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NOVEL SCENERIO FOR MANAGING APHIDS IN *BRASSICA* CROPS

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Introduction

Insect pests are major destabilizers with respect to growth and crop yield of oilseed *Brassicas.* Among them.The aphid causes severe yield losses throughout rapeseed mustard growing areas in India. Aphids suck cell sap from all plant parts and devitalize the plants. The mean yield loss from aphid infestation varies from 35.4% to 73.3% depending on the agro-climatic conditions and averages 56.2% across all of India . In mustard, the oil yield loss is estimated to be 32% . It has not been possible to transfer resistance against insect pests from wild *Brassica species,* which are the only known source of resistance, to cultivated Brassicas by means of conventional breeding techniques. The development of transgenic crops expressing foreign genes that confer protection against insect pests is, therefore, very important. Transgenic canola expressing *Bacillus thuringiensis* endotoxins to give protection against the diamond back moth (*Plutella xylostella*) and corn earworm (*Helicoverpa zea*) have been developed. However, no transgenics for protection against sap-sucking pests such as the mustard aphid have been developed. We report here on the effectiveness of WGA against the mustard aphid, both when incorporated in a synthetic diet or expressed in transgenic mustard plants under the control of the CaMV 35S promoter. Wheat germ agglutinin (WGA), the chitinbinding lectin from wheat germ, has been shown to be antimetabolic, antifeedant and insecticidal to the mustard aphid (*Lipaphis erysimi*. Kalt). A cDNA encoding WGAwas transferred to Indian mustard (*Brassica juncea* cv.RLM-198) through *Agrobacterium*-mediated transformation.Southern analysis of the transgenics showed the integration of the transgene, while Northern and Western analyses demonstrated that the transgene was expressed in the transgenics. Bioassays using leaf discs showed that feeding on transgenics induced high mortality and significantly reduced fecundity of aphids

Novel Technique

1.**Genetic Technique:** Genetic resistance against any insect pest can be achieved through conventional breeding ap-proaches by transferring resistance genes from sexually compatible germplasms. In developing aphid resistance, despite of substantial breeding efforts, resistant genotypes could not be bred mainly because of lack of resistance genes within the crossable gene pool (Yadav *et al.*,1999). To overcome the bottleneck of unavailable resistance source transgenic technology offers new avenues to explore resistance genes even from distant organisms.

Transgenic strategies expressing insecticidal *Bacillus thuringiensis* toxin, have been found to be effective against many insect pests belonging to the order Lepi-doptera and Coleoptera. But for sap sucking hemipteran aphids Bt toxin is ineffective. Engineering of other insecticidal proteins such as protease inhibitors, lectins, amylase inhibitors in crop cultivars also did not yield much resistance and as a result such researches remained confined to laboratory studies only. Therefore, it is imperative to look for new strategies by making use of new biological phenomenon to develop resistance against aphids. Transgenic cultivars were released in the USA that expressed the Leptinotarsa decemlineata (Colorado beetle) specific toxin *Bacillus thuringiensis* var. tenebrionis (Bt) combined with PLRV replicase (Thomas *et al.*, 2000) and other cultivars expressed Bt and PVY coat protein. This technology was far more effective than any presently used tactic, but these cultivars have been withdrawn because of concerns over a public backlash against genetically modified food.

Resistant and tolerant varieties can provide excellent control of aphid-vectored viruses (Clough and Hamm ,1995, Tricoli *et al.*, 1995). Commercially available cucumber, zucchini, and yellow summer squash varieties. That have resistance or tolerance to one or more viruses, including genetically modified varieties that contain the coat protein genes of one or more viruses (Clough and Hamm ,1995, Walters and Surin 2004). A new cantaloupe variety, ‘Hannah’s Choice’, developed in the USA by M. Jahn, a plant breeder at Cornell University, has resistance to WMV, PRSV-W, and ZYMV . Recently, Harris Moran released the first pumpkin (Cucurbita pepo) variety, ‘Magician F1’, with tolerance to ZYMV. In Australia, a ‘Jarrahdale’ type pumpkin (C. maxima) has been released that is highly resistant to ZYMV, PRSV-W, and WMV (Zitter *et al.*,2002) Now, there are no commercially available virus-resistant or virus-tolerant varieties of watermelon in the USA. Resistance to *A. gossypii* and its transmission of viruses has been identified in muskmelon germplasm from India (Fuchs and Gonsalves, 1995) and (Gordon,2004). However, examples of the practical use of this resistance are lacking. In Bangladesh, local genotypes of ash gourd (Benincasa hispida), also known as wax gourd or winter melon, are relatively resistant to *A. gossypii* (Herrington, 2004). The density of trichomes on leaves was negatively correlated with the number of aphidsper leaf.

Many wild potato species are highly resistant to aphids (Herrington, 2004) . Yet only limited use has been made of wild potato species in developing insect-resistant cultivars (Herrington, 2004). Various Agrobacterium-mediated transformations have produced potato lines expressing genes that confer pathogen-derived resistance to viruses. Transgenic lines have been developed that are highly resistant, but not immune, to infection by PLRV, PVY, and PVX Kishoba *et al.,* 1971). While aphids can still acquire virus from low titre plants, efficiency of transmission is greatly reduced . Transgenic cultivars were released in the USA that expressed the Leptinotarsa decemlineata (Colorado beetle) specific toxin *Bacillus thuringiensis* var. tenebrionis (Bt) combined with PLRV replicase (Thomas *et al.*, 2000) , other cultivars expressed Bt and PVY coat protein. This technology of Agrobacterium-mediated transformation was far more effective than any presently used tactic, but these cultivars have been withdrawn because of concerns over a public backlash against genetically modified food crop.Another most important genetic approach is to knock-down the genes responsible for aphid infestation is RNAi method. RNAi is known to be an effective way of gene silencing (Fire *et al*., 1998) in various organisms including plants (Preuss and Pikaard, 2003) and insects (Possamai, 2007). The probability of using RNAi to kill the target insects by down regulating essential gene functions has been appreciated for several years (Price and

Gatehouse, 2008). One of need to explore RNAi technology for growing aphid resistance crop plants is to identify aphid genes which are significantly important for survival and colonization of the insect nymphs on host plants. cDNA sequences of genes or identified ESTs in mustard aphids are still limited in available databases. Additionally, the recognition of genes involved in early stage of infestation and colonization process by aphid insects will give the potential target for RNAi mediated down regulation and resistance. Targeted inactivation of indispensable aphid genes will lead to either retarded breeding cycle or induce lethality to aphids, which could be utilised as a strategy to breed aphid resistant crop cultivars. There are limited reports where RNAi has been strived to develop insect resistance.

2.Biochemical Technique: Plants respond through various morphological, biochemicals, and molecular mechanisms to counter the effects of aphid attack. The biochemical mechanisms of defense against the aphids are wide-ranging, highly dynamic, and are mediated both by direct and indirect defenses. The defensive compounds are either produced constitutively or in response to plant damage, and affect feeding, growth, and survival of aphids. In addition, plants also release volatile organic compounds that attract the natural enemies of the aphids. These strategies may act independently or in conjunction with each other. Although, the understanding of these defensive mechanisms is still restricted. The level of redox enzymes CAT, APX, and SOD, involved in ROS homeostasis in defense signaling, and several defense enzymes viz. POD, PPO, and PAL, remained high in infested plants (Koramutla, 2014). Superoxide dismutase (SOD) protects the cell from oxidation due to reactive oxygen species (ROS) which interferes with the cellular metabolism (McKerise and Lesham, 1994, Tansley,1993) .

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