of kinnow per hectare is nine tons which is lower than some developed countries (Anonymous, 2012). Citrus is frequently attacked

by a variety of pests such as citrus psylla, citrus leaf miner, lemon butterfly, fruit fly, etc. Fruit fly was not a serious pest of citrus before some years because of its thick skin and rind oil, which can kill eggs and larvae but now due to farmers' negligence and poor quarantine policies, fruit fly has become a major threat in citrus growing areas of Pakistan during recent years. Citrus orchards are heavily infested by the attack of fruit fly and estimated fruit losses due to fruit fly at farm level in particular areas are 18% (mango), 30%–35% (guava), 25%–35% (melons) and 6%–11% (citrus). Losses may vary from few percent to 100% depending on severity of the pest (Hardy and Jessup, 2012). About 80% fruit loss has been recorded where citrus and mango

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Structural diversity and functional variability of gut microbial communities associated with honey bees

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ABSTRACT

Microbial consortia accompanied to all eukaryotes can be inherited from ancestors, environment, and/or from various food source. Gut microbiota study is an emerging discipline of biological sciences that expands our understanding of the ecological and functional dynamics of gut environments. Microorganisms associated with honey bees play an important role in food digestion, colony performance, immunity, pollination, antagonistic effect against different pathogens, amelioration of food and many more. Although, many reports about honey bee gut microbiota are well documented, microbiome with other key components of honey bees such as larvae, adults, their food (pollen, bee bread, and honey), honey combs, and floral nectar are poorly understood. Mutual interactions and extent of the roles of microbial communities associated with honey bees are still unclear and demand for more research on the nutritional physiology and health benefits of this ecologically and economically important group. Here in this study, we highlighted all the honey bee microbiome that harbored from different life stages and other relevant components. The anatomical parts of honey bee (larvae, adults), food source (pollen, bee bread, and honey), honey combs, and floral nectar were highly flourished by numerous microorganisms like bacteria (Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria, Actinobacteria, Acidimicrobiales, Bacilli, Bacteroidetes, Corci, Clostridia, Colliforms, Firmicutes, Flavobacteriia, Mollicutes) and fungi (Dothideomycetes, Eurotiomycetes, Mucormycotina, Saccharomycetes, Zygomycetes, Yeasts, Molds). Some distinctive microbial communities of a taxonomically constrained species have coevolved with social bees. This contribution is to enhance the understanding of honey bee gut microbiota, to accelerate bee microbiota and microbiome research in general and to aid design of future experiments in this growing field.

1. Introduction

Honey bees belong to family Apidae, suborder "Apocrita" in the insect order Hymenoptera. Honey bees, a part of the insect family Apidae, are cosmopolitan eusocial insects and are very important for

maintaining biodiversity by pollination of numerous plants. Honey bees are crucial for mankind because of biological and economic reasons [1]. Honey bees not only produce honey and other bee products [2,3] but also add in agricultural productivity and profitability through increase of the quality and quantity of different agricultural crops, horticultural

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Synergetic effects of various plant extracts as bio-pesticide against Wheat Aphid (*Diuraphis noxia* L.) (Hemiptera: Aphididae)

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Human health and environment are greatly affected by extensive used of synthetic insecticide, which is why the alternative way of pesticides, such as botanical pesticide consumption increase with the passage of time to control of insect pests. The insecticidal impact of Moringa (*Moringa oleifera*), Neem (*Azadirachta indica*) and Eucalyptus (*Eucalyptus globules*) leaves extracts were investigated on *Diuraphis noxia* (Hemiptera: Aphididae). This study was aimed to evaluate the efficiency of bio-pesticide instead of synthetic chemicals against *D. noxia*. Experimental results showed that the percentage of mortality fluctuate with the fluctuation of concentration with time intervals and have great impact on the population of *D. noxia*. Single, doubled and tripled combinations of leaves extract were tested at two different concentrations (25 and 50%). The results suggested and concluded that from all the application of single botanicals *A. indica* give better result as compared to other single application while application of double combination *M. oleifera* with *A. indica* give significance result instead of other double mixture, meanwhile the triple combination (*M. oleifera*, *A. indica* and *E. globules*) performed excellent result as the others triple mixtures. The study revealed that combine used of *M. oleifera*, *A. indica* and *E. globules* leaves extract were very effective against *D. noxia* and surge as an alternative way of pesticides instead of synthetic chemicals.

Key words: Wheat aphid, plant extract, mortality, *M. oleifera*, *A. indica*, *E. globule*.

INTRODUCTION

Wheat is the staple food of Pakistan, which is being used 129 kg per head annually and this crop covers more than eighty percent of country. In 2008-2009, wheat were cultivated over an area of 8.459 million hectares giving 23.03 m. tones production (Zeb et al., 2011). Wheat is great source of income in the rural areas of Pakistan and as well as in the world. Yield per acre of wheat in Pakistan is much lower than the other wheat growing countries. Many factors contribute to the low yield of wheat in Pakistan including, method of cultivation, improper irrigation facilities, low level of soil fertility and particularly insect pest attacks.

All over the world, wheat is faced with many problems related to insect pest attacks such as weeds, pathogens and insects, which are main causal agents in yield reduction (25–50%) (Pimentel et al., 1991; Oerke, 2006). Thus, wheat is drastically being affected by sucking insect pests and dominated by wheat aphid, *Diuraphis noxia* (Mordvilko) (Homoptera: Aphididae). There are six species of wheat aphid that include Russian wheat aphid (*D. noxia*), the English grain ear aphid (*Sitobion avenae*), common green wheat aphid (*Schizaphis graminum*), bird cherry-oat aphid (*Rhopalosiphum padi*), and rose grain aphid



Temperature-dependent development of Asian citrus psyllid on various hosts, and mortality by two strains of *Isaria*

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Phylogenetic analysis

ABSTRACT

The Asian citrus psyllid (ACP), *Diuraphis citri* Kuwayama (Hemiptera: Psyllidae) is a devastating pest of *Citrus* spp. The aim of present study was to investigate the development and mortality of ACP on citrus (*Citrus sinensis*) (healthy and Huanglongbing- (HLB) diseased) and jasmine (*Murraya paniculata*) plants at various temperatures. Two new *Isaria* strains were collected from citrus orchards of Fuzhou (China), and HLB-diseased plants were verified by running PCR for 16S gene of *Candidatus Liberibacter asiaticus* (CLas). Development observations were recorded for egg, nymph and adult stages on all plants and three different temperatures (20, 25 and 30 °C) whereas mortality observations were recorded for the nymph (fifth instar) and adults on all plants at 25 °C. Field collected *Isaria* strains were belonged to previously reported Chinese strains under Maximum Parsimony (MP) and Maximum Likelihood methods, as well as, CLas isolates were belonged to previously reported Chinese isolates under MP and Neighbor-Joining methods. The fastest development and mortality was observed on HLB-diseased plants whereas longest time was taken by development and mortality completion on jasmine plants at all temperatures. The fastest developmental times of egg, nymph (first to fourth and fifth instar) and adult stages were ranged from 3.02 to 3.72 d, to 7.63–9.3 d, 5.35–5.65 d and 24.46–28.47 d on HLB-diseased plants at 30–20 °C, respectively. On the other hand, *I. jaysonis* caused the fastest mortality of nymphs and adults (32.21 ± 4.47% and 19.33 ± 4.51%) on HLB-diseased plants with the concentration of 1×10^6 conidia mL⁻¹ after 3 d and 7 d, respectively. It is concluded that there is a need for extensive molecular work to understand the extra-development and mortality of ACP on diseased plants, because, CLas bacterium can be supportive to uptake more sap from plant phloem.

1. Introduction

The Asian citrus psyllid (ACP), *Diuraphis citri* Kuwayama (Hemiptera: Psyllidae), is a major pest of *Citrus* spp. worldwide [1]. ACP feeds on citrus and also is the main vector of the bacterium *Candidatus Liberibacter asiaticus* (CLas), a causative agent of “Huanglongbing” (HLB), which is a serious disease of citrus in the world. Citrus is one of the most important economic crops all over the world, and ACP causes heavy losses to citrus production [2–4]. The loss caused by HLB was recorded 30%–100% in the yield production, depending upon the infected condition of orchards [5,6]. HLB is distributed all

around the world citrus growing areas, including Africa [7], Australia [8], Bhutan [9], Brazil [10], California [11], Ethiopia [12], Florida [13], India [14], Indonesia [15], Iran [16], Japan [17], Kenya [18], Malaysia [19], Mexico [20], Oman [21], Pakistan [22], Somalia, Texas [23], USA [24]. It is recorded all over China, particularly from following provinces: Fujian, Guangxi, Guangdong, Guizhou, Hunan, Jiangxi, Sichuan, Taiwan, Yunnan and Zhejiang [25–27], Hainan, Henan, Hong Kong and Macau [28,29]. ACP feeds on a wide range of hosts, and it is also observed that it feeds for long time on diseased plants as compared to healthy plants [30]. However, the development of ACP has significant consideration under various host plants, but

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The Endosymbiotic *Wolbachia* and Host COI Gene Enables to Distinguish Between Two Invasive Palm Pests; Coconut Leaf Beetle, *Brontispa longissima* and Hispid Leaf Beetle, *Octodonta nipae*

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Abstract

To elucidate taxonomic eminence of identical pest species is essential for many ecological and conservation studies. Without proficient skills, accurate molecular identification and characterization are laborious and time-consuming. The coconut leaf beetle, *Brontispa longissima* (Gestro) (Coleoptera: Chrysomelidae), is biologically and morphologically identical to hispid leaf beetle, *Octodonta nipae* (Maulik) (Coleoptera: Chrysomelidae), and is known as the most harming nuisances of palm cultivation worldwide. The present examination was to establish *Wolbachia* genotyping analysis along with host cytochrome oxidase subunit I (COI) gene for accurate identification between these individuals of the same family (Chrysomelidae). Here, we have cloned and sequenced a gene coding *Wolbachia* surface protein (wsp) and COI gene regions amplified from both species by polymerase chain reaction. The nucleotide sequences were directly determined (~600 bp for wsp and ~804 bp for COI) and aligned using the multiple alignment algorithms in the ESPript3 package and the MEGA5 program. Comparative sequence analysis indicated that the representative of wsp and COI sequences from these two beetles were highly variable. To ensure this bacterial variation, multilocus sequence typing (MLST) of bacterial genes was conducted, and the results vindicated the same trend of variations. Furthermore, the phylogenetic analysis also indicates that *B. longissima* and *O. nipae* being the two different species harbors two distinct *Wolbachia* Hertig and Burt (Rickettsiales: Anaplasmataceae) supergroups B and A, respectively. The present outcomes quickly discriminate between these two species. Considering its simplicity and cost-effectiveness, it can be used as a diagnostic tool for discriminating such invasive species particularly *B. longissima* and *O. nipae* which has overlapping morphologic characters.

Keywords: *Brontispa longissima*, *Octodonta nipae*, *Wolbachia*, multilocus sequence type, invasive species

Understanding the exact taxonomic status of insect pests is fundamental to devise efficient control or management measures against them (Rossman and Miller 1996). Pest management studies rely on the fact that taxonomically, individuals are correctly identified and having a scientific name, and that their ecology along with other biological features is known. Any misidentification or failure to distinguish between closely related species can obscure and obstruct the management of pests (Miller and Rossman 1995). Recently, morphologically indistinct or fundamentally similar insect group (such as a species, clade, or biotype) can be segregated by utilizing DNA succession information (Yassin et al. 2008, Takano et al. 2011, Zhang et al. 2015). Since the availability of reliable methods based on the morphological characters is rare, the molecular methods such

as cytochrome oxidase subunit I (COI) and other come into play as more reliable ways for taxonomic classification (Zhang et al. 2015). Another technique such as polymerase chain reaction (PCR) restriction fragment-length polymorphism is also regularly utilized for this purpose (Scheffer et al. 2001, Takano et al. 2013).

Brontispa longissima (Gestro) (Chrysomelidae: Coleoptera) is a natural intrusive nuisance pest on palm cultivations in Southern China and worldwide (Wan et al. 2015, Zhang et al. 2015). Being, among the most severe pest of coconut palm, *Cocos nucifera* L. (Arecaceae: Arecaceae), *B. longissima*, probably originated from Indonesia and New Guinea and has been reported from many other countries and islands including, Southeast and East Asia and the Pacific region (Nakamura et al. 2006) where the host plant (*C. nucifera*) is cultivated

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A nation-wide genetic survey revealed a complex population structure of *Bemisia tabaci* in Pakistan

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ABSTRACT

The whitefly, *Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae) is a cryptic species complex distributed worldwide. In Pakistan, *B. tabaci* poses a serious threat to agriculture production. To understand its diversity in Pakistan, a large-scale sampling was conducted from various locations of all four provinces of the country and Mitochondrial cytochrome oxidase I (mtCOI) gene sequencing was used to determine the whiteflies genetically. The study revealed the presence of five different cryptic species in Pakistan namely Asia II-1, Asia II-5, Asia II-7, Asia II-8 and MEAM-1, respectively. Among them, Asia II-1, which was previously reported from a few areas in the country, had been found now to be prevalent all over the country covering 88.7% of all the sequenced samples. Based on the mtCOI sequences and genetic distance analyses, the diversity of Asia II-1 was much greater than all other cryptic species, which exist only in small patches.

1. Introduction

Whitefly, *Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae), is one of the most disastrous pests of economically important plants including vegetables, ornamentals and household plants worldwide (Chintakunta, 2015; Outherton and Vänninen, 2015; Islam et al., 2017a). Besides damaging host plants via phloem feeding or secreting honeydews that promote the growth of fungal pathogens, *B. tabaci* transmits several economically important groups of viruses including begomoviruses, which cause great yield losses in tropical and subtropical regions (Arif et al., 2017; Islam and Wu, 2017; Islam et al., 2017b; Varun and Saxena, 2017). Although the transmission of begomoviruses is a complex process, the sucking mouthparts of *B. tabaci* allow or facilitate the acquisition and transmission of the viruses (Czomlek et al., 2017). Whitefly transmits begomoviruses in a circulative persistent manner (Götz et al., 2012). The virus has to cross several barriers and checkpoints successfully, while interacting with the various receptors and other whitefly proteins. However, the bulk of the virus remains associated with the insect midgut and the filter chamber (Ghanim, 2014). In these tissues, viral genomes may be transcribed and may replicate (Czomlek et al., 2017). The whitefly plays considerable role in dispersal of begomoviruses to the nearest agro ecological zones, further spreading them to long distances through trade activities (Islam

et al., 2018a). The reason regarding the evolution of begomoviruses and emergence of new strains can be the existence of other viruses, various hosts, new vector species, changing environmental conditions and varying agricultural practices (Jones, 2014).

Currently, *B. tabaci* is considered as a species complex (Polston et al., 2014; Tay et al., 2012) embedding more than 35 (da Fonseca Barbosa et al., 2015; Qin et al., 2016; Hu et al., 2017; Mugerwa et al., 2018) morphologically indistinguishable, but genetically distinct cryptic species (Li et al., 2012; Wang et al., 2011). Historically, a region is dominated by one or a few native cryptic species. However, the situation is changing rapidly because of the highly invasive nature of some *B. tabaci* cryptic species. The introduction of new cryptic species is sometimes disastrous to a region because the introduced species may be resistant to local management strategies and display novel behavioral features that favor virus transmission (Götz and Winter, 2016). For example, the worldwide fast distribution of Middle East Asia Minor-1 (MEAM-1) has been blamed for the increasing agricultural importance of begomoviruses (Gilbertson et al., 2015; Islam, 2017).

Various DNA-based techniques have been utilized to identify *B. tabaci* cryptic species (Ahmed et al., 2011; Ashfaq et al., 2014; Rabello et al., 2008). However sequence analysis of mitochondrial cytochrome oxidase I (mtCOI) gene have been most widely applied (Alhudaib et al., 2014; Chintakunta, 2015; Prassanna et al., 2015; Qin et al., 2016). The

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Microbial Pathogenesis

journal homepage: www.elsevier.com/locate/micpathA novel bacterial symbiont association in the hispid beetle, *Octodonta nipae* (Coleoptera: Chrysomelidae), their dynamics and phylogenyHabib Ali^{a,b}, Abrar Muhammad^{a,b}, Saif Ul Islam^{a,c}, Waqar Islam^{a,c}, Youming Hou^{a,b,*}^a State Key Laboratory of Ecological Pest Control for Fujian and Taiwan Crops, Fujian Agriculture and Forestry University, Fuzhou, 350002, China^b Fujian Provincial Key Laboratory of Insect Ecology, College of Plant Protection, Fujian Agriculture and Forestry University, Fuzhou, 350002, Fujian, China^c College of Plant Protection, Fujian Agriculture and Forestry University, Fuzhou, 350002, China

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ABSTRACT

The hispid leaf beetle, *Octodonta nipae* (Maulik), (Coleoptera: Chrysomelidae), is a devastating pest of palm cultivation worldwide. Endosymbiotic bacteria in the genus *Wolbachia* are arguably one of the most abundant bacterial group associated with arthropods. Owing to its critical effects on host reproduction, *Wolbachia* has garnered much attention as a prospective future tool for insect pest management. However, their association, infection dynamics, and functionality remain unknown in this insect pest. Here, we diagnosed for the first time, the infection prevalence, and occurrence of *Wolbachia* in *O. nipae*. Experimental evidence by the exploration of *wsp* gene vindicate that *O. nipae* is naturally infected with bacterial symbiont of genus *Wolbachia*, showing a complete maternal inheritance with shared a common *Wolbachia* strain (*wNip*). Moreover, MIST (*gusH*, *flpA*, *coxA*, *ftsZ*, and *hspA*) analysis enabled the detections of new sequence type (ST-484), suggesting a particular genotypic association of *O. nipae* and *Wolbachia*. Subsequently, quantitative real-time PCR (qPCR) assay demonstrated variable infection density across different life stages (egg, larvae, pupae and adult male and female), body parts (head, thorax, abdomen), and tissues (ovaries, testes, and gut). Infection density was higher in egg and female adult stage, as well as abdomen and reproductive tissues as compared to other samples. Interestingly, *Wolbachia* harbored dominantly in a female than the male adult, while, no significant differences were observed between male and female body parts and tissues. Phylogeny of *Wolbachia* infection associated with *O. nipae* rectified from all tested life stages were unique and fall within the same monophyletic supergroup-A of *Wolbachia* clades. The infection density of symbiont is among the valuable tool to understand their biological influence on hosts, and this latest discovery would facilitate the future investigations to understand the host-symbiont complications and its prospective role as a microbiological agent to reduce pest populations.

1. Introduction

The nipa palm hispid, *Octodonta nipae* (Maulik) (Coleoptera: Chrysomelidae), native to Malaysia [1] and initially described from Peninsular Malaysia [2], is one considered of the most damaging pest of palm cultivations in Southeast Asia. In China, this invasive pest was highlighted for the first time in 2001 in the forest nurseries of Hainan province [1], and subsequently the damaging dispersal reached to Fujian province during 2007 [3,4], and several other provinces are likely to be infested. Owing to its gregarious nature, adults and larvae favorably attacks on the middle unopened epidermal parenchyma [5–7] and ultimately leaflets of furled fronds appear withered, and gray-brown with rolled edges, which can result in stunted plant growth and even tree death [8] (Fig. 1). According to the literature, *O. nipae* can infest a total of 15 genera in 4 subfamilies (Coryphoideae, Arecoideae,

Nypoideae, and Calamoideae) belonging to the family Palmae [1,9]. Among them, *O. nipae* preferably attacks California fan palm, *Washingtonia filifera* (Linden ex. Andre), rattan, *Calamus manan* (Miquel) [10], queen palm, *Syagrus romanzoffiana* (Chamisso) Glazman [11], Chinese windmill palm, *Trachycarpus fortunei* (Hooker) H. Wendland and Canary Island date palm, *Phoenix canariensis* (Chabaud) [3,4].

Symbiosis frequently occurs in insect, and there are more than 10% of insects reported entirely relying on symbiotic interactions for their development [12] that they have a high degree of co-speciation and dependence as a consequence of general evolutionary history. It is based mainly on metabolic innovations related to ecological advantages obtained by the host, such as the supplementation of missing or insufficient nutrients in the host diet, tolerance to stress factors, and increased host plant use due to metabolic compensation [13]. Intracellular symbionts are functionally known as obligate or facultative

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A WAY OF REPRODUCTIVE MANIPULATION AND BIOLOGY OF *Wolbachia pipientis*

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ABSTRACT

The endo-symbiotic relationship between microorganism and eukaryotes are very common and has been broadly investigated from all insect species. The genus *Wolbachia* are obligatory intracellular bacteria that induce evolutionary alterations and have been frequently reported in egg cytoplasm's of various invertebrates including insects, spiders, mites, scorpions, crustaceans and nematodes, which exert a profound impact on host biology and behavior through a number of phenotypic alterations. Due to its ubiquitous phenotypic behavior, *Wolbachia* becomes a novel and promising natural micro-biocontrol agent to induce cytoplasmic incompatibility, killing of male embryos, parthenogenesis, and feminization. Autonomous transgenic capability, survival on several host species and the ability to modify the host reproductive systems makes it the most prominent and focusing scientific genomic research from last few decades. The aim of this review is to summarize the significance and recent discoveries of *Wolbachia* related to various arthropods which lead positive directions to be exploited in future for integrated pest management (IPM) strategies. Further, this review also discussed the biology, phylogeny, distributions, classifications and types of *Wolbachia* infections on insect orders and coleopterans in particular.

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ABSENCE OF *WOLBACHIA* IN RED PALM WEEVIL, *RYNCHOPHORUS FERRUGINEUS* OLIVIER (COLEOPTERA: CURCULIONIDAE): A PCR-BASED APPROACH

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Abstract. Among the intracellular bacterial symbionts, the genus *Wolbachia* (Rickettsiales) is one of the most abundant taxa associated with reproductive systems of various insects. It is cytoplasmic inherited endosymbiont that induce various reproductive alterations. Characterization analysis has revealed that a number of arthropods harbour *Wolbachia* sp. estimates from 20-80%, with emphasize on Coleopterous insects (41 species have been reported so far). Therefore, in the context of broad existence and unique phenotypic actions to alter reproductive systems of various insect, we aimed to determine the intriguing possibility of this endosymbiont from one of the deadliest palm pest, i.e. red palm weevil (RPW), *Rynchophorus ferrugineus* (Curculionidae) from different geographical locations using polymerase chain reactions (PCR) with four sets of *Wolbachia*-specific primers (SPs) along with one pair of universal bacterial primer (BP). Parallel analysis was also carried out with *Wolbachia* strain isolated from whitebacked plant hopper, *Sogatella furcifera* (Homoptera: Delphacidae), as a positive control. Our analysis confirmed the absence of *Wolbachia* sp. across the various life stages of RPW reared in laboratory or captured from the field. Moreover, the phylogenetic analysis of all closely related *Wolbachia*-mediated weevils were compiled and retrieved from NCBI database indicates the extent of transfection of this bacterium into RPW for the future work on biological control of RPW. This study may facilitate to understand further evolutionary consequences of *Wolbachia* infection in weevils.

Keywords: RPW, biological control, endosymbiotic bacteria, *Wolbachia*, PCR

Introduction

A number of symbiotic bacteria associated with insects profoundly influence their host physiology. Among these endosymbionts, *Wolbachia* sp. belongs to order Rickettsiales, is widely distributed in invertebrates (Arthropods). The evidences are mounting that *Wolbachia* can infect a number of insect species (20-80%) (Werren and Windsor, 2000; Jiggins et al., 2001; Tagami and Miura, 2004; Hilgenboecker et al., 2008; Zug and Hammerstein, 2012) from different orders including Diptera, Hymenoptera, Coleoptera, Lepidoptera, Orthoptera, and Hemiptera/Homoptera (Werren and Windsor, 2000). Extrapolations of this percentage among the total number of insects and nematode species make *Wolbachia* one of the most abundant endosymbiont. This genus has also been reported from filarial nematodes, the causative agents of river blindness and elephantiasis in human (Fenn et al., 2006). *Wolbachia* has the potential to distort the host reproductive system by various phenotypic effects (Blagrove et al., 2012; Ali et al., 2016) such as cytoplasmic incompatibility (Poinot et al., 2003) (CI), parthenogenesis induction (Stouthamer et al., 1999) (PI), male killing (MK) (Jiggins et



Biological Control and Crop Protection

Comparative bio-efficacy of nuclear polyhedrosis virus (NPV) and Spinosad against American bollworm, *Helicoverpa armigera* (Hubner)



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ABSTRACT

American bollworm (ABW), *Helicoverpa armigera* (Hubner), is considered as a major pest of cotton, *Gossypium hirsutum*, all over the globe. Due to its destructive feeding nature and continuous consumption of the same chemicals, it developed resistant against many insecticides. Therefore, a combined application of bio- and synthetic-pesticide need to evaluate against this pest. The entomopathogenic viruses like nuclear polyhedrosis virus (NPV), a member of baculoviruses, can be the potential candidates for better control against ABW. The present study was conducted to assess the comparative efficacy of NPV and Spinosad 240SC (with the concentration of 250 mL ha⁻¹) against ABW in the controlled environment. The ABW was treated with different concentrations of NPV and Spinosad separately and in a combination of NPV with 0.1% Spinosad. The results revealed that highest concentrations showed highest mortality (95%) followed by 95%, 92%, 84%, 82% and 78% mortality at 1×10^5 , 1×10^4 , 1×10^3 , 1×10^2 and 1×10^1 POBs, respectively. Spinosad when mixed in diet give 100% mortality at 0.8% followed by 50.87%, 42.10%, 29.82%, 26.31% and 22.80% mortality at 0.4%, 0.2%, 0.1%, 0.05% and 0.025% respectively. The results of this study revealed that microbial control of ABW through NPV is an effective tool. The repeated use of synthetic pesticides caused the resurgence of many insect pests, and this study results would provide useful insight to build a framework for future investigations for the management of many major insect pests.

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Introduction

American bollworm (ABW), *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae), has been observed most dangerous pest and yield decliner of the field crops and vegetables in Pakistan since the 1990s and early 21st century. It has become the most hazardous pest due to its polyphagous nature, migratory behavior, high

fecundity rate, multiple generations and can develop the resistance against insecticides. It is estimated that annually, 2.5 million bales losses were caused by this insect pest to the cotton crop (Karim, 2000; Muhammad et al., 2009).

Different levels of resistance (moderate to high) to a wide range of insecticides have been reported worldwide, including Pakistan, among the field population of ABW (Sayyed and Wright, 2006; Damalas and Eleftherohorinos, 2011; Ishtiaq and Saleem, 2011). Therefore, the management of this pest for an extended period with reduced risk of insecticides is the dire need of time and a pragmatically suggested option. Among the various biological control agents used as an alternative to synthetic insecticides (Ali et al.,

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Comparative effectiveness of entomopathogenic nematodes against red palm weevil (*Rhynchophorus ferrugineus*) in Pakistan

Mujahid Manzoor, Jam Nazeer Ahmad, Muhammad Zahid Sharif, Dilawar Majeed, Hina Kiran, Muhammad Jafir and Habib Ali

Abstract

In this investigation the effectiveness of EPNs (entomopathogenic nematode species) including *Steinernema carpocapsae*, *Heterorhabditis bacteriophora* and *Steinernema feltiae* on the *Rhynchophorus ferrugineus* larvae and adults were scrutinized. While during bioassays, plastic boxes of 9x5x5 cm size were used. Whatman filter paper was retained at the base of each culture box and 3rd, 6th and 10th instar larvae of red palm weevil (RPW) (*R. ferrugineus*) were placed. EPNs were inoculated to weevil larvae of mentioned instars and also to the adults at the concentration level of the 100 IJs/larva+adult and then incubated at temperature of 25°C. Later the EPNs inoculation, larval instars and adults were tested after 12 hours of time length and their mortality were noted. This investigation was terminated at the end of 8th day and consequences were assessed. All of the mentioned EPNs applied in this experiment resulted variant mortality on each larval instar and adult stage of red palm weevil. The maximum and minimum %age mortality of *R. ferrugineus* at 3rd and 10th larval instars were 96.5% and 88.17% by inoculation of *S. carpocapsae*, 85.75% and 74.4% by application of *H. bacteriophora* while 38.68% and 35.35% by employing *S. feltiae* respectively. On the other hand, minimum mortality of adult red palm weevil by application of mentioned EPNs was noticed. Furthermore, we observed increasing trend of %age mortality of *R. ferrugineus* populace after time length of 12 to 192 hours (8 days) by utilization of EPN species. At the end, we resulted that the credible application of EPNs against palm weevil should be debated and employed for monitoring this notorious pest.

Keywords: *R. ferrugineus*, Mortality, *S. carpocapsae*, *S. feltiae*, *H. bacteriophora*, EPNs

1. Introduction

The date palm (*Phoenix dactylifera*), an ancient plant was reported before 5000 years ago, heterogeneous in nature and having no propagation ability like other plants [16]. The attack of insect pests and diseases exerts severe (30%) yield losses around the world [11]. It was reviewed that *R. ferrugineus* has been spotted globally in range of 15- 50% in case of coconut producing and date-growing countries respectively [35].

The insect pests of coleopterans are placed among the rapacious pests of economically imperative crops. Among these invasive pests, *R. ferrugineus* is regarded as the extremely dangerous tissue borer, firstly detected on *Cocos nucifera* from southern parts of Asia, later on it became the pest of Middle East [14]. Afterward, infestations of the RPW were reported in India and different districts of Pakistan including Muzaffargarh, Dera Ghazi Khan and Multan [26]. After 2 years, this pest was also identified in the orchards of date palms from Iraq. RPW exert massive losses (\$US 130 million) in Middle East [12, 14]. It has ability to destroy 29 species of palm trees most importantly date palms, the significant crops in Africa, South East part of the Asia and Middle East [7, 13, 32]. Place of original reported home of RPW is particularly southern parts of Asia, destroy a number of coconut plantations [31].

In *P. dactylifera*, control methodologies circle around the usage of food lured pheromone traps, while in case of *P. canariensis* (Canary Island date palm), palm weevil control is mostly undertaken by the utilization of insecticides. Both, the environmental as well as non-target side effects claim for development of management practices which are sustainable and environment friendly [7]. The RPW pest shows cryptic behavior so, it is the reason that synthetic pesticides were found bogus for its management [2]. Alternatively, microbial control agents including entomopathogenic fungi (EPFs) and nematode (EPNs) shows great potential against different insect pests [19, 29, 33].

DISTRIBUTION AND TROPHIC ASSOCIATIONS OF ANT GENUS *MONOMORIUM* MAYR, 1855 (HYMENOPTERA: FORMICIDAE: MYRMICINAE) WITH APHIDS IN POTHWAR REGION OF PAKISTAN

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ABSTRACT

Two species of genus *Monomorium* Mayr, 1855 namely *Monomorium indicum* Forel, 1902 and *Monomorium sagei* Forel, 1902 have been recorded for the first time from Pothwar region of Pakistan. Currently a total of five species of this genus have been documented from Pakistan. Conservation status of two newly recorded species has also been discussed. Both species have been described here using Bingham (1903). Morphological characters of prosoma, mesosoma and metasoma were used for the identification of the species. Description has been supported with micrographs of identification characters, morphometric, world distribution and GPS coordinates of Pakistan. Trophic associations of both species with aphid partners are also reported for the first time from Pakistan. *M. indicum* was found to be associated with 2 aphid species namely, *Aphis gossypii* Glover, 1877 and *Aphis fabae* Scopoli, 1763 while *M. sagei* lived in associations with *Greenidea* (*Trichosiphum*) *psidii* van der Goot, 1917, *Aphis gossypii*, *A. fabae* and *A. fabae solanella* Theobald, 1914.

Key words: *Monomorium*, Myrmicinae, Aphid associations, Pothwar, Pakistan.

INTRODUCTION

Genus *Monomorium* was first described by Mayr in 1855. *Monomorium* is considered as diverse and taxonomically the most difficult ant genus in the subfamily Myrmicinae, having 384 reported species and subspecies throughout the world (Antweb, 2019). *Monomorium* ants mostly occur in Old world, tropics, and temperate zones (Brown, 2000). This genus contains world's most widely distributed and successfully established tramp-species, sharing terrestrial habitats and microhabitats. Species of this genus are generalistic in feeding behaviour, more rarely granivorous and several species have been reported as lepto-biotic or parasitic (Ettershank, 1966; Bolton, 1987). These ants prefer nesting in leaf litter, in the soil, under stone and in rotten wood (Collingwood, 1985; Collingwood and Agosti, 1996; Aldawood and Sharaf, 2011).

Members of this genus can be classified on the basis of following characters: workers monomorphic to polymorphic; 3-5 teeth at mandibles which decrease in size from anterior to posterior margin; distinct median clypeal seta; clypeus raised medially; antennae 10-12 segmented (mostly 12) with 3 segmented club; propodeal

dorsum not spinose; petiole pedunculated anteriorly (Bolton, 1987; Bingham, 1903).

Various scientists have done a lot of work on this genus regarding revision and exploration of these ants like; Afrotropical region (Bolton, 1987), Australian and Malagasy region (Heterick, 2001, 2006), North America (DuBois, 1986), Neotropical region (Fernández, 2007) and for Saudi Arabia (Collingwood, 1985; Collingwood and Agosti, 1996). According to Bolton (2018) this genus is native to Afrotropical, Australasia, Indomalaya, Malagasy, Nearctic, Neotropical, Oceania and Palearctic bioregions. Forty three species belonging to genus *Monomorium* have been reported from Australia (Heterick, 2001; Heterick, 2003), forty species described from Saudi Arabia (Collingwood and Agosti, 1996; Sharaf et al., 2018), four species from Sri Lanka (Antweb, 2019), eight species from Afghanistan (Pisarski, 1970), nineteen species from Iran (Paknia et al., 2010) and twenty species have been reported from India (Bharti et al., 2016; Bolton, 2018). However in Pakistan, ants have been poorly studied. A limited work on ants in Pakistan includes new distributional records, new country records of ants, their associations with sucking insects etc. (Umair et al., 2012; Bodlah et al., 2016; Bodlah et

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Entomopathogenic nematode *Steinernema carpocapsae* surpasses the cellular immune responses of the hispid beetle, *Octodonta nipae* (Coleoptera: Chrysomelidae)

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ABSTRACT

The Nipa palm hispid, *Octodonta nipae* (Muls.) is an important invasive pest of palm trees particularly in Southeast China. How this beetle interacts with invading pathogens via its immune system remains to be dissected. *Steinernema carpocapsae* is a pathogenic nematode that attacks a number of insects of economic importance. The present study systematically investigates the cellular immune responses of *O. nipae* against *S. carpocapsae* infection using combined immunological, biochemical and transcriptomic approaches. Our data reveal that *S. carpocapsae* efficiently resists being encapsulated and melanized within the host's hemolymph and most of the nematodes were observed moving freely in the hemolymph even at 24 h post incubation. Consistently, isolated cuticles from the parasite also withstand encapsulation by the *O. nipae* hemocytes at all time points. However, significant encapsulation and melanization of the isolated cuticles were recorded following heat treatment of the cuticles. The host's phenoloxidase activity was found to be slightly suppressed due to *S. carpocapsae* infection. Furthermore, the expression levels of some antimicrobial peptide (AMP) genes were significantly up-regulated in the *S. carpocapsae*-challenged *O. nipae*. Taken together, our data suggest that *S. carpocapsae* modulates and surpasses the *O. nipae* immune responses and hence can serve as an excellent biological control agent of the pest.

1. Introduction

Nipa palm hispid, *Octodonta nipae* (Muls.) (Coleoptera: Chrysomelidae), is an invasive species of palm trees of the Palmae family in Southeast Asian countries [1,2]. It is native to Malaysia and was first identified as a forest invasive pest in Hainan province of China [3]. It was reported to have spread to other provinces including the Fujian province in 2007 [4]. The palm trees that were reported to be damaged by the attack of this pest include the queen palm, *Syagrus romanzoffiana* (Chamisso) Glassman [5], California fan palm, *Washingtonia filifera* (Linden ex. Andre), Canary island date palm, *Phoenix canariensis* (Chabaud) [4,6], Chinese windmill palm, *Trachycarpus fortunei* (Hooker) H. Wendland and rattan, *Calamus manan* (Miquel) [7]. However, adults and larvae of this pest cause the most remarkable damages by feeding on unopened central epidermal parenchyma and leaf fronds, which leads to scorching and stunted growth and, in some situation, subsequent death of the whole tree [8–11].

The most common control method is the use of insecticides. However, other control agents have reportedly been used [5]. These include *Tetrastichus brontispae* (an endoparasitoid of *O. nipae*) and the entomopathogenic fungus, *Metarhizium anisopliae* [12–16]. Entomopathogenic nematodes (EPNs) are biological control agents for different economically important agricultural pests from laboratory to field levels [16]. The most widely used EPNs are from the families Steinernematidae and Heterorhabditidae. EPNs are reported to be used for control of quarantine leaf eating caterpillars and other insect pests on many crops [17] such as adult western corn rootworm *Diabrotica virgifera* [18], flea beetles *Phyllotreta* spp. [19,20] and Colorado potato beetle *Leptinotarsa decemlineata* found in leaf surfaces [21]. It is generally known that insects defend themselves against bacterial, fungal and nematode infections through their innate immune system which is categorized into cellular and humoral systems [22,23]. The cellular immune reaction includes phagocytosis, lysis, nodulation and encapsulation, whereas the humoral immune reaction includes

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Evaluation of imidacloprid and entomopathogenic fungi, *Beauveria bassiana* against the red palm weevil *Rhynchophorus ferrugineus* (Coleoptera: Curculionidae)

Mubashar Ahmad Malik, Mujahid Manzoor, Habib Ali, Abrar Muhammad, Saif ul Islam, Muhammad Qasim, Nazir Ahmad, Atif Idrees, Arif Muhammad, Hafiz Sohaib Ahmed Saqib

Abstract

The Red Palm Weevil is a serious problem of the date palm in Pakistan. The sole and combine application effects of *Beauveria bassiana* (Balsamo) Vuillemin (Hyphomycetes) and sub-lethal dosages of Imidacloprid against the second and fourth instar larvae of *Rhynchophorus ferrugineus* Olivier was investigated in laboratory. Additionally, the *in vitro* hyphal growth of *B. bassiana* amended with three different concentrations (50, 250, 500 µl L⁻¹) of Confidor® 200 SL was also assessed. The results showed significantly higher mortality for the second and fourth instar larvae of RPW when *B. bassiana* and Imidacloprid applied together compared to their sole application. The highest mortality level (100%) for the second instar was recorded after 15 days of treatment with *B. bassiana* (1×10⁶ conidia/ml) and Imidacloprid (1 µl L⁻¹) in combination, while for the fourth instar larvae it was achieved after 20 days of application. However, the presence of Imidacloprid did not significantly affect the hyphal growth of *B. bassiana* on Sabouraud Dextrose Agar (SDA), and greater colony sizes compared to untreated control were recorded in all cases. The maximum number of sporulation (173, 159.83 conidia ml⁻¹) and percentage (89.15, 79.4%) of mycosed *R. ferrugineus* cadavers was observed at lower dose rate (1×10⁶ conidia/ml) of *B. bassiana*, exhibited the potential to be exploited for the control of RPW.

Keywords: Entomopathogenic fungi, Imidacloprid, Mortality, Mycosis, Sporulation.

1. Introduction

The invasive Red Palm Weevil (RPW) *Rhynchophorus ferrugineus* Olivier (Curculionidae: Coleoptera), is considered as economically important and destructive pest insect of date palm in Pakistan [46]. It was first reported in 1992 from Khairpur Sindh [3], from where the pest spread to other date palm growing areas of Pakistan. At present, the pest has successfully invaded many areas including Asia, Africa, Oceania, and Europe [18], and more recently it has been reported from California and Australia [32, 36]. The female RPW lay their eggs singly in the apertures made with their rostrum at the leaf base [35]. On hatching the larvae made their way into the center of palm and make galleries by feeding inside the trunk, the mature larvae made their cocoons from the chewed fiber and pupate at the leaf base [22]. The adult emerged from the cocoon, resided on the same tree and reproduced till the death of host [13].

The existing methods of RPW management largely rely on the IPM strategies, which include: phyto sanitation, use of conventional insecticide, pheromone traps and bio-control agents. The chemical control method against RPW, include repeated fumigation, spraying and injecting of synthetic insecticides into infected palms [19]. These approaches are not enough as under this category the use of certain chemicals causes the development of resistance in the pest and further have negative environmental and human impacts [1]. Neonicotinoids, comparatively new group of synthetic insecticides, similar to natural product nicotine present in the insect nervous system, agonists the nicotinic acetylcholine receptors (nAChR) [30]. The peculiar mode of action and Chemorational ability of the neonicotinoid insecticides make them a better choice in pest management, especially in case of concealed borers. Imidacloprid [1-(6-chloro-3-pyridylmethyl)-2-nitroimino-imidazolidine] possess both systemic and contact mode of action and is compatible with different application methods for example foliar application, seed treatment [45], soil drench and stem application in different crops and trees [10].



Insect-fungal-interactions: A detailed review on entomopathogenic fungi pathogenicity to combat insect pests

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Highlights

Factors Affecting the Epizootics of Entomopathogenic Fungi-A Review

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Article

Sub-Lethal Effects of *Lecanicillium lecanii* (Zimmermann)-Derived Partially Purified Protein and Its Potential Implication in Cotton (*Gossypium hirsutum* L.) Defense against *Bemisia tabaci* Gennadius (Aleyrodidae: Hemiptera)

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Abstract: Whiteflies, *Bemisia tabaci* Gennadius (Aleyrodidae: Hemiptera), are a polyphagous economically destructive pest of several solanaceous crops around the world. Many secondary metabolites are synthesized by different biotrophic and necrotrophic fungi which are capable of inducing systemic resistance in plants against various phytophagous pests. The present laboratory work demonstrated the anti-insect impact of a protein extracted and purified partially from an entomopathogenic fungus (EPF) *Lecanicillium lecanii* (Zimmermann) against *B. tabaci*. Three different concentrations (i.e., 7.43, 11.15, and 22.31 $\mu\text{g mL}^{-1}$) of this protein were bioassayed to assess its effect on the fecundity rate of *B. tabaci* on cotton (*Gossypium hirsutum* L.) plants. Furthermore, the possible implication of this fungal protein in defense pathways of cotton plants was evaluated by determining the expression profiles of salicylic acid (SA) and jasmonic acid (JA) pathways related to major genes through reverse transcription qPCR (RT-qPCR). According to the results, all protein concentrations exerted a significant ($F_{3,252} = 62.51$; $p \leq 0.001$) and negative impact on the fecundity rate of *B. tabaci* females. At the highest protein concentration (22.31 $\mu\text{g mL}^{-1}$), the minimum rate of fecundity (i.e., 2.46 eggs female⁻¹ day⁻¹) of *B. tabaci* was noted on the seventh day, whereas fecundity rates for the other two protein concentrations (i.e., 11.15 and 7.43 $\mu\text{g mL}^{-1}$) were, respectively, 3.06 and 3.90 eggs day⁻¹ female⁻¹. The maximum rate of fecundity (6.01 eggs female⁻¹ day⁻¹) was recorded in untreated (control) treatments. In addition, the foliar application of *L. lecanii* derived protein significantly upregulated all SA linked genes (OPR3, PPO1 and COI1) and slightly triggered up the JA linked genes (LOX1, UBC7 and AOS) in the cotton plants. These findings revealed that this *L. lecanii* extracted partially purified protein triggered systemic resistance against *B. tabaci* in the cotton plants, proposing its



Analysis of food resources, host availability and insecticidal impacts on the fecundity, longevity and parasitism efficiency of *Diaeretiella rapae* (McIntosh)

Ahmad Nawaz¹ · Habib Ali^{1,2} · Sajid Fiaz³ · Muhammad Sufyan¹ · Miza Abdul Qayyum⁴ · Rashid Azad⁵ · Yasir Niaz² · Muhammad Adnan Bodlah² · Khalid Ali Khan⁶

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Abstract

The use of pesticides may pose a serious threat to soil and water quality, human health, food safety, aquatic species and beneficial insects. Biological control is an effective component of integrated pest management comprising importation, augmentation and conservation. Conservation of natural enemies could enhance or limit the efficiency of herbivorous insect pests. The present study was designed to evaluate the efficiency of *Diaeretiella rapae* (McIntosh) and other food resources impacts on parasitoid's fecundity, survival and efficiency. The effect of different insecticides [Carbosulfan, Imidacloprid, Diafenthiuron and Lufenuron] was also evaluated in a laboratory controlled experiment. The results revealed that females fed on aphid honeydew proved to be of poor quality as compared to other sugar sources, including honey and sugar solution and has a positive impact of mating on monogenesis. The negative impact was observed on oviposition activity by female reared in the laboratory having less number of mature eggs than females collected in the field. All insecticides demonstrated dose-dependent mortality of *D. rapae*. In conclusion, food and host availability and insecticides affect the efficiency of *D. rapae*. These results could lead to the hypothesis that *D. rapae* females have a high mortality rate in the field due to lower aphid hosts.

Keywords Biological control · Natural enemies · *Diaeretiella rapae* · Fecundity · Monogenesis · Insecticides

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Background

Agriculture is the backbone for the economic development of Pakistan, which share 21.4% to GDP, and provides 45% employments of the total labor force (Anonymous 2013). The insect pests are the major factors affecting the yield of cash crops. The development of genetically modified (GM) crops (Fiaz et al. 2020) and climate change has changed the pest status of different insect pests. The GM crops can develop resistance against insects pests having chewing mouthparts; however sucking insect pests remain to be managed. Use of pesticides to control these insect pests could have several disadvantages, including the development of resistance against insecticide, hazardous for non-target organisms. Among sucking insect pests, aphids are the most serious pests of many crops causing huge damage by sucking cell sap and create a sooty mold on its honeydew secretions. All over the globe, around ten thousand insect species were reported to effect the major crops, among them 10% are considered as a major pests (Dhaliwal et al. 2010).

REVIEW



Apomixis and strategies to induce apomixis to preserve hybrid vigor for multiple generations

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ABSTRACT

Hybrid seeds of several important crops with supreme qualities including yield, biotic and abiotic stress tolerance have been cultivated for decades. Thus far, a major challenge with hybrid seeds is that they do not have the ability to produce plants with the same qualities over subsequent generations. Apomixis, an asexual mode of reproduction by avoiding meiosis, exists naturally in flowering plants, and ultimately leads to seed production. Apomixis has the potential to preserve hybrid vigor for multiple generations in economically important plant genotypes. The evolution and genetics of asexual seed production are unclear, and much more effort will be required to determine the genetic architecture of this phenomenon. To fix hybrid vigor, synthetic apomixis has been suggested. The development of *MiMe* (mitosis instead of meiosis) genotypes has been utilized for clonal gamete production. However, the identification and parental origin of genes responsible for synthetic apomixis are little known and need further clarification. Genome modifications utilizing genome editing technologies (GETs), such as clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein (cas), a reverse genetics tool, have paved the way toward the utilization of emerging technologies in plant molecular biology. Over the last decade, several genes in important crops have been successfully edited. The vast availability of GETs has made functional genomics studies easy to conduct in crops important for food security. Disruption in the expression of genes specific to egg cell *MATRI LINEAL* (*MTL*) through the CRISPR/Cas genome editing system promotes the induction of haploid seed, whereas triple knockout of the *Baby Boom* (*BBM*) genes *BBM1*, *BBM2*, and *BBM3* cause embryo arrest and abortion, which can be fully rescued by male-transmitted *BBM1*. The establishment of synthetic apomixis by engineering the *MiMe* genotype by genome editing of *BBM1* expression or disruption of *MTL* leads to clonal seed production and heritability for multiple generations. In the present review, we discuss current developments related to the use of CRISPR/Cas technology in plants and the possibility of promoting apomixis in crops to preserve hybrid vigor. In addition, genetics, evolution, epigenetic modifications, and strategies for *MiMe* genotype development are discussed in detail.

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Hybrid vigor; flowering plants; apomixis; CRISPR/CAS system

1. Introduction

Scientific, logistical, and humanitarian approaches are simultaneously involved to ensure food security, starting with farmers and breeders and further extending to policymakers and governments. A universal solution for sustainable food security is difficult owing to differences in cultural values and geographical regions, environments, and technologies. Although these challenges are substantial, there is great potential to increase the efficiency and productivity of current agricultural

products. The powerful breeding techniques that we have at our disposal are being used to exploit heterosis in commercially important crops. Hybrid seed production is a successful technology, and hybrid seeds produced either by three-line or two-line systems to achieve supreme qualities, i.e., premium yield and quality along with resistance against biotic and abiotic stresses, have been cultivated for a long time.¹ Among the two-line and three-line hybrid development systems, the former is more advantageous than later because in this system, photoperiod-dependent/thermogenic

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Association of Alu-repeat Polymorphism and Myocardial Infarction in Pakistani Population

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Abstract

Polymorphism of tissue plasminogen activator(t-PA), gene-induced myocardial infarction (MI) is not well-defined in patients suffering from high blood pressure. Plasminogen activator generates the active enzyme by limited proteolysis of zymogen plasminogen to plasmin. Plasmin then degrades the fibrin network of a clot to form soluble product in thrombi. This action of t-PA can be suppressed by plasminogen activator inhibitor type1(PAI-1). This study determined the potential insertion/deletion of polymorphism that may contribute to the development of MI in Pakistani population. The study analyzed blood samples originating from three hundred and fifty patients with MI, two hundred and fifty healthy individuals as controls, and hundred hypertensive study subjects. The genomic DNA was extracted from the blood of each individual, and a Polymerase Chain Reaction was carried out to study polymorphism of Tissue plasminogen Activator (t-PA) gene. The Chi-square method was used to reveal the demographic differences among the groups. Cholesterol's higher levels, triglyceride, LDL-cholesterol, and lower HDL-cholesterol levels had been investigated in cases/patients in contrast with controls. In some cases, the input allele frequency ("T") is higher with MI ($p = 0.0354$). Diabetes, high blood pressure, family history, and smoking had a strong association with MI ($p < 0.01$). No significant association between myocardial infarction and Insertion/Deletion (I/D) and Deletion/Deletion (D/D) polymorphism of t-PA gene, significant association found between Insertion/Insertion(I/I) and MI, which supports the results of previous MI studies.



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Bio-diesel production of sunflower through sulphur management in a semi-arid subtropical environment

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Abstract

Energy security is a prime focus of policy makers to support agriculture, industrialisation, and transportation. Due to limited conventional energy sources, there is a need to harness non-conventional energy sources. In this regard, one of the proposed approaches is using biomass (e.g. energy crops) to produce biofuel—a renewable source of energy. Sunflower has several agronomic features to be exploited for a renewable, non-conventional, and environment-friendly source of bioenergy. Sulphur (S) fertilisation holds key for realising sunflower potential for seed and oil yield. In response to variable S supply rates, here we compared and quantified sunflower yield (seed, oil, and biodiesel) and biodiesel quality according to the ASTM international standards. We used a combined approach of field experimentation and rigorous lab analysis. Firstly, in a field experiment laid out in randomised complete block design with split-plot arrangement, response of two local sunflower hybrids (FH-331 and FH-689) to four S supply rates (0, 25, 50, 75 kg S ha⁻¹) was evaluated in terms of agronomic traits. Experimental data showed that fertilisation of S significantly influenced growth and yield (seed, oil) traits; the response was different between two hybrids which also interacted with S supply rate. FH-331 recorded the highest achene yield at S fertilisation of 75 kg S ha⁻¹, whereas FH-689 recorded the highest achene yield at 50 kg ha⁻¹; achene yield of FH-331 was 13.6% higher than FH-689. Compared to control, S at 75 kg S ha⁻¹ increased oil yield of FH-331 by 22% whereas S at 50 kg ha⁻¹ increased oil yield by 23% of FH-689. Seed samples were analysed for different biodiesel quality parameters. The ranges of all quality parameters of sunflower biodiesel such as viscosity, calorific values, acid value, iodine value, saponification value, cetane number, and pour point were in ASTM standard range. We conclude that sunflower is a promising and sustainable option for producing biodiesel, the potential of which can be increased by optimal S management under field conditions.

Keywords Oil content · Oil yield · Biodiesel yield ha⁻¹ · Saponification value · Iodine number · Cetane value

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Comparative genome sequence and phylogenetic analysis of chloroplast for evolutionary relationship among *Pinus* species

Running title: Comparative and phylogenetic analysis of *Pinus* species

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Comparative pathogenicity of four entomopathogenic fungal species against nymphs and adults of citrus red mite on the citrus plantation

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Abstract

Panonychus citri (citrus red mite) is a devastating pest of citrus orchards. The conventional chemical acaricides have been strongly forbidden for the management of agricultural insect pests in China. Therefore, we evaluated the susceptibility of adult and nymphs *P. citri* in laboratory against eight isolates of four fungal species, *Akanthomyces lecanii*, *Metarhizium anisopliae*, *Beauveria bassiana* and *Aschersonia aleyrodis*. Each citrus seedling having 40 adults (2-d-old) and nymphs (on separate plants) were sprayed with isolates at the concentration of 10^4 – 10^8 conidia mL⁻¹ whereas controlled seedlings were sprayed with 0.02% Tween-80. After 9 days of fungal exposure, the four fungal isolates caused more than 50% mortality of mites, such as; 85.6%, 87.9%, 64.6% and 79.7% by *A. lecanii* (V3450), *B. bassiana* (BFZ0409), *M. anisopliae* (MFZ0706) and *A. aleyrodis* (ASG0910), respectively. The nymphal mites were less susceptible to applied fungi compared to adults. The LC₅₀s of the tested isolates were determined by the fitted time-concentration-mortality relationships, which declined over days after spray. LT₅₀s were decreased with a high concentration of isolates. After the 9-d inoculation, two isolates of *B. bassiana* (BFZ0409 and D1344) and one isolate of *A. lecanii* (V3450) were highly effective at the minimal dose of LC₅₀ of 10^4 conidia mL⁻¹ and are promising candidates to control mites, as compared to other tested fungal isolates.

Keywords Citrus seedlings · Time-concentration-mortality · *Panonychus citri* · *Metarhizium anisopliae* · *Beauveria bassiana* · *Aschersonia aleyrodis*

Introduction

Mites belonging to Arachnida class (Ruppert et al. 2004) consist of four stages, i.e. egg, larva, nymph and adult. Nymphs (protonymph, deutonymph, and tritonymph) and adult are most feeding and damaging stages. There are more than

50,000 species of mites, which are predatory, parasitic, saprophagous, herbivores, necrophagous, fungivores, coprophagous as well as phoretic species (Dhooria 2016).

Mites that attack citrus plantations worldwide include *Panonychus citri* McGregor, *Eotetranychus kankitus* Ehara, *Polyphagotarsonemus latus* Banks, *Tetranychus kanzawai*

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RESEARCH ARTICLE

Diet impacts on the biological aspects of pink bollworm, *Pectinophora gossypiella* (Lepidoptera: Gelechiidae) under controlled laboratory conditions

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Abstract

Background

Pink bollworm, *Pectinophora gossypiella* (Lepidoptera: Gelechiidae) is a native pest of Asia and preferably invasion on cotton (*Gossypium hirsutum* L.) crop as a commensal host plant. Commercially, *G. hirsutum* is known as white gold and is an important cash crop all over the globe. Limited studies were published to focus on certain dietary compositions against different cotton pests. Therefore, the present study was undertaken in the laboratory under controlled conditions (temperature: $27 \pm 2^\circ\text{C}$ and relative humidity: $60 \pm 10\%$) to determine the impact of three different treatment diets (wheat germ meal, okra, and chickpea) on the biological aspects (lifetime, developmental period) of *P. gossypiella*.

Results

Results revealed that the shortest larval time of *P. gossypiella* was observed on the okra fed diet while the longest period was recorded on the wheat germ diet. Meanwhile, the



Original article

Effect of constant and fluctuating temperature on the circadian foraging rhythm of the red imported fire ant, *Solenopsis invicta* Buren (Hymenoptera: Formicidae)

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ABSTRACT

Understanding circadian foraging rhythms activity of the red imported fire ant, *Solenopsis invicta* Buren (Hymenoptera: Formicidae) foragers at different temperatures is an important step towards developing control measures in Integrated Pest Management (IPM) programs. In this study, the circadian foraging rhythm activities of *S. invicta* foragers at different temperature were investigated under laboratory and field conditions. Results indicated that the foraging activity increased after sunrise, and maximum foraging occurred at 14:00 (foraging rate was 69.22 ± 0.57 and 72.58 ± 1.15 foragers/min in the first and second year, respectively) in the tea fields of Guangzhou during autumn. Furthermore, foragers demonstrated circadian rhythms and exhibited a unimodal after 24 h. A significant correlation was found between foraging activity and temperature. *S. invicta* colonies were active at moderate soil temperatures (approximately 26.65 °C to 29.24 °C). The preferred temperature of the colonies was 26 °C, followed by 22 °C and 18 °C in the laboratory. The individual *S. invicta* activity was maximum at 17:00 (18.67 ± 1.66 times/10 min) and minimum at 5:00 (8.33 ± 2.51 times/10 min) at 26 °C. The fluctuating temperature had a significant impact on individual locomotor activity ($r = 0.8979$, $P < 0.01$) but did not alter the rhythm activity. Our results demonstrated that temperature might play an important role in circadian foraging rhythms activity of *S. invicta*. These results may have implications for the development of more effective fire ant management strategies.

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1. Introduction

Solenopsis invicta Buren (Hymenoptera: Formicidae) commonly called red imported fire ant, most notorious pest and has invaded more than 20 countries, e.g., Australia, China, Taiwan, India, Japan, South Korea, Myanmar, Laos, and Vietnam (Allen et al., 1994; Vinson, 1997; Wang et al., 2019). In China, *S. invicta* is considered a most notorious ant species and have been invaded more than 15 provinces in China (Zheng and Zhao, 2005; Huang and Huang, 2010; Wang et al., 2019; Zeng et al., 2005; Ma et al., 2018; Wang

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Exogenous Zinc Induced NaCl Tolerance in Okra (*Abelmoschus Esculentus*) by Ameliorating Osmotic Stress and Oxidative Metabolism

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ABSTRACT

Zinc appeared to be an important modulator with specific functions in plant physiology and increase resistance against different abiotic stresses. Many advances regarding role of zinc under abiotic stress in plants have been made since its revelation, therefore, present study was intended to understand the ameliorating role of zinc on morpho-physiological and biochemical responses of the okra varieties (Anmol and Haseena) when exposed to salt stress. A solution culture experiment was conducted to understand the protective role of zinc in relation to growth, physiological, ionic and antioxidant attributes in two okra varieties under saline condition. The growth medium was salinized with three salinity (Control, 80 mM L⁻¹ and 160 mM L⁻¹ NaCl) and one zinc level (30 µM L⁻¹) applied exogenously in combine and sole form. Salt stress significantly reduces plant dry matter accumulation, chlorophyll contents, relative water contents (RWC), membrane stability index (MSI), K⁺/Na⁺ ratio and activities of antioxidant enzymes (POD, SOD, APX, CAT) and these effects are more prominent at high salt level (160 mM). Addition of zinc-alleviated salt toxicity is more conspicuous in okra variety Anmol as compared to variety Haseena as demonstrated by increasing RWC, MSI, K/Na ratio and activities of antioxidant enzymes. Our results suggested that zinc induced salt tolerance in Okra which can serve as a potential source to get sustainable production from salt-affected lands.

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Zinc; salinity; Okra; MDA contents; K⁺/Na⁺ ratio; antioxidant enzymes

Introduction

Soil salinization is the most threaten abiotic aspect to agricultural yield across the globe, particular in arid to semi-arid regions (Zhu et al. 2017). Salinization is escalating rapidly causes loss of 10 million hectares of land annually and will results in 50% loss of arable land up to 2050 (Ahanger et al. 2017). Upon the exposure to saline conditions, the water absorption rate of plants is reduced drastically. This affects inter- and intra-cellular water level and inhibits the cell expansion followed by decline in stomatal activity. High salt concentration in the growth

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FACTORS AFFECTING THE EPIZOOTICS OF ENTOMOPATHOGENIC FUNGI-A REVIEW

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ABSTRACT

Entomopathogenic fungi (EPF) specifically infect and kill insects can serve as a potential biological control agent. Several biotic and abiotic factors affect their occurrence, persistence, and epizootics caused by them. In biotic factors, fungi characters (host range, latency, spore density and dispersal, mainly host-pathogen compatibility), insect host factors (behavioral, morphological, and physiological), plant-mediated effects (plant architecture, surface chemistry, and leaf topology) are included supports or causes the barrier to mycobiopesticide. Abiotic factors which affect the EPF field persistence are mainly environmental (temperature, sunlight, humidity, rainfall) physical and chemical soil properties (soil texture, pH, E.c, moisture, C/N content, and organic matter) that greatly influence the entomopathogenic fungi. To use them as biocontrol agents, we have to overcome these factors by providing them nutrients, protectants, and using different control practices.

Keywords: Entomopathogenic fungi, biotic factors, abiotic factors, epizootics, biocontrol agent.

INTRODUCTION

Entomopathogenic fungi specifically infect and kill insects can serve as a potential biological control agent. EPF are environmentally sustainable because they have goal-specific behaviour, high reproductive abilities, and no effects on beneficial and non-target species. They also have resting stage or saprobic process generation abilities, allowing them to survive for long periods of time without a host (Mudgal et al., 2013). EPF can be present in a variety of environments (from terrestrial to aquatic, arctic to temperate climate, and arid to tropical), infect a wide variety of insects and colonize a wide range of plant species (Mantzoukas et al., 2015). There are several biotic and abiotic factors that affect their occurrence, persistence, and epizootics of EPF (Sharma et al., 2020). Biotic factors directly effects the EPF also includes insect host factors and plant-mediated effects (Cory and Ericsson, 2009; Herlinda, 2010; OrtizUrquiza and Keyhani, 2013). Abiotic factors like environmental factors including temperature (Bayissa et al., 2017), sunlight (Fernandes et al., 2007), humidity, rainfall (Inglis et al., 2001), and soil properties (Quesada Moraga et al., 2007) cause damage directly to the fungal spores. In this review, we focused on all the expected factors in detail which affect the epizootics caused by EPF.

Article

Foliar Spray of Alpha-Tocopherol Modulates Antioxidant Potential of Okra Fruit under Salt Stress

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Abstract: As an antioxidant, alpha-tocopherol (α -Toc) protects plants from salinity-induced oxidative bursts. This study was conducted twice to determine the effect of α -Toc as a foliar spray (at 0 (no spray), 100, 200, and 300 mg L⁻¹) to improve the yield and biochemical constituents of fresh green capsules of okra (*Abelmoschus esculentus* L. Moench) under salt stress (0 and 100 mM). Salt stress significantly reduced K⁺ and Ca²⁺ ion concentration and yield, whereas it increased H₂O₂, malondialdehyde (MDA), Na⁺, glycine betaine (GB), total free proline, total phenolics, and the activities of catalase (CAT), guaiacol peroxidase (GPX), and protease in both okra varieties (Noori and Sabzpari). Foliar application of α -Toc significantly improved the yield in tested okra varieties by increasing the activity of antioxidants (CAT, GPX, SOD, and ascorbic acid), accumulation of GB, and total free proline in fruit tissues under saline and non-saline conditions. Moreover, α -Toc application as a foliar spray alleviated the adverse effects of salt stress by reducing Na⁺ concentration, MDA, and H₂O₂ levels and improving the uptake of K⁺ and Ca²⁺. Among the tested okra varieties, Noori performed better than Sabzpari across all physio-biochemical attributes. Of all the foliar-applied α -Toc levels, 200 mg L⁻¹ and 300 mg L⁻¹ were more effective in the amelioration of salinity-induced adverse effects in okra. Thus, we concluded that higher levels of α -Toc (200 mg L⁻¹ and 300 mg L⁻¹) combat salinity stress more effectively by boosting the antioxidant potential of okra plants.

Keywords: antioxidants; alpha-tocopherol; foliar spray; salinity; okra varieties

1. Introduction

Okra [*Abelmoschus esculentus* (L.) Moench] is the most popular mallow crop and a common food crop in Asia. Its finger-like fruits, called capsules, are mainly consumed as a vegetable. These capsules are a rich source of vitamins, minerals and dietary fiber, and are low in calories. The mucilage's properties have medicinal enormous value [1]. The high mucilage content sets okra apart from other vegetables and makes it suitable for various medicinal and industrial applications [2].

A 90% loss (6.5 dSm⁻¹) in okra yield has been reported under high salt levels [3]. Soil salinity is one of the most prominent obstacles suppressing plant productivity. It remarkably affects the production of crops by disrupting the overall cellular metabolism of



Genetic variability and heritability estimates of morphological traits in F_2 populations of rapeseed (*Brassica carinata* L.)

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Abstract

This study aimed to estimate genetic variability, heritability, and genetic advance for morphological characters in *Brassica carinata* L. A total of 22 genotypes comprised of six parental lines and their 16 bulk F_2 populations were evaluated in a randomized complete block design with three replications at The University of Agriculture Peshawar during 2013-14. Data were recorded on days to 50% flowering, primary branches plant⁻¹, main raceme length, pods on main raceme, pod length, number of seeds pod⁻¹, 100-seed weight, and seed yield plant⁻¹. Significant genetic differences were observed for all the traits studied. Among parental lines, C-93 performed better for primary branches plant⁻¹ (11 branches), main raceme length (59.57 cm), pods on main raceme⁻¹ (33 pods), and seeds pod⁻¹ (15 seeds), C-95 for pod length (4.04 cm) and seed yield plant⁻¹ (12.26 g). Among F_2 populations, C-97 x C-88 performed better for main raceme length (73.20 cm), pods on main raceme (41 pods), C-88 x C-93 for pod length (4.95 cm), seeds pod⁻¹ (18 seeds), and seed yield plant⁻¹ (14.71 g). Moderate ($30 < h^2 \leq 60$) to high ($h^2 > 60$) broad-sense heritability was observed for all morphological traits with maximum genetic advance, hence indicated that selection could be effective in the early generation for the improvements of these studied traits. Generally, cross combinations C-88 x C-93, C-97 x C-95, C-90 x C-93, and C-97 x C-88 performed better than their parental lines for morphological (primary branches plant⁻¹, seeds pod⁻¹, and seed yield plant⁻¹) traits and could be exploited for varietal development in future brassica breeding programs.

Keywords: Genetic variability, genetic advance, heritability, pod length, seeds pod⁻¹

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Immune function differences between two color morphs of the red palm weevil *Rhynchophorus ferrugineus* (Coleoptera: Curculionidae) at different life stages

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Abstract

Several studies demonstrated that in insects cuticle melanism is interrelated with pathogen resistance, as melanin-based coloration and innate immunity possess similar physiological pathways. For some insects, higher pathogen resistance was observed in darker individuals than in individuals with lighter cuticular coloration. Here, we investigated the difference in immune response between two color morphs (black and red) and between the life stages (pupa and adult) of the red palm weevil *Rhynchophorus ferrugineus* (Coleoptera: Curculionidae). Here in this study, cuticle thickness, microbial test (antimicrobial activity, phenoloxidase activity, and hemocyte density), and immune-related gene expression were evaluated at different stages of RPW. Study results revealed that cuticle thickness of black phenotype was thicker than red phenotype at old-pupa stage, while no significant difference found at adult stage. These results may relate to the development processes of epidermis in different stages of RPW. The results of antimicrobial activity, phenoloxidase (PO) activity, and hemocyte density analyses showed that adults with a red phenotype had stronger pathogen resistance than those with a black phenotype. In addition to antimicrobial activity and PO activity, we tested relative gene expression in the fat body of old pupae. The results of hemolymph antimicrobial analysis showed that old pupae with a red phenotype were significantly different from those with a black phenotype at 12 hr after *Staphylococcus aureus* injection, suggesting that red phenotype pupae were more sensitive to *S. aureus*. Examination of gene expression in the fat body also revealed that the red phenotype had a higher immune response than the black phenotype. Our results were inconsistent with the previous conclusion that dark insects had increased immune function, suggesting that the relationship between cuticle pigmentation and immune function in insects was not a direct link. Additional possible factors that are associated with the immune response, such as life-history, developmental, physiological factors also need to be considered.

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In silico analyses of predicted substitutions in fibrinolytic protein ‘Lumbrokinase-6’ suggest enhanced activity

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ABSTRACT

Lumbrokinases (LKs) belong to the group serine proteases capable to prevent thrombosis through the proteolysis of both plasminogen-bound and plasminogen-free fibrin molecules. The article presents improved activity of Lumbrokinase-6 (Lk-6) by suggesting the substitution of a Serine found at position 214 (Lk-6) with three other amino acids namely Glutamic acid, Proline and Valine. To characterize the stability, enzyme-substrate interaction and improved activity of three mutant Lk-6 proteins (Lk-Glu214, Lk-Pro214, Lk-Val214) *In Silico* tools were utilized. Subsequently, Lk-6 wild type and three mutant proteins were subjected to structure prediction, molecular modeling, phylogeny, molecular docking and Protein-Protein Interaction (PPI) using the *In Silico* tools. Collection and analysis of results revealed that substituted mutation at Ser214 with Valine214 can appreciably stabilize the overall structure of Lk-6 protein and makes its interaction with plasminogen activator physically powerful for higher plasmin activation. Similarly, Serine214 to Valine214 substitution resulted the direct activation of plasmin breakage at the Arg561-Val562 bond. The Arg-Val at position 561–562 in plasminogen and its connection at catalytic site have significantly shown that the predicted residue Valine214 could be further examined through genetic engineering of Lk-6 protein. Therefore, such results are potential steps towards the engineering of smart and active Lks.

1. Introduction

Cardiovascular and cerebrovascular disorders (CVDs), especially stroke and coronary heart disease, are leading cause of mortality causing more than 31 % deaths around the globe. Same reason contributed 18 million deaths in 2017 [1]. The blockage of coronary blood supply towards myocardium by the formation of thrombosis is the major cause of

this disease [2]. In various countries, stroke is the leading cause of death which is major economic burden on healthcare system. CVDs are growing health concern in the Middle Eastern region and the Gulf Council Countries (GCC). It is reported that during 2015, the Kingdom of Saudi Arabia had 42 % deaths attributed to CVDs [3]. In USA, approximately 750,000 stroke incidences take place every year. Among these incidences approximately 80–85 % strokes are ischemic and cause

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RESEARCH

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In vitro virulence of three *Lecanicillium lecanii* strains against the whitefly, *Bemisia tabaci* (Genn.) (Hemiptera: Aleyrodidae)

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Trinh Duy Nam⁵ and Dewen Qiu^{1*}



Abstract

Background: Whitefly, *Bemisia tabaci* (Genn.) (Hemiptera: Aleyrodidae), is one of the most harmful pests in greenhouses and in open fields.

Main body: The present study aimed to assess in vitro virulence of 3 entomopathogenic fungal strains (EPFs) of *Lecanicillium lecanii* 3 (V-3), 4 (V-4), and 5 (V-5) against the whitefly, *Bemisia tabaci* (Genn.) (Hemiptera: Aleyrodidae), by spray method. The 3 disparate bioassays were performed encompassed of conidial concentrations and fungal filtrate of the strains, V-3, V-4, and V-5, and also their binary combinations. In the filtrate bioassay, 2 ml of fungal filtrate of each strain was used. In the conidial bioassay, 3 disparate concentrations (1×10^5 , 1×10^6 , and 1×10^7 conidia ml^{-1}) were used for each fungal strain, while in the binary combinations (1 ml filtrate + 1 ml conidia) of V-3 $\times$ V-3, V-4 $\times$ V-4, and V-5 $\times$ V-5 were used. Mortality rates against the whitefly were recorded on the 7th day. In the conidial bioassay, maximum mortality rates were found at V-3 strain (90.6%), V-4 strain (78.4%), and V-5 strain (83.6%) at the highest concentration (1×10^7 conidia ml^{-1}) on the 7th day. In the filtrate bioassay, V-3 strain revealed a maximum mortality (93%), V-4 strain (85%), and then V-5 strain (87%) on the 7th day. Moreover, in the bioassay of binary combinations, the highest mortality rate of the whitefly was counted in V-3 $\times$ V-3 strain (84.6%), V-4 $\times$ V-4 strain (70.6%), and V-5 $\times$ V-5 strain (79.8%) on the 7th day.

Conclusion: All treatments had the potential to control *B. tabaci* significantly. In all bioassays, the V-3 strain was the extreme virulent, and the filtrate application of V-3 strain was the utmost impressive against *B. tabaci*.

Keywords: Entomopathogenic fungi, Virulence, *Lecanicillium lecanii*, *Bemisia tabaci*, Biological control

Background

Whitefly, *Bemisia tabaci* (Genn.) (Hemiptera: Aleyrodidae), is one of the most serious pests of crops that causes great losses (Oliveira et al. 2001). It causes direct destruction by sucking the plant sap, and also indirect destruction by encouraging the growth of black sooty mold on their honeydew secretions (Varma and Malathi

2003). It causes economic losses in many crops, including leafy vegetables, cotton, beans, and tomatoes (Fontes et al. 2012). The application of chemical pesticides leads to increase resistance and has a negative environmental impact that encourages the development of alternative pest management strategies, where microbial control plays a key role (Nazir et al. 2019b). Application of microbial control agents mainly entomopathogenic fungi isolates (EPFs) has been examined to control various orchard and field crop pests (Lacey and Shapiro-Ilan 2008). The use of EPFs in biological control is developing extremely due to food safety concerns, environmental awareness, and the failure of conventional chemicals

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Insect-fungal-interactions: A detailed review on entomopathogenic fungi pathogenicity to combat insect pests

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Highlights



Original article

In-vitro and In-vivo management of *Meloidogyne incognita* (Kofoid and White) Chitwood and *Rhizoctonia bataticola* (Taub.) Butler in cotton using organic's

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ABSTRACT

Root-knot nematodes *Meloidogyne incognita* (Kofoid and White) Chitwood and *Rhizoctonia bataticola* (Taub.) Butler, fungus, are very dangerous root damaging pathogens. Present study was planned to establish a chemical control of these root deteriorating pathogens under lab conditions as well as in field. Maximum death rate of nematode juveniles and minimum numbers of nematode eggs hatched were recorded in plates treated with Cadusafos (Rugby® 100G) @12 g/100 ml and Cartap® (4% G) @9g/100 ml. Chemical treatment of *Rhizoctonia bataticola* with Trifloxystrobin + Tebuconazole (Nativo®) @0.2 g/100 ml and Mancozeb + Matalaxyl (Asiom) @0.25 g/100 ml significantly controlled the mycelial growth in plates. The best treatments tested in laboratory were applied in field as protective and curative treatments. Results proved that chemical control of root-knot nematode and root rot fungi by tested chemicals at recommended time and dose is a significant management technique under field conditions. © 2020 The Author(s). Published by Elsevier B.V. on behalf of King Saud University. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

1. Introduction

Sedentary endoparasitic plant parasitic nematodes (PPNs) of genus *Meloidogyne* are highly polyphagous and root deteriorating

agents of various crops worldwide (Jones et al., 2013; Mantelin et al., 2017). Among PPNs, *M. incognita* is economically important with high rate of reproduction and broad host range (Abad & Williamson 2010). Second stage juvenile (J₂) attacks vascular bundles in plants and produce multinucleated giant cells for uninterrupted supply of nutrients (Vovlas, 2005; Jones, 2011). Interaction of PPNs with fungi has been numerous reported by several scientists in different crops (Bond et al., 2004; Back et al., 2006; Khan & Haque, 2013). The most damaging and catastrophic plant parasitic nematodes belong to genus *Meloidogyne* especially *M. incognita* having more than 90 species (Taylor & Sasser, 1978; Hunt and Manzanilla-López, 2005). In Pakistan, various researchers have

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Molecular identification of seven new Zygopteran genera from South China through partial cytochrome oxidase subunit I (COI) gene



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Damselflies
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Genetic diversity
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ABSTRACT

The study was carried out in eight provinces of China, with 16 different localities. A total of 150 adult damselflies were collected for the study from the area. Mitochondrial (COI) was the target gene to sequence from all 51 representative samples of the entire collection. From the resulted sequences, ten species belonging to seven genera and four families were identified. The families are Calopterygidae, Coenagrionidae, Chlorocyphidae and Libellulidae. After alignment through BioEdit v6, MEGA7 was used for phylogenetic tree construction as well as the calculation of genetic divergence, whereas genetic diversity was calculated by DnaSP v5. All species from the respective families had clustered together within the groups, but divided into sub-groups. Maximum genetic divergence (9.31%) was found in the genus *Rhincocypha*, followed by *Heterotarsus* with (8.23%), while, minimum divergence was observed for the genus *Coenagrion* (0.32%) followed by *Ischnura* (0.48%). However, significant genetic diversity was found for all sequences at 346 mutations confirmed by two tests, Tajima's D and Pi's Fs. Maximum genetic diversity among genera was also observed, the genus *Heterotarsus* has maximum genetic diversity (1.81 mutations) followed by *Rhincocypha* (68 mutations) while minimum genetic diversity was observed for the genus *Coenagrion* followed by *Ischnura*. The presented results showed a higher diversity of damselflies in the south China regions.

1. Introduction

From the Permian period, Odonates are the most conspicuous, ancient and winged insects on the planet (Grimaldi and Engel, 2005). Existing Odonata considered as monophyletic (Davis et al., 2011), including suborders Zygoptera (damselflies) and Anisoptera (dragonflies) having more than 6000 species in each subgroup, and the third suborder is Anisozygoptera (damselfly dragons) with merely four species (Kalkman et al., 2008; Dijkstra et al., 2013). Though, wing venation

was considered as a classification tool of Odonata, which leads towards a complicated relationship in suborder Anisoptera (Dijkstra and Vick, 2006; Ware et al., 2007; Fleck et al., 2008; Pilgrim and Von Dohlen, 2008; Blanke et al., 2013) as well as Zygoptera. Thus, some systematic challenges remain in the groups specifically with the maximum basic venation (O'Grady and May, 2003; Carle et al., 2008; Pessacq, 2009).

The first organism that acquired the functional wings was the invertebrate, approximately 400 million years ago (mya) in the Devonian period (Engel and Grimaldi, 2004). To colonize in both terrestrial and

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Review

Perspectives on Bioenergy Feedstock Development in Pakistan: Challenges and Opportunities

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Abstract: Pakistan faces challenges in both food and energy security. Indeed, extensive literature suggests that food and energy security are interdependent. While acknowledging that food security is still a primary concern for Pakistan, energy security is also a major issue. It is crucial to develop sustainable energy sources for energy production. Among sustainable sources, biomass is a promising source that can be effectively used for environmentally friendly energy production. This article addresses the energy issues and potential solutions using crop residues, non-edible energy crops, and animal and municipal solid wastes in Pakistan. The current research challenges, relevant industries, opportunities, and the future share of energy production derived from renewable and sustainable sources are emphasized with a focus on the potential of biomass energy. This article shows that Pakistan has considerable potential to develop bioenergy crops on marginal lands without compromising food security, with considerable greenhouse gas (GHG) benefits. Pakistan has vast biomass resources, including crop residues, animal waste, municipal solid waste, and forest residues, which collectively produce 230 billion tons of biomass annually. There are about 72 million bovines (cows and buffaloes), 81 million tons per year of crop biomass, and about 785 million birds in poultry farms across the country. Land that is currently non-productive could be used for energy crops, and this has the potential to produce 2500–3000 MW of energy. The utilization of waste cooking oil and fats is the most economically feasible option for obtaining biodiesel due to its easy and almost free availability in Pakistan. Systematic management is needed to collect this huge quantity of waste cooking oil and efficiently convert it to biodiesel. Similarly, molasses may be a promising source for bioethanol production. Furthermore, this study suggests that Pakistan's energy policies need to be amended to ensure that the energy supply meets the demand. In the future, massive energy projects on biomass-based bioenergy need to be implemented in Pakistan. To achieve its bioenergy potential, Pakistan needs to develop incentive-based bioenergy technologies. Moreover, this objective can only be achieved in the country by initiating R&D projects to promote advanced biomass conversion technologies, such as biogas plants and combustion systems.



Phyto-derivatives: an efficient eco-friendly way to manage *Trogoderma granarium* (Everts) (Coleoptera: Dermestidae)

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Abstract

Worldwide, stored products are attacked by a large number of pests resulting in significant economic losses. Among these stored grain pests, khapra beetle, *Trogoderma granarium* (Everts) (Coleoptera: Dermestidae) is one of the top ranked pests that has ability to survive under diverse climate conditions. The management of this pest is mainly done by using synthetic chemicals which have side effects on consumers, ecosystem and non-target organisms. However, phyto-derivatives were found to be the effective environment friendly alternatives against *T. granarium*. Therefore, in this review, success of phyto-derivatives against khapra beetle from conventional means to modern research has been critically analyzed, summarized and discussed. In context, the different life stages of the insect starting from egg laying to adult development have been briefly explained. The review focuses upon recent research conducted on the evaluation of dozens of phyto-derivatives. In addition, the article has also highlighted some limitations of plant derived compounds and concludes via hoping that the future formulated pesticides will be safer, economical, least toxic to human and our planet ecosystem.

Keywords Botanicals · Phyto-chemicals · Food security · Extracts · Stored products

Introduction

Agricultural and animal stored products are attacked by more than 20,000 field insects including six hundred species of beetles, more than 70 moth species and around 355 species of mites, resulting in quantitative and qualitative losses

worldwide (Nagpal and Kumar 2012; Rajendran 2005). These losses are more associated with developing countries because of poor sanitary conditions during commodity procurement, processing, non-hygienic transportation, conventional storage techniques and technically and poorly maintained storage units (Dubey et al. 2008; Hubert et al. 2004).

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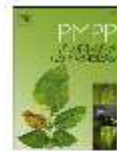
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journal homepage: www.elsevier.com/locate/pmppRegulation of *Capsicum* immunity against microbial pathogens: Transcription factors in focusAli Noman^{a,*}, Sameer Hasan Qari^b, Muhammad Aqeel^{c,**}, Noreen Khalid^d, Saad Alamri^{e,f}, Nahla Alsayed Bouqellah^g, Mohamed Hashem^{a,k}, Mona H. Soliman^h, Habib Aliⁱ, Ijaz Haider^j^a Department of Botany, Government College University, Faisalabad, Pakistan^b Biology Department, Al-Jamran University College, Umm Al-Qura University, Makkah, Saudi Arabia^c State Key Laboratory of Grassland Agroecosystems, School of Life Sciences, Lanzhou University, Lanzhou, 730000, Gansu, PR China^d Department of Botany, Govt. College Women University Sialkot, Pakistan^e King Khalid University, College of Science, Department of Biology, Abha, 61413, Saudi Arabia^f Prince Sultan Bin Abdulaziz Center for Environmental and Tourism Research and Studies, King Khalid University, Abha, Saudi Arabia^g Taibah University, Science College, Biology Department, 42317-8599, Al Madinah Al Munawwarah, Saudi Arabia^h Botany and Microbiology Department, Faculty of Science, Cairo University, Giza, 12613, Egyptⁱ Department of Botany, University of Agriculture, Faisalabad, Depolier Campus, Okara, Pakistan^j Entomological Research Institute, Ayub Agriculture Research Institute, Faisalabad, Pakistan^k Assiut University, Faculty of Science, Botany and Microbiology Department, Assiut, 71516, Egypt

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ABSTRACT

Induction of stress-related genes is primarily reckoned as a major step in plant responses to any stress. The plant genomes possess multiple types of transcription factors (TFs) belonging to diverse families and many of them are unique to plants. The TFs are regulators of transcriptional reprogramming linked with stress responses in plants. TF gene(s) frequently reverts to numerous stresses and then their respective polypeptides may contribute in the positive or negative regulation of apparently dissimilar processes. Many plant TFs are induced by biotrophic as well as necrotrophic pathogens. Functional characterization of TFs provides solid evidence of their role in the regulation of plant defense responses. This advocates the fact that TF does not work alone, but the immune response to pathogens mediated by it, is strongly linked to enhancing the effect of TF on the transcription of defense associated genes. The plant-plant difference in transcriptional responses of TF and Defense associated genes highlights that a TF may regulate defense positively or negatively. This diversity in responses is proof of functional diversity among TFs. Their functions in plant immunity and abiotic stress tolerance in plants have been characterized but still need investigations in *Capsicum annuum*. This article focuses upon recent progress in our understanding of the role of the TFs involved in *Capsicum* defense against Microbial pathogenesis especially against bacterial pathogens. We have dissected collateral interactions between TFs, proteins and nuclear receptors working for *Capsicum* defense. Besides, we have also reviewed and discussed the topical concepts of transcriptional intervention for controlling the activity of TFs.

1. Introduction

Plant stress-tolerance to abiotic and biotic stresses is controlled by multifarious signaling cascades [1]. Significant overlaps among gene expression patterns during different stress conditions reveal the induction of genes individually as well as collectively. This coordinated induction of genes and regulation of plant responses unveil complex

regulatory mechanisms, necessary for survival in changing environment [2,3]. Induction of stress-related genes primarily takes place at the transcriptional level and is reckoned as a major step in plant responses to any stress [4]. Therefore, plant genomes are blessed with multiple types of transcription factors (TFs), belonging to diverse gene families and many of them are unique to plants [5]. The TFs regulate transcriptional reprogramming, linked with stress responses in plants [6]. Any

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Resistance Assessment of Different Cultivars of Okra (*Abelmoschus esculentus*) Against Whitefly (*Bemisia tabaci*)

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Abstract

Whitefly, *Bemisia tabaci* (Hemiptera: Aleyrodidae), is a substantial pest of Okra crop, *Abelmoschus esculentus*, that caused substantial losses through sucking cell sap. On the basis of susceptibility, a field experiment was carried out to assess the different genotypes of *A. esculentus* during growing season (July–August) against *B. tabaci*. Plant genotype resistance and vigor were also evaluated in this study. Meanwhile, morphological parameters were also recorded as well as whitefly-infested and healthy leaves were collected for biochemical analysis. The obtained results revealed that the Okra varieties *A. esculentus* OK-1304 and Pen Beauty were less infested (mean populations 4.10, 4.97 adults/leaf, respectively) while OK-1307 was highly susceptible to *B. tabaci* (10.22 adults/leaf). Meanwhile, comparative analysis revealed that *B. tabaci* population was negatively correlated to relative humidity and positively correlated with low and high temperature. The low concentrations (0.5%) of salicylic acid and citric acid effectively reduced the infestation to 4.63 adults/leaf and 4.87 adult/leaf, respectively, as compared to control (10.17 adult/leaf). The biochemical analysis represented that the catalase concentration decreased in infested leaves and the concentrations of peroxidase, phenolics and superoxide dismutase that act as resistant compound against insect feeding increased.

Keywords *Bemisia tabaci* · Biochemical analysis · *Abelmoschus esculentus* · Resistance · Genotypes · Environmental factors

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NOTICE

RESPONSE OF LEADING BER (*ZIZYPHUS JUJUBA*) VARIETIES AGAINST FRUIT FLIES (TEPHRITIDAE: DIPTERA) AND ESTIMATION OF THEIR LOSSES

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ABSTRACT

A study was carried out during 2014-15 and 2015-2016 at Government Agriculture Farm, old Shujabad Road, Multan, Punjab-Pakistan to assess the preference of five ber varieties for fruit flies. Fruit flies, *Carpomaya vesuviana* and *Bactrocera* spp. (Diptera: Tephritidae) are considered as the serious pest of ber that breeds on fruit and create interruption in fruit quality. So the choice of fruit flies to ber varieties from fruit setting to maturity is crucial. Four grafted and one 'Tukhmi' ber (*Zizyphus jujuba*) viz., 'Kheeri', 'Umri', 'Ajooba', 'Dokil' and 'Tukhmi' were evaluated for their suitability for fruit flies. The results indicate that maximum damaged fruits were recorded on 'Umri' and 'Dokil' i.e. 18.8 and 14.6 percent as compared with other varieties and lower on 'Ajooba' and 'Kheeri' with 10.40 and 9.7 percent damaged fruits. The minimum damaged fruits were recorded on 'Tukhmi' varieties of ber i.e. 6.8 percent. Further, it was also recorded that in the month of November, December and January only *C. vesuviana* attacked the ber fruits but in the month of February along with *C. vesuviana*, *Bactrocera sonata* and *Bactrocera dorsalis* attacked the ber fruits. The ranking positions towards their suitability as host plant for *Bactrocera* spp was observed: 'Umri' > 'Ajooba' > 'Dokil' > 'Kheeri' > and 'Tukhmi'. So it is concluded that the special attention should be given on 'Umri' a high value variety, for the management of fruit flies when devising an IPM program for the control of fruit flies as well as a guideline for the horticultural scientists/ breeders when developing new ber varieties.

KEYWORDS:

Ber varieties; Feeding preference; Population density; Host plant resistance; Fruit flies; Sex ratio; Punjab-Pakistan

INTRODUCTION

The fruits of *Zizyphus jujuba* (Rhamnaceae), known as jujube or red date or Chinese date, are being consumed globally due to their healthiness benefits, both as food and herbal medicine. Usually, one of the main functions of jujube is in herbal medicine, which benefits our brain by calming down the mind and improving superiority of sleep [1,2]. Jujube have neuro-protective actions which includes protection of neuronal cells against neuro-toxin stress, stimulating neuronal differentiation, increasing expression of neurotrophic factors, and promoting memory and learning. These findings imply that jujube is a potential candidate for development of health supplements for prevention and/or treatment of neurological diseases [3,4, 5]. It is widely consumed in Asian countries owing to the remarkable health activities of its fruits. It is considered as the King of arid zone fruits, due to its adaptations to tolerate the biotic and abiotic stresses prevailing under rain fed conditions [6,7]. But in Pakistan the quantity and quality of the fruit is low as compared with other ber producing countries of the world [8]. There are so many reasons of low production like pruning, fertilization, intercropping and insect pest. The occurrence of insect pest is the key risk to lessen the quantity and quality of fruits, finally causing marvelous economic loss to the

The genus *Cryptogonus* Mulsant (Coleoptera: Coccinellidae) from Pakistan, with description of a new species and a new record

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Abstract

The new species of a ladybird beetle, *Cryptogonus bhaiwalensis* Azad & Wang sp. nov. is described from Pakistan. *Cryptogonus nepalensis* bielowski is recorded for the first time from Pakistan. Both species of *Cryptogonus* from Pakistan are diagnosed and illustrated; a distribution map is also presented.

Keywords: Coccinelloidea, Aspidimerini, ladybird beetle, new species, new record, taxonomy

Introduction

Mulsant (1850) erected the genus *Cryptogonus* with *C. orbiculus* (Gyllenhal, 1808) as the type species. This genus can be distinguished from related genera of the tribe Aspidimerini by the following combination of characters: prosternum with two pairs of carinae; antennal club clavate, as long as width of scape; trochanter and femur form a continuous rounded line on inner margin Huo *et al.* (2015a). Owing to their predatory habit, members of this tribe are used as biological control agents under various integrated pest management strategies against insect pests like mealybugs, aphids, whiteflies and scale insects (Kapur, 1948; Huo *et al.* 2015b). Prior to the present study, 49 species have been described in the genus *Cryptogonus* mainly from South and Southeast Asia. In the present paper, one new species along with a new country record have been described and diagnoses of the genus with distribution patterns are provided.

Materials and methods

Specimens examined in this study were collected from various localities in Pakistan. Type specimens designated in the present paper are deposited at the Department of Entomology, South China Agricultural University, China. Zeiss Stereo Discovery V20 microscope was used for the morphological photographs. Genitalia photographs were generated using Olympus BX51 microscope. The procedure for dissection of male and female genitalia as in Azad *et al.* (2017). Morphological terminology follows Ślipiński (2007) and Ślipiński & Tomaszewska (2010). The following measurements were made with an ocular micrometer:

CRISPR/Cas9 regulations in plant science

3

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3.1 Introduction

The global population is predicted to reach 10 billion by 2050, whereas the available cultivable land and water resources are declining, requiring approximately 20%–70% of increase in food demand from present production level (Hunter et al., 2017; Fiaz et al., 2019). Therefore, to ensure food security to such a huge population, especially under drastic climate change is a big challenge. To tackle issue, it is much important to make improvement in classical food production system through sustainable agriculture development (Fiaz et al., 2020). In past the plant breeding was mainly based on selection as there was seldom knowledge of genome, mutations, and genomic manipulations but selection based on unique traits was vital for crop production. All the modern cultivable crops are the product of repetitive cycles of selection based on conducive traits from wild relatives.

To resolve limitation of classical breeding, new plant breeding techniques (NPBTs) have been developed by researchers which are faster, predictable, and can be utilized to wide range of plant species (Ahmad et al., 2020). Genome editing through endonucleases are the most widely adapted technique in plant sciences. These programmable endonucleases holds ability to cause double-strand breaks in targeted gene (Ahmad et al., 2020) repaired through nonhomologous end joining or homology-directed repair. The targeted mutagenesis may cause Insertion, deletions, substitutions, and DNA recombinants in targeted gene of interest (Puchta, 2005; Symington and Gautier, 2011). The revolutionary role of

Genome Engineering for Crop Improvement

Chapter 21

Genome Engineering for Food Security

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Mahmoud Mohamed Gaballah, Galal Bakr Anis

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Summary

Global food security is increasingly challenging in the light of population increase, the impact of climate change on crop production, and limited land available for agricultural expansion. Plant breeding and other agricultural technologies have contributed considerably to hunger reduction over the last few decades. The classical breeding approaches lack the ability to meet global food requirements. The complexities of different cultural or geographic systems, combined with changing needs, technologies and environments have made the application of broad, universal solutions for sustained food security difficult. Therefore, genetic-engineering approaches are powerful tools that we have at our disposal to overcome substantial obstacles in the way of efficiency and productivity of current agricultural practices. The genome edited crops have shown economic benefits in terms of yield, nutritional quality and resistance for biotic and abiotic stresses. The genome-editing technology involves targeted changes in the genetic makeup in a very small and well-defined manner. The knowledge of genomics has further improved our understanding for modern and genome-editing techniques available to plant breeders. In this chapter, a detailed highlight is given for genetic-engineering approaches widely utilized in the crop production system. Moreover, the discussion has taken place on the importance of genomics for the crop improvement programs. The knowledge of both classical and modern breeding efforts can help to understand the underlying mechanism important to ensure food security. The novel plant-breeding techniques (NPBTs) can help to accelerate breeding programs with high efficiency and reliability.

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