Emir. J. Food Agric. 2012. 24 (5): 462-469
http://ejfa.info/

REGULAR ARTICLE

Potential applications of gene silencing or RNA interference (RNAi) to control disease and insect pests of date palm

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Abstract

Gene silencing or RNA interference (RNAi), a recently-discovered regulatory and defense mechanism in plants, animals and other organisms, has great potential to control plant pests. A gene essential for survival or development of the plant pest is targeted, and an inverted repeat construct of the gene is transformed into susceptible host plants. Plant transcription produces a double-stranded RNA (dsRNA), which the plant recognizes as a foreign molecule. Dicer, the plant's protective ribonuclease enzyme, hydrolyzes the dsRNA to small interfering RNAs (siRNAs). The feeding pest ingests the siRNAs, causing the pest's RNAi mechanism to hydrolyze the messenger RNA of its own essential gene. This "silences" that essential gene in the pest, which either dies or is debilitated, and the transgenic plant is resistant to that pest. RNAi, having been shown to provide resistance against insects (Diabrotica, Helicoverpa), bacteria (*Agrobacterium, Staphylococcus*), nematodes (Heterodera, Meloidogyne) and parasitic plants (*Orobanche, Striga, Triphysaria*), should provide effective, durable resistance to red palm weevil (*Rhynchophorus ferrugineus*), Bayoud disease (*Fusarium oxysporum* f. sp. albedinis), Al-Wijam, and other serious pests of date palm.

Key words: Disease, Gene silencing, Insect, RNAi

Introduction

Date palm (*Phoenix dactylifera*) has been a cultivated tree crop for at least 5,000 years (Johnson, 2011). It is a very important plant throughout the world, and is perhaps the most important plant in Saudi Arabia and throughout the Middle East. It has high socioeconomic importance, due not only to its food value, but also its capacity to provide many other products such as shelter, fiber, clothing, aesthetic beauty and furniture (Mousavi et al., 2009). It has high natural tolerance to very adverse growing conditions, including drought, salinity and high temperatures (Bakheet et al., 2008). In 2007 nearly 1.1 million ha of date palm were harvested, yielding 6.91 million tonnes. The major producers were Egypt (19%), Iran (15%) and Saudi Arabia (14%) (http://faostat.fao.org 2007).

Each year plant pests cause serious economic losses throughout the world in palm species, especially in date and coconut palms. In date palm up to 30% of production can be lost to pests and diseases (El-Juhany and Loutfy, 2010), including the red palm weevil, (RPW *Rhynchophorus ferrugineus*), the Bayoud disease (*Fusarium oxysporum* f. sp. *albedinis*) (Quenzar et al., 2001; Zaid et al., 2002) and phytoplasma diseases (Nixon, 1954; Alhudaib et al., 2007; Harrison and Elliott. 2009). Depending on the level of infestation, the RPW can cause losses up to $130 million annually in the Middle East countries alone, and additional millions of dollars of losses on coconut and other palm species (Faleiro, 2006; El-Sabea et al., 2009). Because date palm is a long-lived plant and because it is genetically heterogeneous and difficult to propagate, it is essential to develop durable resistance against these important pests and to incorporate it into horticulturally desirable cultivars.

Recent advances in biotechnology, both in date palm and in new disease resistance strategies, provide encouraging opportunities for developing pest resistant date palms. Tissue culture from somatic embryos of date palm has been advanced significantly, and large scale micro-propagation (Al-Khayri, 2010) is now possible. In addition, transformation of embryonic date palm callus cells has been achieved (Mousavi et al., 2009; Saker et al., 2007) using biolistics (particle bombardment). Successful transformation was demonstrated both by β-glucuronidase (GUS) expression (histochemical staining) and direct detection by polymerase chain reaction (PCR). However,

Received 22 January 2012; Revised 26 February 2012; Accepted 05 March 2012

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transgenic plants have not yet been regenerated. Even greater success has been achieved in the oil palm (*Elaeis guineensis*), where stable transformation has been achieved both by biolistics and by *Agrobacterium tumefaciens* (Ismail et al., 2010), and transgenic plants have been regenerated.

RNAi is a recently discovered mechanism for regulating gene expression. It functions in diverse organisms including plants and fungi (Van West et al., 1999; Hammond and Keller. 2005; McDonald et al., 2005; Eamens et al., 2008), bacteria (Escobar et al., 2001; Hannon, 2003; Katiyar-Agarwal et al., 2006; Yanagihara et al., 2006), insects (Baum et al., 2007; Mao et al., 2007) nematodes, (Fire et al., 1998; Huang et al., 2006; Sindhul et al., 2009), hydra (Matzke, and Matzke, 2004), humans (Hannon, 2003), parasitic plants (Tomilov et al., 2008; Aly et al., 2009), and plant viruses (Ahlquist, 2002; Baulcombe, 2005; Dietzgen and Mitter, 2006). It holds great promise to control human, plant and animal diseases (Matzke and Matzke, 2004). RNAi is considered an ancient defense mechanism whereby the host organism recognizes as foreign a double-stranded RNA (dsRNA) molecule and hydrolyzes it with a ribonuclease named dicer. This hydrolysis produces small and specific RNA fragments of 21–28 nucleotides called small interfering RNAs (siRNAs). The siRNAs then combine with constituitive proteins to form the RNA-induced silencing complex (RISC). The RISC diffuses in the cell, and its resident siRNA hybridizes to the specific messenger RNAs (mRNAs) with sequences complementary to that of the siRNA. The new double-stranded region stimulates the hydrolysis of that mRNA by dicer to produce more siRNAs. This process is repeated each time the siRNA hybridizes to its complementary mRNA, effectively destroying and preventing that mRNA from being translated, thus "silencing" the expression of that specific gene (Eamens et al., 2008).

Stimulated by these discoveries, Venganza, Inc. developed a new technology for plant disease control called host-mediated silencing of pest genes (HMSPG). Venganza has filed a patent on HMSPG (www.venganzainc.com), and has used HMSPG to develop plants resistant to several fungal pathogens in addition to the oomycete *Phytophthora* species shown in the figures below. The molecular approach is to target an essential gene of the pest by producing a DNA construct containing an inverted repeat of that essential gene. The susceptible host plant is transformed with the inverted repeat construct, and transcription in the plant produces a dsRNA with the sequence of the targeted and essential pest gene. The plant recognizes the dsRNA as a foreign molecule, and the plant's protective dicer enzyme hydrolyzes the dsRNA into siRNAs. When the pest attacks the transgenic plant, it ingests those siRNAs, which then cause the RNAi mechanism within the pest to hydrolyze the mRNA of the pests' own essential gene. Silencing the pest gene stops the infection because the pest dies or is no longer pathogenic (depending on the choice of the gene), and the transgenic plant is now resistant to that disease. Venganza first demonstrated HMSPG in tobacco using the cutinase gene from *P. nicotianae*, because cutinase is essential for pathogenicity in Phytophthora (Munoz and Bailey, 1998). Interestingly, this essential gene sequence from *P. nicotianae* also was effective in conferring resistance against several related pathogenic fungi.

Materials and Methods

The materials and methods used in the following research are described in the Niblett patent application (Niblett, 2006), in the individual references cited, and in "Molecular Cloning: A Laboratory Manual" (Sambrook et al., 1989).

Results and Discussion

As shown in Figure 1, plant A, typical of those transformed with the cutinase gene construct (pVZA100), is resistant to *P. nicotianae*, whereas the untransformed or wild type plant B is susceptible. This resistance was effective against both Races 0 and 1 of *P. nicotianae*. Similar results have been obtained with other fungal pathogens on dicot and monocot hosts.

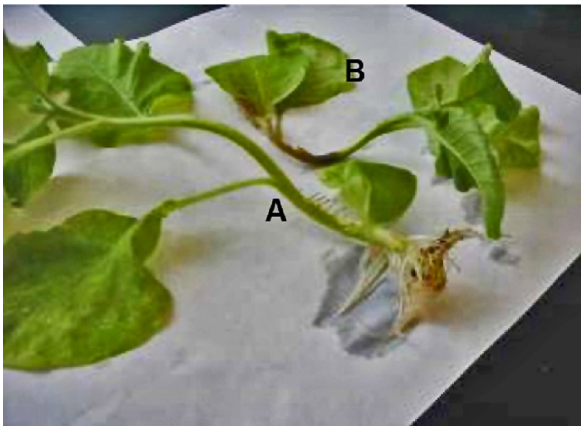

Figure 1. Resistance to P. nicotianae conferred to tobacco by transformation with pVZA100 (plant A), and a susceptible untransformed control plant (B).

Figure 2 demonstrates that the molecular mechanism of the resistance is RNAi. Panel A

shows the effect of pVZA100 transformation on *P. nicotianae*. Lane 1 is a 35 nt marker and Lane 2 shows the intact 620 nt cutinase mRNA from a wild type culture. RNAs from four transformed cultures (A-D; lanes 3-6) contain 21-25 nt siRNAs that hybridized to the cutinase probe, demonstrating that the cutinase mRNA has been hydrolyzed in the transformed cultures. Furthermore, the *P. nicotianae* isolates containing the intact mRNA were pathogenic, whereas those transformed with pVZA100 and containing the siRNAs were nonpathogenic. Cultures of *P. nicotianae* re-isolated from resistant transgenic tobacco plants showed the same siRNA profiles as those in Figure 2 A-D. The nonpathogenic cultures of *P. nicotianae* that had been transformed with pVZA100 or re-isolated from resistant transgenic tobacco were transferred monthly on growth media for three years and never regained pathogenicity, indicating that the RNAi is long-lasting, if not permanent.

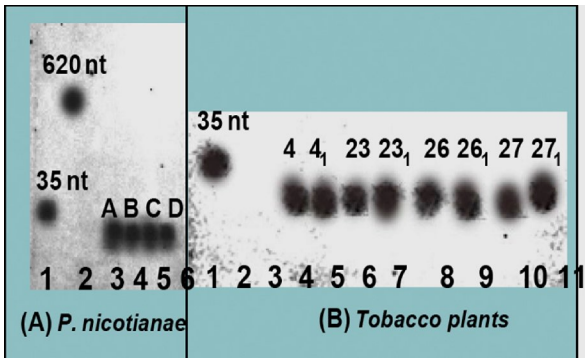

Figure 2. Hybridization of a of cutinase probe to PAGE-separated RNA extracted from transgenic and wild-type cultures of P. nicotianae and tobacco plants.

Panel B of Figure 2 shows direct evidence for RNAi activity in tobacco plants transformed with pVZA100. Lane 1 is the 35 nt size marker. RNAs from wild type and pCAMBIA1201 control transformed plants (lanes 2 and 3) showed no hybridization because cutinase is a fungal gene, not present in plants. However, the four tobacco lines transformed with pVZA100 (lines 4, 23, 26 and 27, and their T1 seed progeny; 4_1 etc.) all contained the cutinase siRNAs, and were resistant to *P. nicotianae*, as in Figure 1. These lines were randomly selected from about 50 individual transformation events.

Figure 3 shows that the tobacco plants transformed with pVZA100 also were resistant to the blue mold disease caused by *Peronospora tabacina*. *Peronospora* and *Phytophthora* are related taxonomically as members of the same Order (Peronosporales), but they are in different families (*Peronospora* = Peronosporaceae and *Phytophthora* = Pythiaceae). Therefore the pVZA100 construct also confers broad resistance to a distantly related fungal pathogen. Figures 4 and 5 provide additional evidence that HMSPG confers a broad type of resistance. In Figure 4 the cutinase gene from *P. nicotianae* (pVZA100) provides resistance against *P. sojae*, in soybean. This resistance was effective against all seven races of *P. sojae* tested.

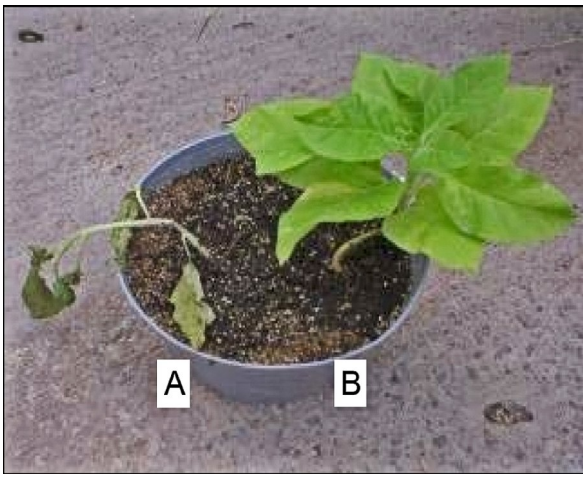

Figure 3. Resistance to tobacco Blue Mold (Peronospora tabacina) conferred by transformation with pVZA100 (plant B). Plant A is an untransformed control plant.

Figure 4. Resistance to P. sojae in soybean plants by transformation with pZA 100.

In Figure 5, pVZA100 (Row 2) confers resistance against *P. infestans* in potato as compared with Row 1, which contains wild type plants and those transformed with pCAMBIA1201 alone). Rows 3 and 4 show resistance in potato plants transformed with pVZA300 and pVZA400,

which contain the elicitin and ribosomal RNA (rRNA) genes, respectively, from *P. infestans.* Note that the level of resistance conferred against *P. infestans* by pVZA100 is not as high as with pVZA300 and pVZA400. This may reflect the lower sequence identity (82%) between the cutinase gene of *P. nicotianae* and that of *P. infestans.* The resistance conferred by pVZA100, 300 and 400 was effective against both mating types A1 and A2 of *P. infestans.* The pVZA300 construct used here contains a gene from both *P. infestans* and a plant insect pest, indicating that a construct containing two genes remains functional and is effective against its target gene in *P. infestans*. We have recently used "gene stacking" on a single construct to confer resistance to three different fungal pathogens.

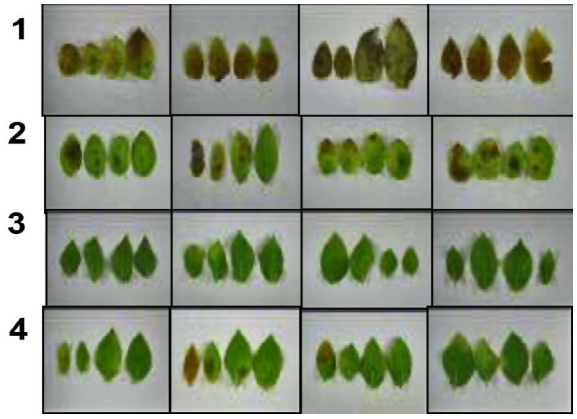

Figure 5. Resistance to late blight (P. infestans mating type A2) conferred to potato by transformation with pVZA100 (Row 2), pVZA300 (Row 3) or pVZA400 (Row 4), compared to wild type plants, and those transformed with pCAMBIA 1201 (Row 1).

HMSPG and similar strategies are widely applicable to a broad spectrum of plant pests. For example, inverted repeats of genes from multiple plant viruses were used to obtain resistance to four different viruses (Dietzgen and Mitter, 2006). HMSPG also is effective against insects, nematodes, parasitic plants, and bacteria, which is the capability we propose to implement here against the pests of palm. Corn plants transformed with a construct containing an inverted repeat from a vacuolar ATPase gene from Western corn rootworm showed significant reduction in damage from this insect (Baum et al., 2007), and cotton plants were protected from the cotton bollworm when transformed with an inverted repeat from a bollworm cytochrome P450 gene (Mao et al., 2007).

Inverted repeats of nematode parasitism genes also have been effective in controlling both root knot (Huang et al., 2006) and cyst nematodes (Sindhul et al., 2009). Working with the parasitic plant *Triphysaria versicolor*, a species of broomrape, (Tomilov et al., 2008) demonstrated that lettuce plants containing an inverted repeat of the GUS gene could silence an active GUS gene in the *T. versicolor* when it fed on the lettuce. Furthermore, feeding that same "silenced" *T. versicolor* on lettuce expressing the GUS gene, silenced the GUS gene in lettuce. This demonstrated that the "silencing principle" (siRNA) moves back and forth between lettuce and *T. versicolor.* Using *Orobanche aegyptiaca,* another species of broomrape, Aly et al. (2009) demonstrated that tomato plants transformed with an inverted repeat of the mannose 6-phosphate reductase gene of *O. aegyptiaca* showed a 58% greater mortality of the broomrape tubercles that developed on the transgenic tomatoes.

With bacteria, siRNAs have been effective *in vivo* and *in vitro* against the coagulase enzyme of the human pathogen *Staphylococcus aureus* (Yanagihara et al., 2006), and the crown gall disease of plants caused by *Agrobacterium tumefaciens* was controlled in tobacco, Arabidopsis and tomato plants transformed with inverted repeats of the *A. tumefaciens* genes *iaaM* and *ipt*, which encode precursors for auxin and cytokinin biosynthesis (Escobar et al., 2001).

Because HMSPG is effective against bacteria there is reason to be optimistic that it will be effective against phytoplasmas. Because phytoplasmas cannot be cultured we performed a preliminary experiment to test the efficacy of HMSPG against *Xanthomonas campestris* pv. *campestris* (Xcc), a serious bacterial pathogen of cabbage and other vegetables. We used the *in vitro* incubation assay that we developed for fungi (Bailey and Niblett, 2010) to identify candidate genes for HMSPG. With fungi, spores or mycelium are incubated in the dsRNAs and siRNAs, and viability is measured by colony formation or infectivity. Here we prepared dsRNAs from the Xcc 23S rRNA and enolase as candidate genes. The bacteria were incubated directly in the dsRNAs or in siRNAs prepared from the dsRNAs by digestion with ribonuclease III and then plated on YDC medium to measure viability by colony formation. The control dsRNA and siRNAs were prepared from the β-glucuronidase (GUS) gene of *Escherichia coli.* Our data (not shown) indicates

that Xcc dsRNAs and siRNAs reduced colony formation by 24 to 55%, as compared to about 10% reduction in the controls treated with GUS ds- or siRNAs. Similar reductions in viability were obtained with candidate fungal genes that subsequently provided strong resistance in transgenic plants. Therefore, as demonstrated with fungi, both the 23S rRNA and enolase genes have high potential for conferring resistance to Xcc *in planta*, and likely to other bacterial and phytoplasma pathogens.

Advantages of RNAi and HMSPG

A major concern voiced against transgenic plants is the possible expression of a protein that might cause an allergic response in consumers. Therefore, a major asset of our RNAi strategy is that no protein is expressed. We further ensure this in our construct design by avoiding both 5' terminal and internal ATG initiation codons and by inserting one or more stop codons in all six possible reading frames. Potential off-target effects on host plant genes or other species are minimized by designing constructs to produce siRNAs with a maximum of 15 contiguous base pairs of identity to known coding sequences. This is a conservative strategy, given that an upper limit of 18 contiguous base pairs is generally considered adequate to avoid off-target effects (Xu et al., 2006).

Using conventional breeding techniques it may be difficult or impossible to achieve disease resistance in an important crop species when genes for resistance to a particular pest do not exist or that crop is difficult or very time-consuming to breed for resistance because of sterility, ploidy differences or incompatibilities (e.g. date palm, bananas, potatoes, etc.). Also, when plant resistance genes are identified and transferred into desirable varieties, that resistance may not be durable because of the presence of diverse genotypes of the pest, or because the pest may mutate and rapidly "defeat" that resistance gene.

HMSPG has now been demonstrated to be effective against all fungi for which it has been tested. Evidence for Oomycetes is presented above. From ongoing projects we have evidence in transgenic monocots and dicots for resistance to a Basidiomycete and three species of Ascomycetes, while others have shown RNAi activity in the ascomycetes *Fusarium graminearum* (*Gibberella zeae*) and *Aspergillus flavus* (McDonald et al. (2005), Nowara et al. (2010) and Yin et al. (2011) have recently shown it to be effective against the obligate parasites *Blumeria* and *Puccinia*, respectively. As noted above, HMSPG also is effective against insect, bacterial and nematode pests.

Durability of RNAi-Derived Resistance or HMSPG

Venganza and others have demonstrated that sequence identity to the target gene must be about 70-80% to provide high level resistance (Fig. 5). Therefore, it would require mutations altering 20-30% of an essential gene sequence for a pest to overcome or develop resistance to HMSPG. Such mutation in an essential gene would likely be lethal to the pest. Furthermore, even if a pest does mutate sufficiently to overcome the action of a single siRNA, the many other siRNAs also produced from that same essential gene construct (Ho et al., 2006) will be present to hydrolyze the mRNA transcript of that essential gene at many additional sites, and only a single hydrolysis is necessary to cause the desired silencing of that essential gene. Hence, this form of resistance is recalcitrant to mutation and should provide durable resistance to all mating types, races, strains, biovars and pathovars of the important pests of date palm that we have described. Our recent discovery of near identity among the sequences of essential genes in fungal pathogens from the US and Africa strongly supports this concept.

Because of their interest in HMSPG, the National Agricultural Research Organization of Uganda negotiated a contract with Venganza, Inc. in 2009 to identify candidate genes potentially effective for the control of *Fusarium oxysporum cubense* (FOC) and *Mycosphaerella fijiensis* (MF), which cause Fusarium wilt and black sigatoka, respectively, in bananas. Using our *in vitro* assay we identified candidate genes for conferring resistance to these pathogens. Genes known to be essential for fungal survival were selected. Because the FOC genome has not been sequenced, the sequenced genome of the closely related *Fusarium graminearum* (*Gibberella zeae* = GZ) was used to identify sequences for PCR oligonucleotide primers to amplify gene segments from genomic DNA of FOC and MF, which like GZ are both ascomysetes. The well-annotated genomic sequence of *Neurospora crassa*, another ascomycete, and accessible through the National Center for Biotechnology Information (NCBI), also was used for comparison, as were the expressed sequence tags (ESTs) for FOC and MF available through NCBI. PCR primers were designed for 14 FOC and 12 MF candidate genes, and the amplicons obtained in all 26 cases from genomic DNA of both Ugandan and Florida isolates of FOC and MF. The

dsRNAs transcribed from the gene segments were effective against both Ugandan and Florida isolates of FOC and MF. Sequencing of the cloned amplicons from both Ugandan and Florida isolates of FOC and MF revealed that the nucleotide sequences of the same essential genes were essentially identical, demonstrating the highest possible level of conservation among these essential genes, and therefore the broad applicability of HMSPG to disease control on both continents, and probably worldwide.

Conclusions

HMSPG now provides to agriculture and plant breeders an entirely new and unique source of genes for pest resistance - the essential genes of the pests themselves. Using HMSPG to develop resistant plant varieties is potentially much more rapid than conventional breeding. HMSPG inserts specific genes for resistance into proven and accepted plant varieties with no phenotypic changes. Several genes may be stacked on a single or on multiple constructs, thereby conferring durable resistance to several pests with a single transformation event. This benefits plant breeders because transgenes are inherited as single dominant genes. Where sequences of essential genes are not available for a particular pest, we have demonstrated that sequences of closely related pests can be used to prepare PCR primers and those amplicons sequenced to confirm the gene's identity. Therefore, we conclude that HMSPG should be tested immediately and could provide durable resistance to serious pests affecting date palm.

Acknowledgments

The authors thank Drs. Jameel Al-Khayri, Mohan Jain and Dennis Johnson for helpful suggestions in improving the manuscript.

References

Ahlquist, P. 2002. RNA-dependent RNA polymerases, viruses, and RNA silencing. Science 296:1270-1273.

Alhudaib, K., Y. Arocha, M. Wilson and P. Jones. 2007. "Al-Wijam", a new phytoplasma disease of date palm in Saudi Arabia. Bull. Insectol. 60:285-286.

Al-Khayri, J.M. 2010. Somatic embryogenesis of date palm (*Phoenix dactylifera* L.) improved by coconut water. Biotechnology. 9: 477-484.

Aly, R., H. Cholakh, D. M. Joel, D. Leibman, B. Steinitz, A. Zelcer, A. Naglis, O. Yarden and A. Gal-On. 2009. Gene silencing of mannose 6-phosphate reductase in the parasitic weed *Orobanche aegyptiaca* through the production of homologous dsRNA sequences in the host plant. Plant Biotechnol. J. 7:487-498.

Bailey, A. M. and C. L. Niblett. 2010. U. S. Patent Application (Bioassay for Gene Silencing Constructs). Published October 7, 2010 as US 2010/0257634A1.

Bakheet, S. A., H. S. Taha, M. S. Hanafy and M. E. Solliman. 2008. Morphogenesis of sexual embryos of date palm cultured *In vitro* and early identification of sex type. J. Appl. Sci. Res. 4:345-352.

Baulcombe, D. 2005. RNA silencing. Trends Biochem Sci. 30:290-293.

Baum, J. A., T. Bogaert, W. Clinton, G. R. Heck, P. Feldmann, O. Ilagan, S. Johnson, G. Plaetinck, T. Munyikwa, M. Pleau, T. Vaughn and J. Roberts. 2007. Control of coleopteran insect pests through RNA interference. Nature Biotechnol. 25:1322-6.

Dietzgen, R. G., and A. N. Mitter. 2006. Transgenic gene silencing strategies for virus control. Austr. Plant Pathol. 35:605-618.

Eamens, A., M. B. Wang, N. A. Smith and P. M. Waterhouse. 2008. RNA silencing in plants: Yesterday, today, and tomorrow. Plant Physiol. 147:456-68.

El-Juhany, M. and I. Loutfy. 2010. Degradation of Date Palm Trees and Date Production in Arab Countries: Causes and Potential Rehabilitation. Aust. J. Basic Appl. Sci. 4:3998-4010.

El-Sabea, A. M. R., J. R. Faleiro and M. M. Abo El Saad. 2009. The threat of red palm weevil *Rhynchophorus ferrugineus* to date plantations of the Gulf region of the Middle East: an economic perspective. Outlook Pest Manage. 20:131–134.

Escobar, M. A., E. L. Civerolo, K. R. Summerfelt, and A. M. Dandekar. 2001. RNAi-mediated oncogene silencing confers resistance to crown gall tumorigenesis. Proc. Nat. Acad Sci. USA. 98:13437-42.

Faleiro, J. R. 2006. Review Article. A review of the issues and management of the red palm weevil *Rhynchophorus ferrugineus* (Coleoptera: Rhynchophoridae) in coconut and date palm during the last one hundred years. Internat. J. Trop. Insect Sci. 26:135–154.

Fire, A., S. Xu, M. K. Montgomery, S. A. Kostas, S. E. Driver and C. C. Mello. 1998. Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*. Nature 391:806-11.

Food and Agriculture Organization of the United Nations, http://faostat.fao.org 2007.

Hammond, T. M., and N. P. Keller. 2005. RNA silencing in *Aspergillus nidulans* is independent of RNA-dependent RNA polymerases. Genetics 169:607-17.

Hannon, G. 2003. RNAi A Guide to Gene Silencing. Cold Spring Harbor Laboratory Press. pp.436.

Harrison, N. A. and M. L. Elliott. 2009. Lethal Yellowing (LY) of Palm. Circular pp.222. Institute of Food and Agricultural Sciences, University of Florida. Gainesville, FL.

Ho, T., D. Pallett, R. Rusholme, T. Dalmay and H. Wang. 2006. A simplified method for cloning of short interfering RNAs from *Brassica juncea* infected with Turnip mosaic potyvirus and Turnip crinkle carmovirus. J. Virol. Methods 136:217-223.

Huang, G., R. Allen, E. L. Davis, T. J. Baum and R. S. Hussey. 2006. Engineering broad root-knot resistance in transgenic plants by RNAi silencing of a conserved and essential root-knot nematode parasitism gene. Proc. Natl. Acad. Sci. USA. 103:14302-6.

Ismail, I., N. F. Iskandar, G. M. Chee and R. Abdullah. 2010. Genetic transformation and molecular analysis of polyhydroxybutyrate biosynthetic gene expression in oil palm (*Elaeis guineensis* Jacq. var Tenera) tissues. Omics J. 3:18-27.

Johnson D.V. 2011. Introduction: date palm biotechnology from theory to practice. In: S.M. Jain, J.M. Al-Khayri and D.V. Johnson (Eds). pp. 1-11. Date Palm Biotechnology. Springer Science+Business Media BV. Dordrecht.

Katiyar-Agarwal, S., R. Morgan, D. Dahlbeck, O. Borsani, A. J. Villefas, J-K. Zhu, B. Staskawicz and H. Jin. 2006. A pathogen-inducible endogenous siRNA in plant immunity. Proc. Nat. Acad. Sci. USA. 103:18002-18007.

Mao, Y. B., W. J. Cai, J. W. Wang, G. J. Hong, X. Y. Tao, L. J. Wang, Y. P. Huang, and X. Y. Chen. 2007. Silencing a cotton bollworm P450 monooxygenase gene by plant-mediated RNAi impairs larval tolerance of gossypol. Nature Biotechnol. 25:1307-13

Matzke, M. A. and A. J. Matzke. 2004. Planting the seeds of a new paradigm. PLoS Biol. 2:E133.

McDonald, T., D. Brown, N. P. Keller and T. M. Hammond. 2005. RNA silencing of mycotoxin production in *Aspergillus* and *Fusarium* species. Mol. Plant Microb. Interact. 18:539-545.

Mousavi, M., A. Mousavi, A. A. Habashi, and K. Arzani. 2009. Optimization of physical and biological parameters for transient expression of *uidA* gene in embryogenic callus of date palm (*Phoenix dactylifera* L.) via particle bombardment Afr. J. Biotech. 8:3721-3730.

Munoz, C. I. and A. M. Bailey. 1998. A cutinase-encoding gene from *Phytophthora capsici* isolated by differential-display RT-PCR. Curr Genet. 33:225-230.

Niblett, C. L. 2006. U. S. and PCT Patent Application (Methods and Materials for Conferring Resistance to Pests and Pathogens of Plants. Published May 4, 2006 as US 2006/0095987 A1 and WO 2006/047495 A2.

Nixon, R.W. 1954. Date culture in Saudi Arabia. Ann. Date Growers' Instit. 31: 15-20.

Nowara, D., A. Gay, C. Lacomme, J. Shaw, C. Ridout, D. Douchkov, G. Hensel, J. Kumlehn and P. Schweizer. 2010. HIGS: Host-Induced Gene Silencing in the Obligate Biotrophic Fungal Pathogen *Blumeria graminis*. The Plant Cell. Vol. 22: 3130–3141.

Quenzar, B., M. Trifi, B. Bouachrine, C. Hartmann, M. Marrakchi, A. A. Benslimane and A. Rode. 2001. A mitochondrial molecular marker of resistance to Bayoud disease in date palm. Theor. Appl. Genet. 103:366–370.

Saker, M. M., M. A. Allam, A. H. Goma and M. H. Abd El-Zaher. 2007. Optimization of some factors affecting genetic transformation of semi-dry Egyptian date palm cultivar (Sewi) using particle bombardment. J. Genetic Eng. Biotech. 5: 57-62.

Sambrook, J., E. F. Fritsch and T. Maniatis. 1989. Molecular Cloning: A Laboratory Manual

(Second Edition). pp.1659. Cold Spring Harbor Laboratory Press. N.Y.

Sindhul, A. S., T. R. Maier, M. G. Mitchum, R. S. Hussey, E. L. Davis and T. J. Baum. 2009. Effective and specific in planta RNAi in cyst nematodes: Expression interference of four parasitism genes reduces parasitic success. J. Exp. Bot. 60:315-324.

Tomilov, A. A., N. B. Tomilova, T. Wroblewski, R. Michelmore and J. I. Yoder. 2008. Trans-specific gene silencing between host and parasitic plants. Plant J. 56:389-397.

Van West, P., S. Kamoun, J. W. van't Klooster and F. Govers. 1999. Internuclear gene silencing in *Phytophthora infestans*. Mol. Cell 3:339-348.

Xu, P., Y. Zhang, L. Kang, M. J. Roossinck and K. S. Mysore. 2006. Computational estimation and experimental verification of off-target silencing during posttranscriptional gene silencing in plants. Plant Physiol. 142:429-440.

Yanagihara, K., M. Tashiro, Y. Fukuda, H. Ohno, Y. Higashiyama, Y. Miyazaki, Y. Hirakata, K. Tomono, Y. Mizuta, K. Tsukamoto and S. Kohno. 2006. Effects of short interfering RNA against methicillin-resistant *Staphylococcus aureus* coagulase *in vitro* and *in vivo*. J. Antimicrob. Chemother. 57:122-6.

Yin, C., J. E. Jurgenson, S. Hulbert. 2011. Development of a host-induced RNAi system in the wheat stripe 2 rust fungus *Puccinia striiformis* f. sp. *tritici.* Mol. Plant-Microbe Interact. 24:554-561.

Zaid, A., P. F. De Wet, M. Djerbi and A. Oihabi. 2002. Chapter XII: Diseases and pests of date palm, In: A. Zaid. Paper 156, Rev. 1. Date Palm Cultivation. FAO Plant Production and Protection. Rome.