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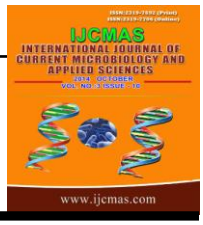

Original Research Article

RNA interference (RNAi) tool for Insect Pest Management Patenting analysis their impact on incentives and disincentives to undertake research and development

Prakash M Navale[1], V.Krishna[2], Hanchipura Mallesh Mahadeva Swamy[1*], Sowmy H D[1], Ramasamy Asokan[1], M. Manamohana[1], Riaz Mahmood[2] and K. Prasd Babu[1]

[1]Division of Biotechnology, Indian Institute of Horticultural Research (IIHR), Hessaraghatta Lake Post, Bangalore 560089, Karnataka, INDIA
[2] Post-Graduate Department of Studies and Research in Biotechnology and Bioinformatics, Kuvempu University, Jnanasahayadri, Shankaraghatta, Shimoga 577451, Karnataka, INDIA
**Corresponding author*

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A B S T R A C T

RNA interference is a natural process that affects the level of activity of genes in animals and plants. Flexibility, adaptability, and demonstrated effectiveness of RNA interference technology mean it will have an important place in the future of pest management. As with all patents, there are opportunities, challenges and trade-offs when interim absolute rights are required to spur innovation - RNA interference patents are no different. It has gained importance over the decade which is used in this review as a case study and analysis of the patents granted on RNAi insect pest management was carried out. This study categorizes a number of patents related to RNAi in insect pest management and the development of patentable technologies. The analyses were done using various criteria like patenting trends over the years, assignees playing a major role, comparison of the technology used in different patents and the patenting activity across the insect orders. Despite significant achievements have been made RNAi in insect pest management strategies, the patents and patentable technologies were very limited confined to certain geographical regions. Emphasis must be given to the patents and patentable technologies and this will enable the RNAi technology for the effective control of economically important insect pests. Patent documents related to bacterium RNAi insect pest management contain a trove of technical and commercial information and thus, patent analysis is considered as a useful tool for R&D management and techno economical development. Undeniably, there is broad potential for the application of RNAi technology in pest control, mainly if combined into integrated pest Management strategies.

Introduction

Commercial crops are often the targets of insect attack. Chemical pesticides have been very effective in eradicating pest infestations. However, it is well known that there are several disadvantages to using chemical pesticidal agents. First of all, chemical pesticidal agents are not selective, therefore, on the same time of controlling

target insect, because of the lack of selectivity, they also exert their effects on non-target fauna, often effectively sterilizing a field for a period of time over which the pesticidal agents have been applied. Second, chemical pesticidal agents persist in the environment and generally are slow to be metabolized, if at all. They accumulate in the food chain, and finally in the high predator species, such as human being, where these pesticidal agents act as a mutagens and/or carcinogens, to cause irreversible and deleterious genetic modifications. This kind of accumulation causes to higher predator pest resistance. Thus there has been a long felt need for environmentally friendly methods for controlling or eradicating insect infestation on or in plants, i.e., methods which are selective, environmental inset, non-persistent, and biodegradable, and that fit well into pest resistance management schemes. These environmental safe compositions, including *Bacillus thuringiensis* (*Bt*) bacteria and transgenic plants expressing one or more genes encoding insecticidal *Bt* protein, have been remarkably efficient in controlling insect pest infestation. However, with the increased use of *Bt* crops, such as corn and cotton, comes the threat that target pests may develop resistance to these toxins. Although Bt-resistant insect populations have not yet been observed in the field, resistant strains have been developed in the laboratory by selection with toxin-impregnated diet (McGaughy, 1985). Thus, beside to work out ways to delay *Bt* resistance development, it is greatly valuable to find a different mode of action to control pest infestations by single use or combined use with Bt expression strategy (Jian Ye et al. 2012).

RNA Interference (RNAi) provides a potentially powerful tool for controlling gene expression because of its specificity of target selection and remarkably high efficiency in target mRNA suppression. RNAi refers to the process of sequence-specific post- transcriptional gene silencing mediated by short interfering RNAs (siRNAs) (Zamore, *et al.,* 2000; Fire, *et al.,* 1998); Hamilton *et al.,* 1999; Lin *et al.,* 1999). While the mechanics underlying RNAi are not fully characterized, it is thought that the presence of dsRNA in cells triggers RNAi by activating the ribonuclease III enzyme Dicer (Zamore, *et al.,* 2000; Hammond *et al.,* 2000). Dicer processes the dsRNA into short pieces called short interfering RNAs (siRNAs), which are about 21 to about 23 nucleotides long and comprise about 19 base pair duplexes (Zamore *et al.,* 2000; Elbashir *et al.,* 2001).

Following delivery into cells, the siRNA molecules associate with an endonuclease complex, commonly referred to as an RNA-induced silencing complex (RISC), which brings together the antisense strand of the siRNA and the cellular mRNA gene target. RISC cleaves the mRNA, which is then released and degraded. Importantly, RISC is then capable of degrading additional copies of the target mRNA. Although the technique of RNAi has been generally known in the art in plants, *C. elegans* and mammalian cells for some years, to date little is known about the use of RNAi to down-regulate gene expression in insects. The objective of the present study is to assess the patenting trends of RNA interference (RNAi) as one of the new alternative strategies to reduce damage from insect pests offers new opportunities to increase the production by using more sustainable and ecological friendly agriculture system. This patent analysis intern helps researchers to become familiar with RNAi research, a rapidly growing field where new avenues and techniques are being used to investigate

insect RNAi mechanisms for the development of pest control in eco friendly manner for sustainable crop production. This also insights on how to reduce the pescticidal residues in environment.

Methodology

In 2002, RNA interference (RNAi) was proclaimed by *Science* as the "breakthrough technology of the year" and by *Fortune* as a "billion dollar breakthrough." (Chi-Ham *et al.*, 2010). The recognition of RNAi-mediated gene suppression as an important experimental tool and its potential commercial application is further reflected in the patent landscape related to RNAi-mediated gene suppression, with an increasing number of patent applications seeking exclusive rights to RNAi-based discoveries. To analyze technological innovation, patent analysis was used, because it provides innovative information of individual, organizational, regional and national scientific levels. Patent analysis was also used to map the technological activities at various levels. This article attempts to analyze the patenting activity in the field of RNAi new sound pest management strategy. This report also try to highlight the important technological directions and gaps in our knowledge in order to allow further pursue of R&D, using data from different databases, namely European Patent and trade mark office database (EPO) (http://www.epo.org/), Google patents (https://www.google.com/?tbm=pts), Indian patent database (IPO) (http://www.ipindia. nic.in/), United States Patent office database (USPTO) (http://patft.uspto.gov /netahtml/PTO/search-bool.html), World Intellectual Property Organization (WIPO) (http://www.wipo.int/tools/en/gsearch. html). All searches and data have been culled from 1970 to 2013 to cover active patenting authorities throughout the world. International search for patents on a specific subject used the following key word: RNAi in insect pest management. Searches were made using WIPO, USPTO, EPO, PCI, JPO, KIPO, INPADOC, IPO and all electronic database used the advanced Boolean search on issuing date, country and international classification number and bibliographical references of all the patents. This was done in order to understand the technical approaches taken by different research groups throughout the world. It also provided an insight into emerging technologies and key areas for R&D.

Results and Discussion

Gene silencing has been suggested as one of the new alternatives to reduce damage from insect pests. RNA interference (RNAi) is first described by Fire *et al*., 1998, and its mechanism lies in that a double-stranded RNA (dsRNA) introduced in an organism has the capacity to silence post-transcriptional genes (Hannon, 2002; Geley and Muller, 2004). RNAi is highly conserved in eukaryotic organisms (Fire, 2007). It is considered as a specific type of defence mechanism (Terenius *et al*., 2011). Four different types of RNAi have been described including short interfering RNAs (siRNAs), piwi-interacting RNAs (piRNAs), endogenous siRNAs (endo-siRNAs or esiRNAs), and microRNAs (miRNAs) (Terenius *et al*., 2011). To date, RNAi has been proven promising for research on gene function determination and gene knockdown in eukaryotes and medical control of cancers and viral disease (Huvenne and Smagghe, 2010).

Widespread increase in the application of RNAi technology in insect research has facilitated the identification of insect gene function. Research has shown that while dsRNA is particularly conservative, there

are various functions and development factors among insect species. Such variations are yet to be fully understood but certainly can serve as a basis for determining their capacity to control insect genes. The main challenge for moving towards larger scale projects remains the development of effective delivery mechanisms. Feeding is very popular in insect RNAi research and may have the most promising future in pest control, especially with the creation of transgenic plants producing dsRNA. Overtime, the use of transgenic insects will also lead to more efficient pest control (Guang Yang *et al.*, 2011).

The present study data was examined with respect to (a) trends in the growth of patenting activity (b) organizations/ industries active in research, and (c) the focus of research pertaining to present situation. The analysis has been used to indicate the emerging technological opportunities and trends by highlighting the important technological directions and gaps. The gist of results obtained from the analysis is presented here.

Patent Analysis

Patent analysis is a unique management tool for addressing the strategic management of the firm's technology and product or service development process. Translating patent data into competitive intelligence allows the firm to magnitude its current technical competitiveness, to forecast technological trends, and to plan for potential competition based on new technologies (Fleisher *et al.*, 2003). Patent analysis is an extremely versatile tool, with many implications for businesses strategic planning, mergers, acquisitions, licensing opportunities, R&D management, human resources, competitive intelligence, business intelligence, etc. Since this study was developed as part of an intelligence studies course, my primary aim is to examine patent analysis as a tool for intelligence particularly competitive intelligence. Analytical tools are used for searching patent and associated with scientific literature to show the gap in research areas.

RNAi insect pest management

RNA interference (RNAi), the sequence-specific suppression of gene expression, offers great opportunities for insect science, especially to analyze gene function, manage pest populations, and reduce disease pathogens. At present, we have a limited capacity to predict the ideal experimental strategy for RNAi of a particular gene/insect because of our incomplete understanding of whether and how the RNAi signal is amplified and spread among insect cells. Consequently, development of the optimal RNAi protocols is a highly empirical process.

This limitation can be relieved by systematic analysis of the molecular physiological basis of RNAi mechanisms in insects. An enhanced conceptual understanding of RNAi function in insects will facilitate the application of RNAi for dissection of gene function, and to fast-track the application of RNAi to both control pests and develop effective methods to protect beneficial insects and non-insect arthropods, particularly the honey bee (*Apis mellifera*) and cultured Pacific white shrimp (*Litopenaeus vannamei*) from viral and parasitic diseases (Scott *et al.*, 2013). From figure 1 it can be seen that the first patent was initiated in the year 2000 even though the technology was well described by Fire et al. in 1998. It can be seen that there is a gradual increase in the number of patents during 2000 to 2013. The number of patents

reached maximum of 117 in the year 2011 and 2012. In the present year the number of patents granted is 114 till 25.10.2013.

The patent claiming Methods for controlling pests using RNAi, EP2347759 describes the methods for controlling pest infestation using double stranded RNA molecules. This invention also provides methods for producing transgenic cells expressing the double stranded RNA molecules, as well as compositions and commodity products containing or treated with such molecules (Els Van Bleu *et al.,* 2008).

Comparison of Patenting Activity by the Major Assignees

Patent analysis in terms of level of activity gives general information on companies that are active in R&D of these bio-insecticides. The assignees across the globe are shown in figure 2 and following observations were made. (i) Monsanto and syngenta companies hold the maximum number of patents 26.09% and 24.47% respectively, followed by Divergene representing 13.46%. (ii) Among agricultural universities and research Institutions University of California (1.92%) has the maximum number of patents followed by University of Georgia, University of Copenhagen and finally University of Dortmund. Most of the assignees concentrate on the RNAi as the alternative strategy for the effective pest managements. In conclusions the search and analysis of patents drives research strategy and support innovation. With this in mind patent analysis was done to find industrial trend and to understand the competitiveness across the globe. It also provides an insight into emerging technologies and key areas of R&D.

Among current assignee evolution it has been observed that the Monsanto has the maximum and consistent evolution followed by Basf Crop, Dow Agroscience and Bayer crops in RNAi insect pest management R & D activities (Fig. 3).

RNAi across insect orders

Lepidopterans, Coleopterans and Sap sucking pests are the major pests of agricultural crops, and it is becoming increasing urgent to find new methods to control them. From the figure 4A, B and C it has been observed that the patenting trend there is a gradual increase in patents over the period of time. The maximum number of patents were observed in the order Lepidoptera followed by Coleoptera and Hemipteran pests. This data shed lights on the R & D gaps in RNAi insect pest management strategies. This basic information helps in identifying the research gaps and brings out a scope for generating intellectual property through focused research since considerable agricultural crop losses incurred by different insect pest orders where in effective control measures are not available so far.

RNAi transgenic plants

RNA interference (RNAi) is a sequence specific gene silencing mechanism, triggered by the introduction of dsRNA leading to mRNA degradation. It helps in switching on and off the targeted gene, which might have significant impact in developmental biology. Discovery of RNAi represents one of the most promising and rapidly advancing frontiers in plant functional genomics and in crop improvement by plant metabolic engineering and also plays an important role in reduction of allergenicity by silencing specific plant allergens.

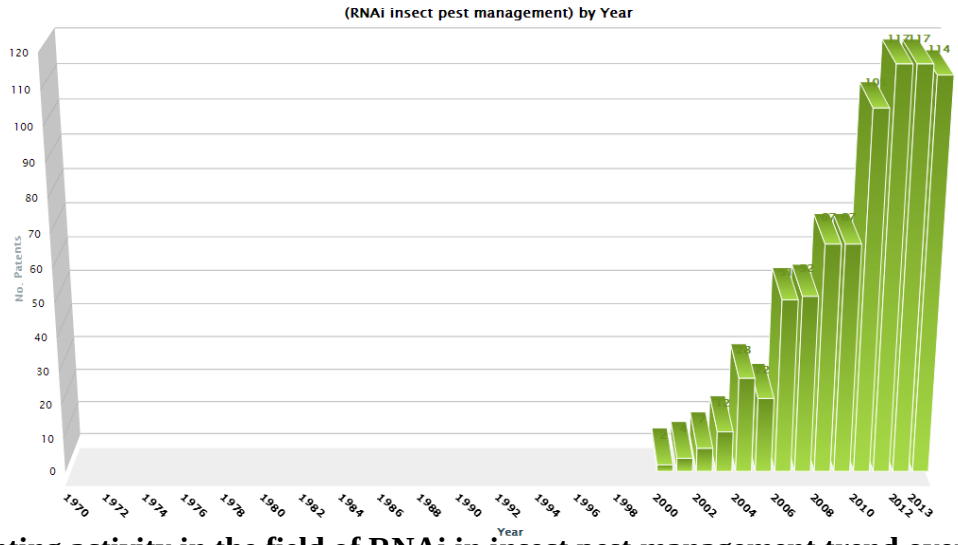

Fig.1 Patenting activity in the field of RNAi in insect pest management trend over the years (1970-2013). IP laws vary between countries and patents have national boundaries, it is essential to perform an FTO IP analysis for each country in which products will be developed and deployed.

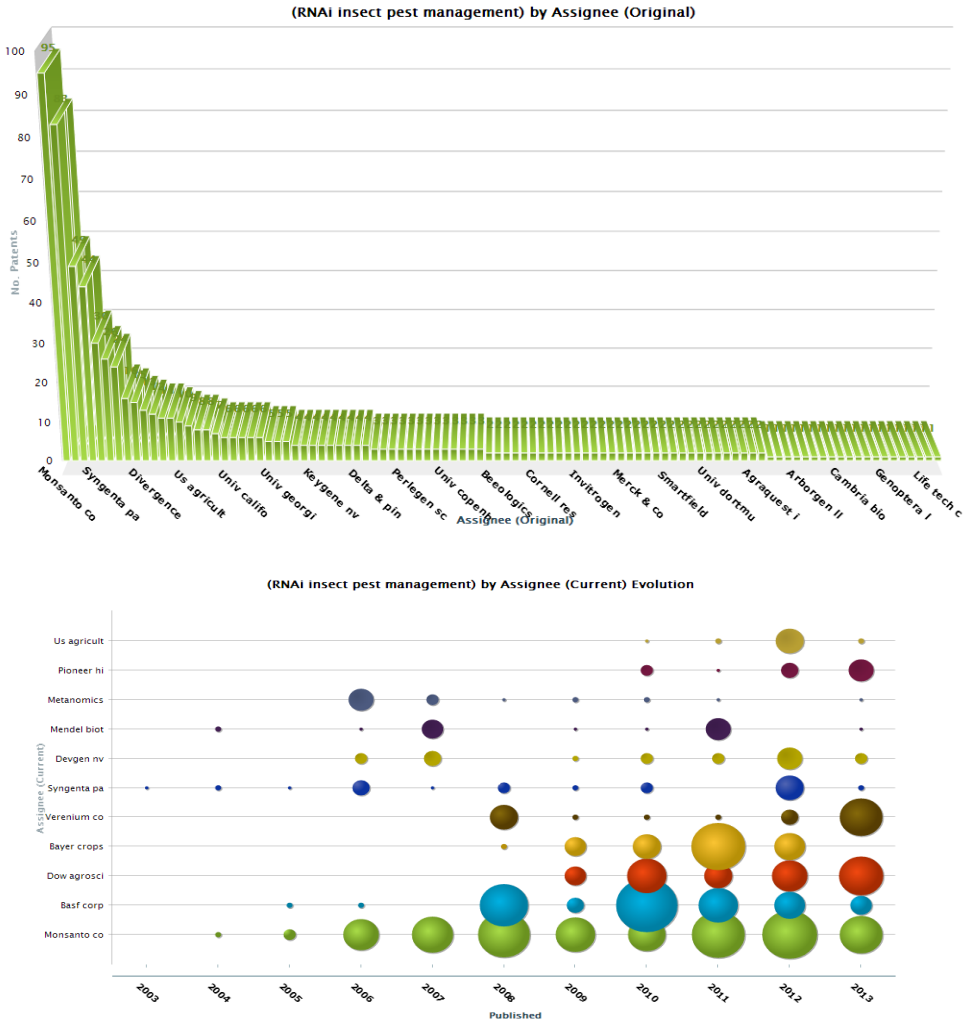

Fig.2 Patenting activity in the field of RNAi in insect pest management major assignees involved in the R & D research

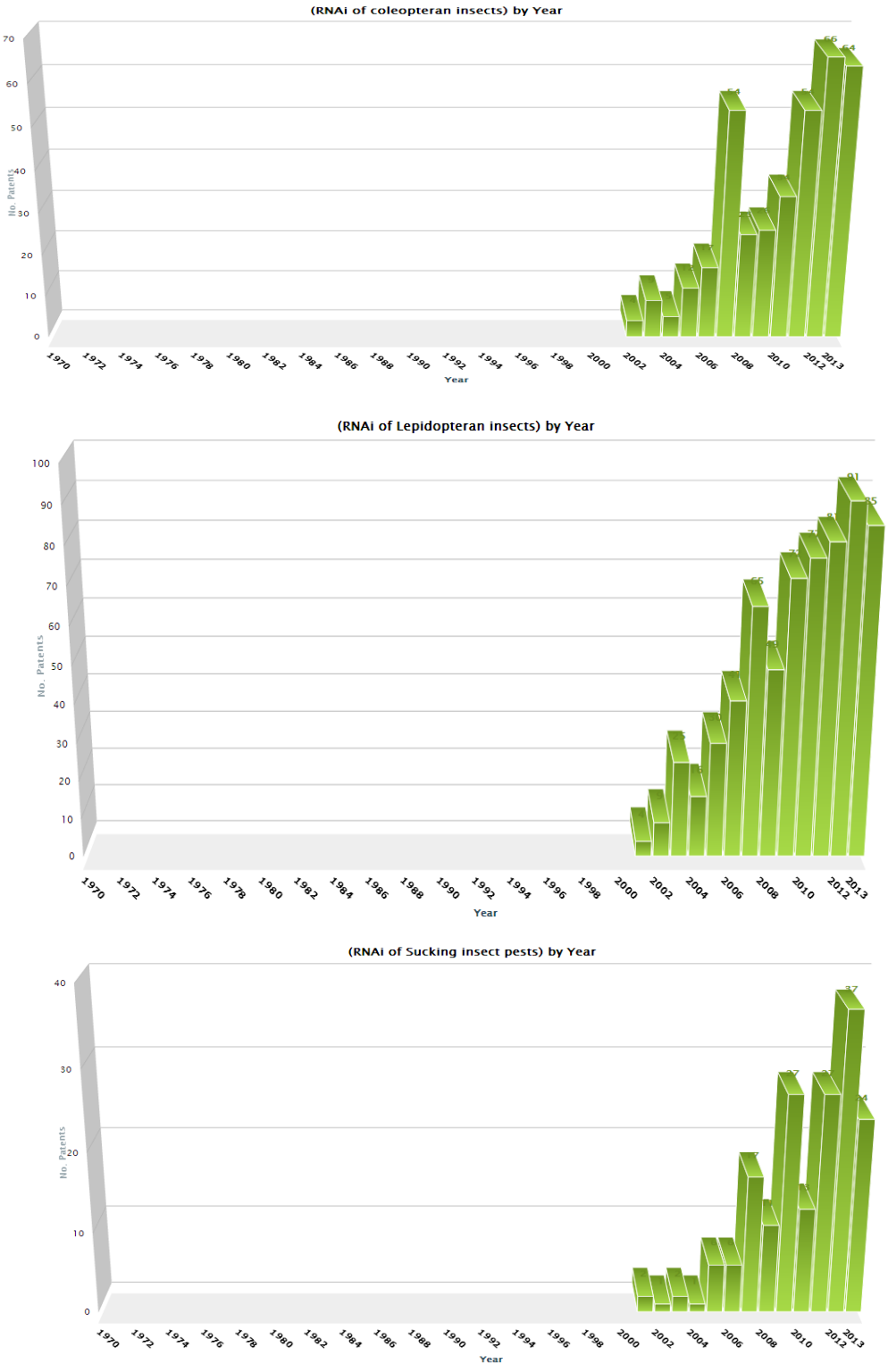

Fig.3 Patenting activity in the field of RNAi insect pest management active against different insect orders. Maximum number of patents observed in the order Lepidoptera following Coleoptera and Hemipteran, indicating that emphasis must given to other insect orders also.

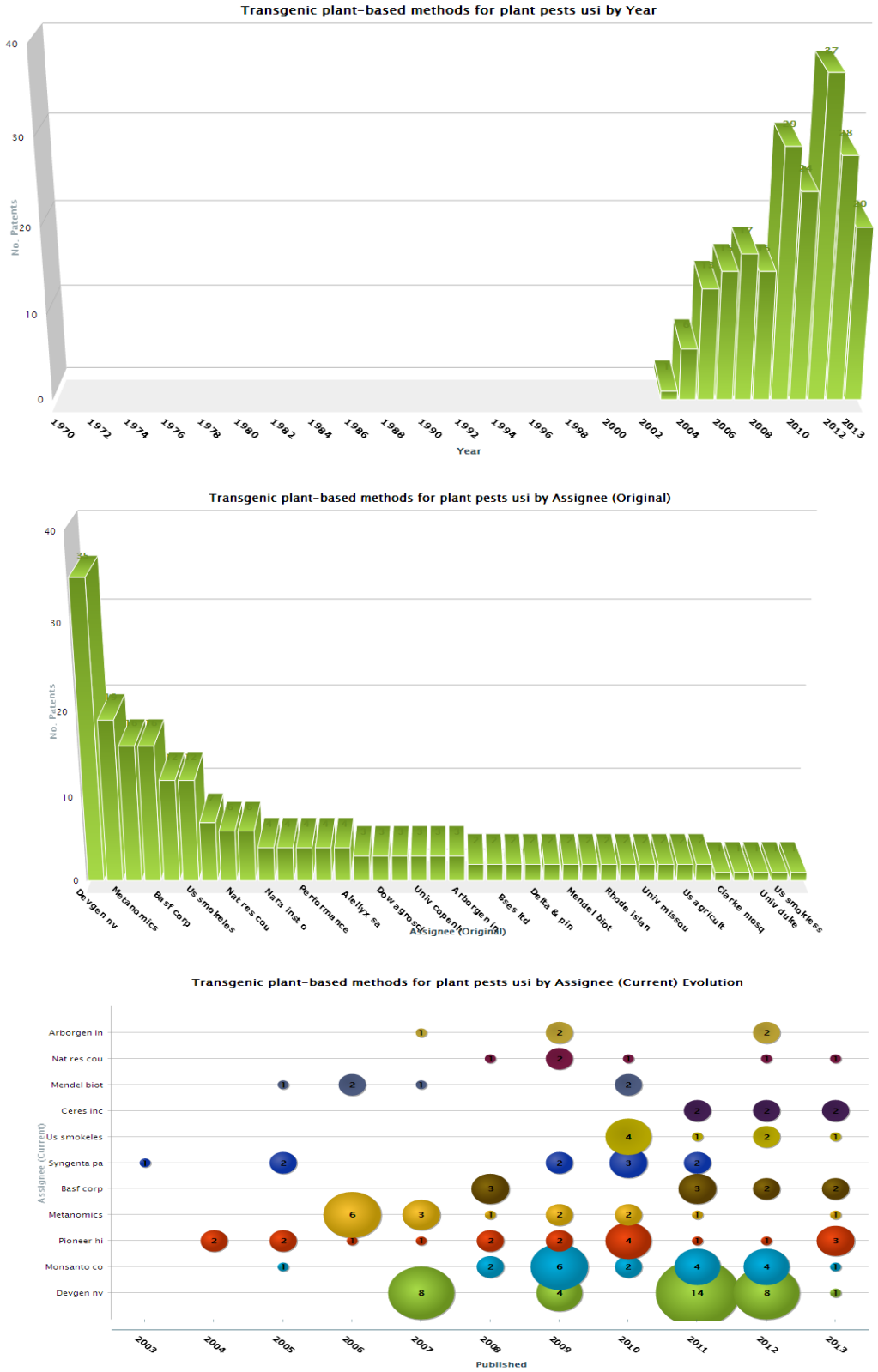

Fig. 4. Patenting activity to RNAi insect pest management transgenic plant-based methods for plant pests. A-Patenting trend over the years, B-Major assignees involved in the development of RNAi based transgenic plants and C-major assignees holding the number of patents.

In plants the RNAi technology has been employed successfully in improvement of several plant species- by increasing their nutritional value, overall quality and by conferring resistance against pathogens and diseases (Ali *et al.,* 2010). The patenting activities in this field over the years depicted in Fig. (4). It can be observed that the year 2009-2011 has the maximum patenting activity. It is also observed that there was increasing trend in the number of patents since 2004 to 2013. This indicates that the patenting activity has been very recent and that this area has gained importance in recent years. The application of RNA-mediated gene suppression to produce GM organisms evolved from strategies based on expression of target genes in antisense orientation, to co-suppression by over expressing sense transcripts and then to producing dsRNA. There now exist both emerging as well as expiring patents in the United States for the general use of RNAi in plants, and DNA constructs that mediate dsRNA production (Chi-Ham *et al.,* 2010).

RNAi is continuing to develop as a fundamental tool in both plant and animal biotech and an ongoing assessment of the patent landscape will be important to equip scientists and investors with knowledge for evaluating freedom to operate (FTO) in this technology sector (Chi-Ham *et al.,* 2010) . Widespread increase in the application of RNAi technology in insect research has facilitated the identification of insect gene function. Research has shown that while dsRNA is particularly conservative, there are various functions and development factors among insect species. Such variations are yet to be fully understood but certainly can serve as a basis for determining their capacity to control insect genes. The main challenge for moving towards larger scale projects remains the development of effective delivery mechanisms. Feeding is very popular in insect RNAi research and may have the most promising future in pest control, especially with the creation of transgenic plants producing dsRNA. Overtime, the use of transgenic insects will also lead to more efficient pest control (Guang Yang *et al.,* 2010). In conclusion analyzing the RNAi insect pest management patents on various criteria can provide valuable information which can be put into use in different ways. These indexes can be used to analyze up-to-date trends of technologies and identifying promising venues for new product development. Patent search and analysis of technological strategies of formulation may lead to higher probability of success in new technological ventures. Finally the specific goal of RNAi insect pest management patent analysis is to discover, develop, and understand new products and methods for safe and effective control of pests, thereby maximizing food production and public health. It has also been observed that patenting activities in the emerging fields of technology are increasingly growing. This quickens the technology evaluation process for businesses and helps to highlight the important technological directions and gaps for further R&D decisions with greater confidence.

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