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Host Plant Resistance (HPR) to Insect Pests

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ABSTRACT: Conventional breeding in conjunction with molecular techniques and transgenic approaches have a great promise to reduce pest associated crop losses, and accelerate the progress in developing cultivars with resistance to insects. Although, considerable progress has been made over the past two decades in manipulating genes from diverse sources to develop plants with resistance to insect pests, rapid and cost effective development and adoption of biotechnology derived products will depend on developing a full understanding on the interaction of genes within their genomic environment, and with the environment in which their conferred phenotype interact. A good beginning has been made in developing genetic linkage maps of many crops, but the accuracy and precision of phenotyping for resistance to insect pests remains a critical constraint in many crops.

Key words: resistance to insect, Genetic Resistance, Cytoplasmic Male-Sterility, RNAi

INTRODUCTION

A. Identification sources of resistance to insect

Over the past five decades, a large proportion of the world sorghum germplasm collection has been evaluated for resistance to insect pests, and a number of lines with resistance to major insect pests have been identified (Sharma *et al*. 1992, 2003). However, cultivars with resistance to insect pests are cultivated by farmers only on a limited scale due to an overemphasis by national programs on grain yield as a criterion for release of cultivars. Several new sources of insect resistance have been identified and supplemented to the existing resistance sources against corn earworm, *H. zea*, corn borer, *O. nubilalis*, sugarcane borer, *Diatraea grandiosella* (Dyar), fall armyworm, *Spodoptera frugiperda* (Smith), and spotted stem borer, *Chilo partellus* (Swinhoe) in maize (Kanta *et al.*, 1997); brown plant hopper, *N. lugens*, gall midge *Orseolea oryzae* (Wood-Mason), and stem borers, *Scirpophaga incertulas* (Walker) and *Chilo suppressalis* (Walker) in rice (Smith *et al.*, 1994); Hessian fly, *M. destructor* and greenbug, *S. graminum* in wheat (Smith, 2005); sorghum shoot fly, *Atherigona soccata* (Rondani), spotted stem borer, *C. partellus*, sorghum midge, *S. sorghicola*, and head bug, *Calocoris angustatus* (Lethiery) in sorghum (Sharma *et al.*, 2003, Sharma *et al.*, 2005c); and Oriental armyworm, *Mythimna separata* (Walker) in pearl millet (Sharma and Sullivan, 2000). Sources of resistance have also been identified against cotton bollworm, *Helicoverpa armigera* (Hubner), and leafhopper *Amrasca biguttula biguttula* Ishida in cotton; legume pod borer, *H. armigera* in chickpea and pigeonpea (Sharma *et al.*, 2005a); spotted pod borer, *Maruca vitrata* (Geyer) in pigeonpea and cowpea (Sharma *et al.*, 1999, Sharma and Franzmann, 2000); and pea weevil, *Bruchus pisorum* L. in pea (Clement *et al.*, 1994).

B. Techniques to screen for resistance to insect pests

The ability to develop insect resistant cultivars, use of marker-assisted selection, and development of transgenic plants with insect resistance depends on the precision of resistance screening techniques. Infester row, cage and leaf disc screening techniques have been standardized to evaluate sorghum germplasm, breeding material, and mapping populations for resistance to insect pests under field and greenhouse conditions (Sharma *et al*., 1992, 2003).

C. Genetic Resistance to Pests

Pests are persistent threats to successful cotton growing. Substantial economic losses are incurred. Yield loss estimates due to insect pests and diseases in the Philippines (computed from experimental data between 1989 to 1994) ranged from 41 to 47 percent In the United States, available field data indicated an annual average yield loss of about 7 to 8 percent Earlier, the National Academy of Sciences (1975) put it at 14 to 15 percent. Many genes have been identified in rice that contribute for resistance to brown plant hopper, green leaf hoppers, gall midge, white backed plant hopper, and yellow stem borer (Khush and Brar, 1991).

Resistance to shoot fly is inherited by additive gene action (Dhillon *et al*., 2006e), while additive and non additive gene effects govern resistance to spotted stem borer in sorghum (Sharma *et al*., 2007). Resistance to sorghum midge is inherited as a recessive trait, and is controlled by additive gene effects (Sharma *et al*., 1996).

D. Cytoplasmic Male-Sterility Systems and their Reaction to insects

Several CMS systems have been identified in sorghum for diversifying hybrid production. However, only the A1 CMS system has been deployed for producing sorghum hybrids worldwide, with the exception of A2 CMS based hybrids in China (Shan *et al*., 2000). The use of a single source of male-sterility (A cytoplasm) has narrowed the genetic base of sorghum hybrids. As a result, there is considerable risk of insect pest and disease outbreaks in cultivars based on a single source of male-sterility (Sharma *et al*., 2004).

The expression of non preference and antibiosis components of resistance to *D. grandiosella* and *D. saccharalis* was higher in resistant inbred lines based hybrids than the inbreds (Kumar and Mihm, 1996). Expression of different mechanisms and traits associated with resistance to shoot fly, midge, shoot bug, and sugarcane aphid have been found to be significantly lower in CMS as compared to the maintainer lines of sorghum (Dhillon *et al*., 2006b, c, d). Hybrids based on shoot bug, sugarcane aphid, midge, and shoot fly-resistant CMS and restorer lines suffered less damage than the hybrid based on susceptible CMS and resistant or susceptible restorer lines, suggesting that expression of resistance to these insects is influenced by the genetic background of the CMS lines, and resistance is needed in both the parents to produce insect-resistant hybrids (Sharma *et al*., 1996; Dhillon *et al*., 2006c; Sharma *et al*., 2006).

Development of CMS and restorer lines for resistance to insect pests. The maintainer lines harbor the factors that influence expression of resistance to insects (Sharma *et al*., 2004b). Therefore, there is a need to develop a range of CMS, maintainer, and restorer lines with resistance to insect pests, and diversify the CMS systems in sorghum. The A4 M cytoplasm is slightly less susceptible to shoot fly than the other CMS systems. Recovery from shoot fly damage is better in A4 M, A3, and A2 cytoplasm's than the A1 cytoplasm. Shoot fly survival and development is also poor on A4 M and A4 VzM CMS systems. The A4 M cytoplasm being less susceptible to shoot fly and having better recovery resistance can be exploited for developing shoot fly-resistant hybrids in future. However, as a first step, it may be better to transfer the traits associated with resistance to shoot fly into the hybrid parents in A1 cytoplasm. Another alternative would be to explore opportunities for using male gametocytes and/or temperature and photoperiod induced male-sterility for sorghum hybrid seed multiplication as these might allow exploitation of the favorable effects of normal maintainer line cytoplasm (s) on expression of resistance to insects in this crop. Of course, the simplest alternative would be to focus on open-pollinated varieties that do not require use of male- sterility for seed multiplication. Much of the area under high-yielding sorghum cultivars is sown to hybrids in Asia, Australia, and the Americas. Therefore, it is apparent that for host plant resistance to be an important component of pest management in sorghum, we need to transfer the insect resistance genes into male-sterile, maintainer, and restorer lines that can be used by the public institutions and private seed industry to develop insect-resistant hybrids. Much of this material has been shared with public institutions and private seed industry over the past decade for use in sorghum improvement, and for developing high yielding hybrids with resistance to insects. To develop insect resistant hybrids, the genes conferring resistance to insect pests need to be transferred into both CMS and restorer lines (Sharma *et al*., 2004b).

E. Insect resistance genes from wild relatives of crops

Various herbivorous insect species are major pests in agriculture (Schoonhoven *et al*., 2005) and many of them are mainly controlled through the use of insecticides. Alternative means of insect control are needed because of environmental concerns and negative effects of pesticides on beneficial insects such as pollinators and insectivorous insects (Lewis *et al*., 1997). Host plant resistance is one of the most effective forms of insect control and offers a very good alternative to the use of insecticides. To be able to develop insect resistant varieties, it is essential to identify, characterize and categorize effective sources of resistance. In nature, devastating pests only rarely occur despite the abundant presence of herbivorous insects. This is because of a multitude of defences that plants can use to protect themselves against herbivorous insects (Schoonhoven *et al*., 2005). Within plant species, there is considerable variation in defence mechanisms that has been shaped by differences in selection pressure (Thompson, 2005). However, only very little of this natural variation has been exploited in agriculture. Exploring natural variation among wild relatives of crop plants, or even accessions of crop plants themselves, May yield resistant varieties.

The development of powerful molecular genetic tools allows genome-wide association studies to dissect the molecular variation underlying variation in insect resistance (Chan *et al*., 2010; Kump *et al*., 2011).

Such analyses facilitate the development of molecular markers and enhance marker-assisted breeding in order to introgress resistance traits into economically important cultivated crops (Varshney *et al*., 2005; Bergelson and Roux, 2010). Alternatively, insect-resistance genes may be introduced into crops using transgenics (Gust *et al*., 2010). Moreover, the potential of transgenic approaches, involving Bt-genes or RNAi constructs, has been reviewed by others (Huvenne and Smagghe, 2010; Sanahuja *et al*., 2011).

Variation in plant traits conferring resistance to insects. In general, plant resistance to insects can be based on direct and ⁄ or indirect defence mechanisms, which can be constitutively present or induced upon herbivore attack (Schoo nhoven *et al*., 2005). Direct defence involves physical and ⁄ or chemical plant traits that by themselves interfere with the physiology and ⁄ or behaviour of the herbivore and are the main determinant of plant resistance. For several plant species, natural variation in plant traits related to direct defence has been found and this includes, for example, differences in trichome density (Kaplan *et al*., 2009) or specific secondary metabolites (Wu *et al*., 2008). Indirect defence includes physical and ⁄ or chemical plant traits that attract natural enemies of herbivores and promote their effectiveness in the control of herbivore populations. Herbivore induced plant volatiles (HIPV) are the most important compounds serving as cues in indirect defences (D'Alessandro and Turlings, 2006) and have been shown to vary among populations ⁄ accessions from the same plant species (Schuman *et al*., 2009; Snoeren *et al*., 2010). Although variation in plant traits related to direct and indirect mechanisms against herbivorous insects has been recorded, there is limited knowledge on the molecular background of these differences.

Effect of gene expression on resistance traits. Because the expression of genes and post-transcriptional processing determine which proteins or secondary compounds are produced, ELPs provide valuable initial information towards understanding resistance mechanisms and identifying the genes involved (Kliebenstein *et al*., 2006; Wu *et al*., 2008). Micro- arrays, next-generation RNA sequencing and quantitative PCR technologies are excellent tools that have been used to monitor transcript levels in plants from different accessions to detect ELPs (Morozova and Marra, 2008; Kliebenstein, 2009a). For several plant species, the effect of variation in the transcription of genes and plant traits related to direct defence against insects has been studied (Poelman *et al*., 2009; Schuman *et al*., 2009). In *A. thaliana*, transcriptional variation in genes involved in the biosynthesis of glucosinolates, which are important defence compounds in the Brassicaceae, could be linked to glucosinolate concentrations and to aphid performance (Kusnierczyk *et al*., 2007). Also in *Nicotiana attenuata*, ELPs in herbivore responsive genes have been correlated with the production of defensive compounds and with herbivore performance (Wu *et al*., 2008). However, one has to realize that almost all studies to date are studies correlating gene expression with insect resistance. Studies providing direct proof of the involvement of certain genes, using for example mutants or lines containing RNAi constructs that knockout gene functions, are urgently needed (de Ilarduya *et al*., 2003; Kessler *et al*., 2004). Although given much less attention than transcriptional variation underlying direct defence, ELPs for genes encoding proteins involved in the biosynthesis of plant volatiles that result in variation in the attraction of natural enemies of the attacking herbivore have also been found (Schuman *et al*., 2009; Snoeren *et al*., 2010). For example, herbivore-induced expression of TPS23 (terpene synthase 23) in a maize variety resulted in the production of the volatile compound (E)-b-caryophyllene that caused a stronger attraction of the natural enemies of the herbivore compared with a maize variety that did not induce TPS23 expression (Kollner *et al*., 2008). More recently, variation in the herbivore-induced expression of genes involved in volatile biosynthesis among A. thaliana accessions has been connected to differences in the emission of the corresponding volatile compounds and subsequently to discriminative behaviour of a parasitic wasp (Snoeren *et al*., 2010).

F. RNA interference (RNAi) technology in host plant resistance

Recently, the RNAi technology has been demonstrated to be helpful in understanding the functional genomics of valuable crop traits for resistance against insect pests (Gordon and Waterhouse, 2007). In RNAi technology, the dsRNA of insect's gene is expressed in plants by using transgenic technique, and then the interfering RNAs are formed in the plants. The interfering RNAs then enter into insects' bodies after being ingested by the insect that eats the plant, and conduct RNAi against the target gene, thereby expression of the target gene is suppressed by RNAi. Cytochrome P450 gene (CYP6AE14) the first gossypol-inducible P450 gene from bollworms, is directly involved in the ability of cotton bollworm to tolerate gossypol. When CYP6AE14 expression is suppressed, as achieved by plant mediated RNAi, the larval tolerance to gossypol is greatly reduced (Mao *et al*., 2007). The ability to down-regulate CYP6AE14 and GST1 expression in the midgut by feeding cotton bollworms dsRNA-producing leaves, suggests that plant mediated RNAi may be a general approach for gene- silencing in herbivorous insects.

However, the passage of years without reports of success using this approach seemed to suggest that simply expressing hairpin RNA in plant material to be ingested by an insect would not provide sufficient levels of intact dsRNA to trigger potent RNAi in the pest.

RNAi Mechanism. RNAi is an apparently ancient defense mechanism against invading viruses, prevents deleterious efect of transposon movement, regulation of gene expression and chromatin modification. It is a powerful tool to suppress gene expression and analyze gene function. RNAi operates at transcriptional (called as transcriptional gene silencing-TGS), as well as post-transcriptional (called as post transcriptional gene silencing- PTGS) for gene silencing, which has been previously reported as cosuppression in plants (Napoli *et al*., 1999), and quelling in fungus (Romano and Macino, 1992). In fact, these three techniques appeared to be remarkably well conserved in several eukaryotes, which is initiated by the action of dicer enzyme on dsRNA, leading to the production of small interfering RNAs (siRNAs), and these siRNAs along with the RNA induced silencing complex (RISC) are involved in sequence specifc silencing of the target mRNA (Fire *et al*., 1998; Hannon, 2002).

CONCLUSION

A good beginning has been made in developing genetic linkage maps of many crops, but the accuracy and precision of phenotyping for resistance to insect pests remains a critical constraint in many crops. Improved phenotyping systems will have substantial impact on both conventional and biotechnological approaches to breed for resistance to insect pests, in addition to the more strategic research that feeds into these endeavors. Marker assisted selection has had a dramatic impact, particularly in the private sector, in breeding for disease resistance and quality traits where simply inherited components could be readily identified.

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