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RNAi

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RAPID COMMUNICATION

RNAi: What is its position in agriculture?

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Abstract
RNA interference (RNAi) is being developed and exploited to improve plants by modifying endogenous gene expression as well as to target pest and pathogen genes both within plants (i.e. host-induced gene silencing) and/or as topical applications (e.g. spray-induced gene silencing). RNAi is a natural mechanism which can be exploited to make a major contribution towards integrated pest management and sustainable agricultural strategies needed worldwide to secure current and future food production. RNAi plants are being assessed and regulated using existing regulatory frameworks for GMO. However, there is an urgent need to develop appropriate science-based risk assessment procedures for topical RNAi applications within existing plant protection products legislation.

Keywords RNAi · dsRNA · Biosafety · Agriculture · Regulations · HIGS · SIGS

Key message

- RNAi is a natural mechanism found in most eukaryotic organisms in nature and can be exploited to improve plant health.
- RNAi-based technology is already being exploited, and the realized examples confirm its great potential in a range of areas of crop production and protection.
- Plants modified to express target dsRNAs are being assessed and regulated using existing regulatory frameworks for GMO.
- However, there is an urgent need to develop appropriate science-based risk assessment procedures for topical RNAi applications within existing PPP legislation.

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Introduction

Science has taught us about nature's elegant genetic regulation occurring in eukaryotic organisms like plants and animals, where double-stranded RNA (dsRNA) molecules interfere with homologous alien RNA to fine-tune gene expression and subsequent protein production in a process called RNA interference (RNAi). Emerging RNAi tools are increasingly showing potential major impacts on agriculture with applications in crop protection and production, since its discovery led to a Nobel Prize in medicine, but with possible applications in other fields of biology (Fire et al. 1991; Zotti et al. 2018). RNAi is being exploited to adapt endogenous gene expression in plants as well as to target pest and pathogen genes both within plants (i.e. host-induced gene silencing, HIGS) and as topical applications (e.g. spray-induced gene silencing, SIGS). At the molecular level, the pathway works through processing long dsRNA into so-called small interfering RNA (siRNA) molecules, which specifically recognize the target messenger RNA (mRNA), leading to its neutralization. In this way, plant genes can be targeted to remove unwanted metabolites or increase beneficial nutrients in crops. In pests and pathogens, essential genes can be suppressed leading to effective protection of plants. Since siRNAs recognize target gene mRNAs based on sequence complementarity, systems can be designed with high specificity where genes with homologous sequences can be targeted in a narrow range of species. The exponential increase in available genomic and transcriptomic sequence data allows the design of highly specific targeting dsRNAs, minimizing the risk of off-target effects or silencing effects in non-target organisms (Christiaens et al. 2018).

The importance of RNAi in sustainable agriculture

Research on a range of potential applications of RNAi in crop protection is increasing, and it is becoming apparent that RNAi-based approaches could make a major contribution towards integrated pest management and sustainable agriculture. One of the activities is conducted by the European COST action "iPlanta" (CA15223)[1] was based on the consideration that, as literature on RNAi-based control in crop protection continues to expand, it is timely to evaluate both the trends and influence of its development and to provide an indication of the research and development landscape, the prolific centres of research and their collaborations. Sourcing over 76 million records from the most comprehensive database, the Thompson Reuters Web of Science (WoS) using the query string TS = (pest* OR pathogen* NEAR plant) AND TS = (RNAi OR "RNA interference" OR "RNA-interference"), revealed a rapid global increase in the number of publications on RNAi research since year 2002. The top ten countries contributing to RNAi research span Europe, North America and South-East Asia. Leaders are in China, USA, India, Germany, Belgium, Japan, Canada, UK, South Korea and France, with researchers in China leading the number of publications (Fig. 1). Rapid developments in RNAi research are led by a diverse set of collaborative actors from both academia centres and industry, who provide both leadership and globalized contributions to the field across disciplines, space and time (Figs. 2 and 3). Industry pioneers are Devgen N.V. and Monsanto Co. (now: Bayer CropScience) with their landmark paper on RNAi to control the western corn rootworm (Baum et al. 2007). Using an alternative plant-mediated RNAi approach, Mao and co-workers, in another landmark paper, reported the possibility to control the cotton bollworm, by suppressing its detoxification P450 monooxygenase gene, thereby impairing its tolerance to gossypol, a natural toxic phytochemical accumulated by plants to resist or evade herbivores (Mao et al. 2007). The development and number of publications [including patents (see more in Frisio and Ventura 2019)] with RNAi as a tool in crop protection are expected to keep rising in coming years, supporting further R&D and implementation in practice.

In the field of plant biotechnology, RNAi has several unique features which offer additional opportunities to breeders for varietal improvement compared to genome editing technologies such as CRISPR/Cas or TALENs. One of these characteristics is that RNAi can lead to a gene knockdown effect, rather than a complete knockout, depending on the choice of the dsRNA (length and sequence) (Wagner et al. 2011). This is important when reduced levels of gene expression are required, as for certain cases of metabolically engineered plants with modified fatty acid profiles. Other unique aspects of RNAi are that siRNA molecules have high mobility through the plant's vascular system and can move inside the plant from the point of production to other parts of the plant (Molnar et al. 2011). Therefore, dsRNA produced in part of the plant (e.g. rootstock or interstock) has the potential to spread into the grafted parts of the plant so as to confer resistance to disease to the whole plant, including fruit. This results in fruits that are not genetically modified (GM), but protected by the presence of target-specific degradable small RNA molecules (De Francesco et al. 2020; Limera et al. 2017; Zhao and Song 2014).

[1] iPlanta is a multi-actor platform of excellence on RNAi mechanisms, applications, biosafety, socioeconomic issues and communication in many EU and nearby countries, and cooperating researchers in associated countries in North and South America, Australia and Asia. https://iplanta.univpm.it/

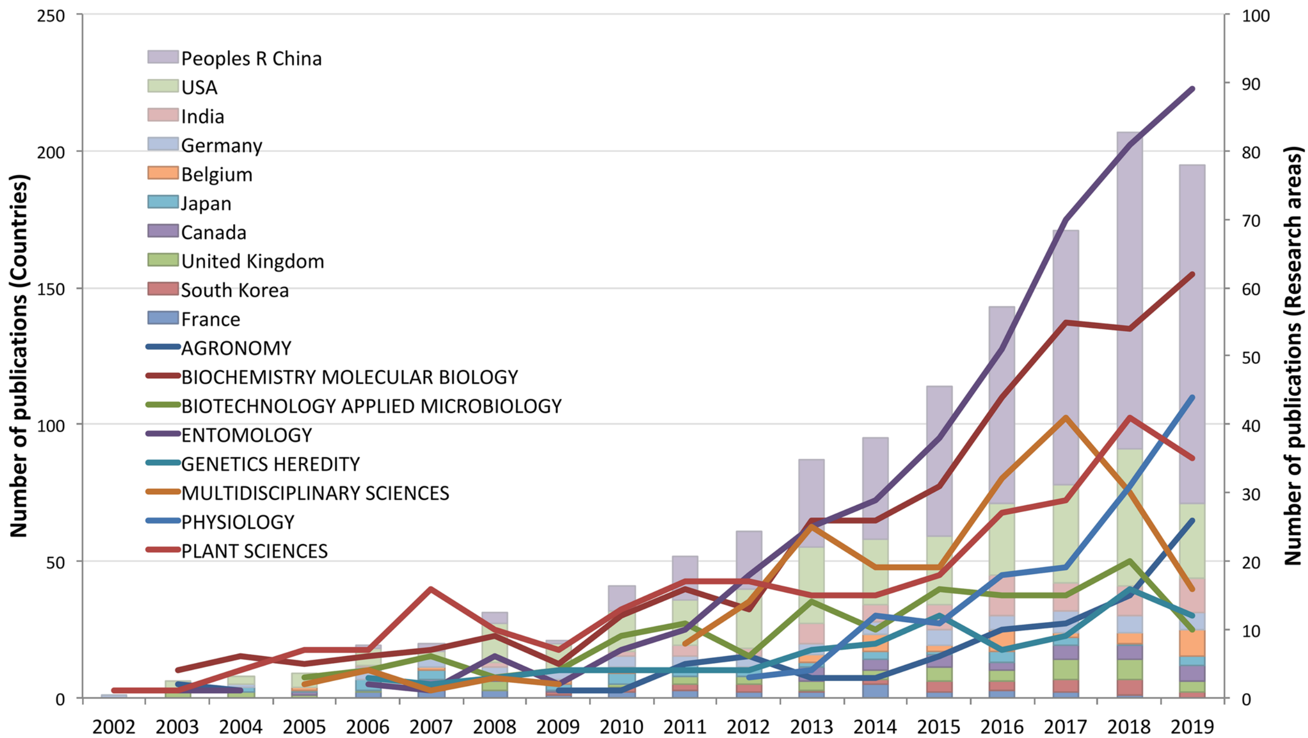

Fig. 1 Number of publications in RNAi research disciplines since 2002 and the ten countries with the most authored or co-authored publications

Stable expression of dsRNAs in a GM plant allows exposure to dsRNA by different types of plant feeding arthropods and pathogens in a range of plant tissues as the plant grows (Zotti et al. 2018). GM plants expressing interfering RNAs are regulated as other GM plants but are expected to potentially raise less safety concerns because no new protein is produced in the plants (Casacuberta et al. 2015; Ramon et al. 2014) and because of the highly sequence-specific mode of action of RNAi (Tan et al. 2016; Bachman et al. 2013). In the EU, the EFSA has given biosafety opinions on food and feed for several crops [potato EH92-527-1 (including cultivation in the EU), soybeans MON87705 and MON87705 × MON89788 (excluding cultivation)], with enhanced nutritional characteristics and more recently on corn rootworm-resistant maize MON87411 and maize MON87427 × MON89034 × MIR162 × MON87411 (EFSA 2019). Worldwide, several virus-resistant plants have been approved for cultivation outside the EU (e.g. plum, squash and papaya) and many more virus control applications are being developed (Khalid et al. 2017; Limera et al. 2017). In addition, plant resistance to a wide range of pests and fungal pathogens is being studied, particularly to insect vectors of pathogens and a range of diseases such as cereal rusts or fruit grey mould (Andrade and Hunter 2016; McLoughlin et al. 2018; Wang et al. 2016). As with other technologies, pest and pathogen resistance management is important and new crop protection applications need to be accompanied by effective stewardship and resistance management plans.

A more recent innovation is the use of topical applications of dsRNA to induce gene silencing as a new strategy for plant protection or growth regulation (San Miguel and Scott 2016; Worrall et al. 2019). Technical advances in the production of dsRNA and formulations to improve the efficacy, stability and persistence of extracellular dsRNA mean that it is now realistic to consider using dsRNA for biological protection ("biopesticide"). It can be applied as foliar sprays, root drenching, seed treatments or trunk injections, and there is considerable commercial interest in this because of the cost of production, the specificity and improved biosafety compared with chemical pesticides and some alternative biocontrol strategies (Rodrigues and Petric 2020; Bramlett et al. 2019; Cagliari et al 2019; Zotti et al. 2018). Spray-induced gene silencing (SIGS) is also being considered for weed control by targeting specific genes in a weed that do not occur in crops or other weed genera. Such a strategy would be very useful for controlling grass weeds in a range of graminaceous crops such as wheat and rice, though formulations and techniques that allow entry into weed cells are currently very challenging (Jiang et al. 2014; Dalakouras et al. 2016).

Topical applications would typically contain dsRNAs which are produced in microbes or synthesized enzymatically in vitro. Thus, they are not like synthetic agrochemicals

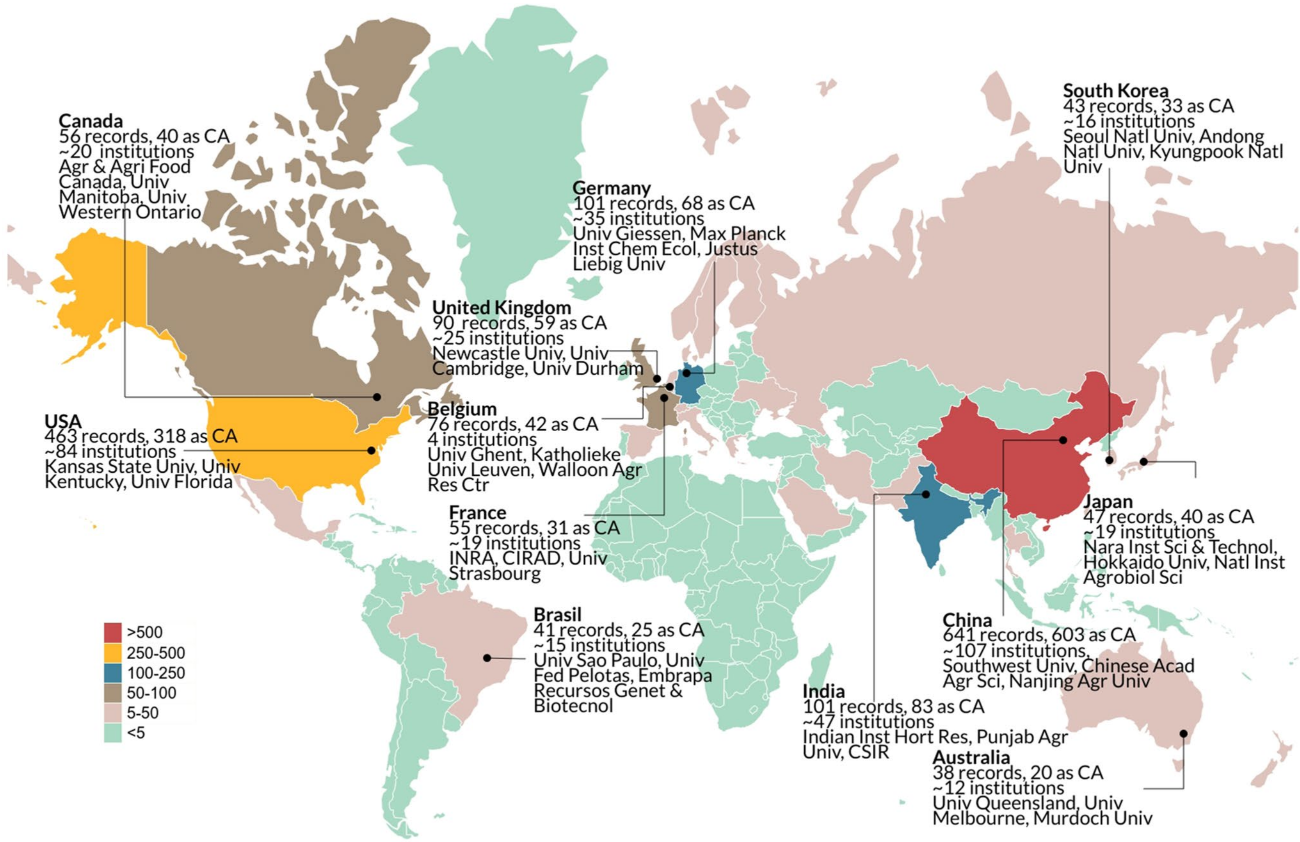

Fig. 2 Global geographical contributions to RNAi research. Leading countries and key research institutions in RNAi are profiled, indicating a global interest in the field. Records represent the number of authored and co-authored articles. CA, corresponding author; Univ, university; Inst, institute

and are different from other biocontrol agents which exploit proteins such Cry toxins. The dsRNA molecules may be produced using bacteria and yeasts, but also cell-free mass production systems are now available. These advances have lowered the production costs significantly in recent years to an estimate of 0.5–1 USD per gram, which is now making RNAi competitive in the market place (Zotti et al. 2018; Taning et al. 2020). Considering that dsRNA is a natural biological molecule that is readily degraded in nature and biological systems, specific formulations to ensure its stability and effective delivery to targets will be required on a case-by-case basis (Taning et al. 2020). Thus, it represents a novel type of biological protection/"biopesticide" and it is important that safety assessments for plant protection products (PPPs) are adapted to allow introduction of this technology. Existing PPP risk assessment approaches can be reliably used to evaluate dsRNA-based products for topical application, with adaptations only required on a case-by-case basis where additional research might be necessary to assess risk.

Virus vectors can also be used to enable an efficient RNAi response in plants and insects. These viruses can be engineered to contain a fragment of the target gene which leads to the production of specific dsRNAs in the host cell (Kurth et al. 2012). The wide range of host-specific viruses offers an elegant way to modify plant characteristics and to target insect pests.

Current RNAi-based applications in pest control aim to kill the target insect pests. However, there is also potential for both HIGS and SIGS to exploit non-lethal modes of action to result in a more sustainable and integrated approach to the management of field pests. For example, when two pheromone-binding proteins were silenced in the agricultural pest, *Helicoverpa armigera* by RNAi, male moths were significantly less able to detect the female sex pheromone, which reduced mating behaviour (Dong et al. 2017). In another example, RNAi was used to silence spermatogenesis genes in *Bactrocera tryoni,* a major horticultural pest in Australia, and resulted in dsRNA-treated males producing 75% fewer viable offspring than negative controls (Cruz et al. 2018). This opens up the possibility of exploiting RNAi to generate new IPM strategies based on altered feeding or reproductive behaviour of pests.

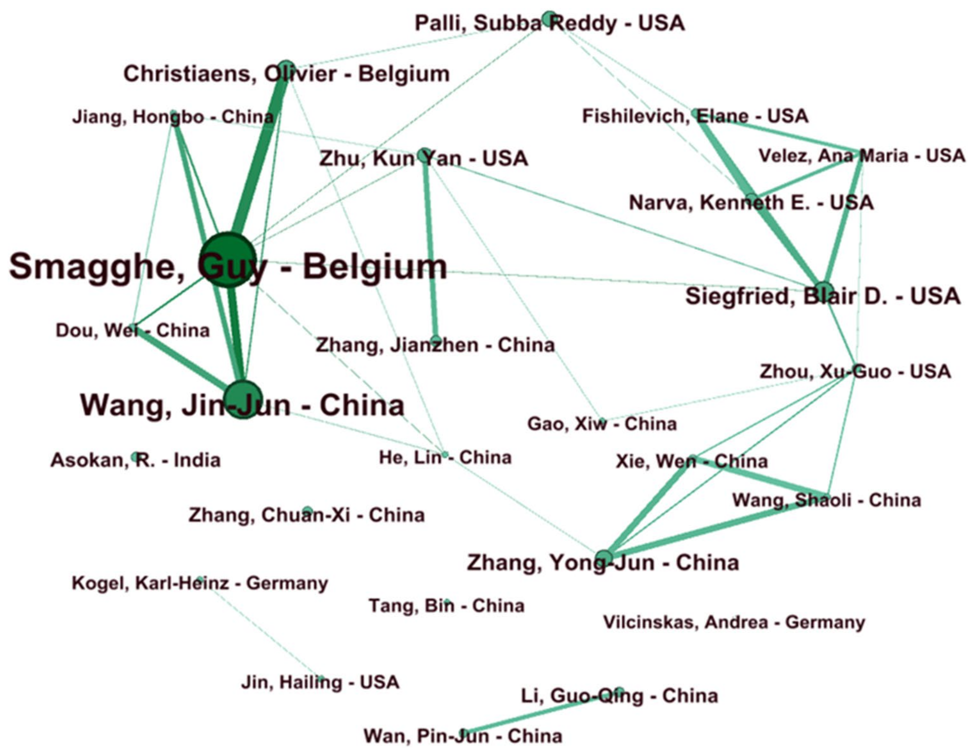

Fig. 3 Collaborative patterns between the 25 most prolific RNAi research authors. Co-authorship patterns reveal a general collaborative spirit in the field. The size of a node indicates the total number of records published by the related author, the weight of an edge represents the frequency of the collaborations between the two authors, and the collaborators listed under each node are the top three co-authors with the largest number of co-publications. Where only two collaborators are shown, there was a multi-way tie for third

Concluding remarks and perspective

In summary, RNAi is a natural mechanism found in most eukaryotic organisms. RNAi-based technology is already being exploited, and the marketed products confirm its great potential in a range of areas of crop production and protection. It can make a major contribution towards integrated pest management and sustainable agricultural strategies needed worldwide for current and future food safety and security. GM RNAi plants are being assessed and regulated using existing regulatory frameworks. However, there is an urgent need to adapt existing PPP legislation so that it incorporates appropriate science-based risk assessment procedures for topical RNAi-based applications. This is reflected in the current activities of the OECD working group on pesticides (OECD 2019).

Looking forward, although Europe is at the forefront of research on RNAi, the developments and applications may be constrained by failure of regulators and policymakers in EU member states to effectively implement current GMO regulations and by inappropriate and restrictive PPP risk assessment methods. If this happens, there is likely to be a disincentive to investment in R&D on agricultural applications of RNAi-based technology in the EU, a declining trend already attested by the reduction in patent applications. In addition, European farmers will be denied access to this technology and so lose productivity and competitiveness compared with non-EU countries, just at a time when sustainable agriculture, integrated pest management and agricultural biodiversity are in the global spotlight. This will also result in knock-on effects for consumers, affecting food availability, choice and price. Thus, policymakers have to adapt if we are to be part of the solutions.

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Compliance with ethical standards

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