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ARTICLE

Evaluation Of Biomass and Vegetative Propagation Of *Spilanthes oleracea* Jacq. (Asteraceae)

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ABSTRACT

Spilanthes oleracea Jacq. is an herbaceous plant whose scientific literature attributes among others, anti-malarial and anti-bilharzia properties. These virtues justify the placing on the market of drugs based on the plant. Our study consisted on the one hand to evaluate the biomass of the plant on a soil of dune amended and on soil of unamended dune and to test its vegetative multiplication by transplanting, cuttings and layering. The results show that the growth of the species is greater on dune soil amended with an average biomass of 106.06 g compared to 71.06 g for un-amended soil plants. The transplanting of the plants and the layering were techniques that made it possible to multiply the plants. *Spilanthes oleracea* Jacq. can be produced using this agronomic data.

1. Introduction

Spilanthes oleracea Jacq. is an annual or biennial herb known as *S. acmella* (India, Indonesia), *S. uliginosa* (Sudan, Cameroon, Mali), according to continents and authors [9,12]. It is a branched plant, 25 to 50 cm high.

This plant has been the subject of numerous studies bearing its chemical composition [2,3,4,13] and its pharmacological properties [5,6,7], which showed a real interest in this species in the management of certain pathologies such as malaria [2] and bilharziasis [6,7]. This interest is at the origin of the authorizations of its placing on the market under various names of specialties based on extracts of the plant, with like properties:

(1) analgesic in cases of sports-related injuries in France and Germany;

(2) antimalarial in Mali [2].

Because of these interesting prospects in the fight against malaria, we have undertaken to carry out trials of natural reproduction of this species.

2. Material and Methods

2.1 Framework of Study

The tests were carried out in the Niayes area of Dakar. It is an area of inter-dune depressions where the water table outcrops most often.

The climate is characterized by a long dry season and

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a short rainy season with average annual rainfall hardly exceeding 500 mm. The annual thermal average is of the order of 24 ° C.

2.2 Plant Material

(1) Seeds of *Spilanthes oleracea* Jacq.
(2) Sandy soil of unamended dune
(3) Sandy soil of dune amended
(4) Watering Cans
(5) Rakes
(6) Picks
(7) Excavators
(8) Precision balance
(9) Horse poop.

2.3 Conduct of the Test

The objective of the test is to evaluate the following parameters: the variation of biomass with soil type, the vegetative propagation of the species and the behavior of the plants at transplanting.

2.3.1 Study of the Variation of the Biomass According to the Soil

This study was conducted in January, which corresponds to a dry season and temperatures between 18 ° and 23 ° C.

For this test, 3 boards of 4 m^2 surface were prepared as follows:

(1) Planks 1 and 4: sandy soil of unamended sand dunes

(2) Planks 2 and 3: sandy soil of dunes amended with 5 kg of horse dung.

The earth of these planks was first drawn, crumbled before being watered with 20 liters of water. Shallow linear streaks were then made using a rake before sowing on the fly, at a rate of 1.06 g seeds per plank. Daily watering is done with the same amount of water.

After germination, the number of plants in each plank is decreased, so as to obtain 64 plants spaced 20 cm apart.

After 98 days, a period of maturity characterized by a flowering of the plants, a sample of 10 plants is taken and each plant is weighed using a precision scale, in order to determine its weight. The average of each sample is expressed in grams.

2.3.2 Study of Vegetative Propagation

This study deals with cuttings and layering of plants in Plank 1.

(1) Cutting trials

On a third sheet of sandy soil amended (Plank 3), prepared under the same conditions as Plank 2, 16 cuttings of the plants of Plank 1 were transplanted. These cuttings are obtained by taking from the mother plants cuttings of branches carrying knots.

The appearance of roots and buds is then followed during the test.

(2) Layout tests

At the level of the Plank 1, we buried in the ground, the stems of 16 other plants without detaching them from the mother plants. A follow-up was then made to observe the appearance of roots and buds on these stems. The separation of the mother plant is carried out when their size and rooting are sufficient. The results obtained from these tests are expressed as a percentage.

(3) Plant transplanting tests

The transplanting took place on the 73rd day of the planting of the plants of the plank 1. For that, 16 other plants of the plank 1 were transplanted on a fourth board with the sandy soil not amended (plank 4), respecting a distance of 20 cm between two plants. The percentage of surviving plants was expressed.

3. Results

The different tests concerning the evaluation of biomass and the study of vegetative regeneration of *Spilanthes oleracea* Jacq. gave the following results:

3.1 Study of the Variation of the Biomass According to the Soil

The evaluation of the biomass on an unamended sandy soil and on the same type of soil amended showed a remarkable variation in the weight of the plants obtained. In fact, an average weight of 71.06 g is obtained on the unamended soil plank against

106.06 g for the amended soil plank, a weight difference of 35 g. In addition, all the plants in the amended soil plank have a weight greater than that of the plant with the highest weight in the unamended soil plank (Table 1).

Table 1. Variation of plant biomass by soil type

Plant number	Plate n ° 1 (weight in grams)	Plate n° 2 (weight in grams)
1	71,09	100,87
2	70,12	108,03
3	73,17	100,11
4	70,43	106,38
5	70,16	106,53
6	71,06	110,91
7	72,45	98,63
8	71,23	112,16
9	68,27	105,48
10	72,76	111,47
Average	**71,06**	**106,06**

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3.2 Study of Vegetative Propagation

Compared to vegetative regeneration of the species, only layering and transplanting gave satisfactory results. In fact, the layering tests made it possible to obtain a regeneration rate of 68.75%, compared to 87.50% for transplanting (Table 2).

Table 2. Test results for transplanting and vegetative propagation of seedlings

Tests	Number of regenerated seedlings (N = 16)	Regeneration rate (%)
Transplanting	14	87,50
Cuttings	0	0
Layering	11	68,75

The cuttings trials were inconclusive.

4. Discussion

The objective of this present work was to compare the growth of *Spilanthes oleracea* Jacq. on two types of soil and to evaluate its vegetative multiplication capacity.

The comparative study of growth consisted of comparing the biomass of plants obtained after soil amendment to that of plants from unamended soil. The results obtained showed a notable difference in weight which favors plants from the amended soil. In fact, the average weight of the plants in the amended soil is 101.06 g against 71.06 g for the plants of the unamended soil. The weight difference of 35 g could be related to the soil characteristics of the Niayes area. Indeed, Ndoye & al [10] showed that Niayes soils are characterized by the salinity of surface horizons. Although they are rich in humus, they require nitrogen and phosphate amendments. The horse dung used as an amendment, made it possible to obtain a much higher biomass than that resulting from unamended soil.

Planting trials yielded an average success rate of 87.50%. This result suggests that large-scale production of the species requires the establishment of nurseries from which the feet will be harvested for transplant.

The layering tests were also successful with an average success rate of 68.75%. This result confirms the thesis of Koster [9] for whom, the plant can take root at the level of the lowest nodes and recover secondarily. The ability of the species to tease is an asset in the production of the plant.

Attempts to cut the species have been in vain. However, because of the therapeutic interest of *Spilanthes oleracea* Jacq., several works of multiplication of the species from cuttings, were carried out with very satisfactory results. This work focuses on *in vitro* micropropagation culture techniques in Murashige and Skoog (MS) medium [1,8,11].

5. Conclusion

The study showed that the biomass of *Spilanthes oleracea* Jacq. is significantly improved on dune soil amended with horse dung only on an unamended dune soil, with a difference of 35 g in favor of the seedlings of the amended soil.

Transplanting plants and layering with success rates of 87.50% and 68.7%, respectively, are techniques for multiplying seedlings. This method is therefore recommended for the production of this species.

References

[1] Ang B.H., Chan L.K. Micropropagation of Spilanthes acmella L., a bio-insecticide plant, throught proliferation of multiples shoots. J. Appl. Hort., 2003, 5(2): 65-68.

[2] Gasquet M., Delmap F., Timon D.P., Keita A., Guindo M., Koita N., Diallo D., Doumbo O. - Evaluation *in vitro* and in vivo of a traditional antimalarial, Malarial 5. Fitoterapia, 1993, LXIV(5).

[3] Gerber E. Uber die chemischen bestandteille de parakresse (*Spilanthes oleracea* Jacq.). Arch. Der Pharmazie, 1903, 236: 270-289.

[4] Ghokhale V.G., Bhide B.V. Chemical investigation of Spilanthes acmella. Journal of Indian Chemical Society, 1945, 22: 250-252.

[5] Heal R.F., Rogers E.F. A suvey of plants for the insecticidal activity. Llodia, 1950, 13: 89-162.

[6] Hostetmann K., Marston A. Plants molluscicides. Phytochemistry, 1985, 24: 639-652.

[7] Johns T., Graham K., Neil Towers G.N. Molluscicidal activity of affinin and other isobutylamids from Asteraceae. Phytochemistry, 1982, 21: 2737-2738.

[8] Joshi V., Tiwari K.L., Jadhav S.K. *In vitro* propagation of Spilanthes acmella (L) Murr. using semisolid and liquid medium. Indian Journal of Biotechnology, 2015, 14: 112-116.

[9] Koster J.T.H. Nomenclatural changes in Spilanthes and Blainvilles with remarks and a key to the species of Spilanthes in the malay archipelago. Busmea, 1950, 6: 349-354.

[10] Ndoye S., Ndiaye B., Diop C. Analyse pédologique de la région des Niayes au Sénégal. Journal des Sciences Pour l'Ingénieur, 2006, 6: 47-55.

[11] Sarita K.V., Prakash E., Ramamurthy N., Naidu C.V.. Micropropagation of Spilanthes acmella Murr. Biologia Plantarum, 2002, 45(4): 581-584.

[12] Trecisson C. Spilanthes acmella Murr., Thèse de doctorat d'état de pharmacie, Université Paul Sabatier, Toulouse, 1988, 88.

[13] Verykokidev V. E., Becker H. Flavonoide aus *Spilanthes oleracea* Jacq. Arch. Der Pharmazie, 1983, 316: 815-816.

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REVIEW

The Use of RNA Interference in Enhancing Plant Resistance against Nematodes

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ABSTRACT

Plant-parasitic nematodes caused severe yield loss in major crops all over the world. The most wild-used strategies to combat the nematodes is the chamical nematicides, but the overuse of synthetic nematicides threaten sustainable agriculture development. Other strategies, like resistance cultivars and crop rotation, have limited efficiency. Thus, the utilization of molecular biotechnology like RNA interference (RNAi) would be one of the alternative ways to enhance plant resistance against nematodes. RNAi has already used as a tool for gene functional analysis in a wide range of species, especially in the non-parasitic nematode, *Caenorhabtidis elegans*. In plant-parasitic nematodes, RNAi is induced by soaking nematodes with double strand RNA(dsRNA) solution mixed with neurostimulants, which is called *in vitro* RNAi delivery method. In another way around, *in planta* RNAi method, which is Host-mediated RNAi approach also showed a great success in conferring the resistance against root-knock nematodes. Two main advantages of RNAi-based transgenics are RNAi technology do not produce any functional foreign proteins and it target organisms in a sequence-specific way. Even though the development of RNAi-based transgenics against plant-parasitic nematodes is still in the initial phase, it offers the prospect into a novel nematode control strategy in the future.

1. Introduction

Plant Parasitic Nematodes (PPN) are one of the significant constraints for crop production worldwide. Up to now, more than 4,100 species of PPNs have been recorded [24], causing about $173 billion of damage to world agriculture every year [14]. However, this damage is likely to be underestimated because many growers are not aware of these parasites in the soil [1]. The symptoms caused by PPNs are easily confused with the symptoms of other pathogens. Sedentary endoparasites such as root-knot nematodes (*Meloidogyne spp*.) and cyst nematodes (*Globodera* and *Heterodera spp*.) are the most damaging PPNs [24]. Even though the life cycles of these nematodes are different from each other, both cyst nematodes and root-knot nematodes inject their saliva into plant cells [28]. The cyst nematodes withdraw nutrients through feeding sites called syncytia, while root-knot nematodes form giant cell to take nutrients from the host. After nematode infection, plants are slowly damaged by the interruption

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of the water transportation and nutrients from the roots to the branches. In addition, the plants also become more susceptible to other diseases [15]. Also, migratory endoparasites are highly damaging as these endoparasites interact with other pathogens to enhance crop damage and yield loss [24].

Many methods can be applied to control PPNs, such as resistant cultivars, nematicides and crop rotation. However, many crops lack nematode resistance genes, and crop rotations are inadequate to manage PPNs while nematicides have adverse environmental effects [15]. Under the circumstances, defending the plant against the PPN with novel strategies such as RNA interference (RNAi) is necessary. RNAi refers to the phenomenon of efficient and specific degradation of homologous mRNA induced by double-stranded RNA (dsRNA). Fire et al. [18] first described the mechanism of RNAi using the nematode *Caenorhabditis elegans*, and a similar mechanism has been revealed in different species and kingdoms. If the sequence of the genes which are essential for PPN infection, feeding or reproduction is known, RNAi could be used as a powerful tool to enhance the resistance in plants against nematodes by silencing those pathogenic genes.

The objective of this paper is to review the research published on the use of RNAi in enhancing plant resistance against Plant-Parasitic Nematodes. The first part of this review introduces the mechanisms of RNAi. The second section presents two ways of inducing RNAi, *in vitro* RNAi and *in planta* RNAi. In *in vitro* RNAi method, the dsRNA is delivered into worms directly, while the dsRNA is transferred to the plant in *in planta* RNAi method. The third section discusses the problems of enhancing the resistance conferred by *in planta* RNAi and their solution. In this section, the off-target effect, non-specific target effect and the dilemma of RNAi phenotype detection are discussed. This review aims to identify the prospect of RNAi being utilised as a powerful tool to develop transgenic plants against PPNs. The research papers included in this review are the result of a search in the online literature database at scopus.com using the keywords 'RNAi', 'nematode', 'resistance', and the reference is limited to 'not older than 2008'.

1.1 Mechanism of RNAi

RNAi refers to the phenomenon of efficient and specific degradation of homologous mRNA induced by double-stranded RNA (dsRNA). Fire et al. [18] firstly described this post-transcriptional silencing of endogenous genes in *Caenorhabditis elegans*. RNAi also is important in the immune response to foreign genetic material [49]. When an organism recognises dsRNA, a cascade of sequence-specific silencing processes will initiate leading to the dsRNA and its homologous mRNA degraded [47]. The RNAi pathway can be considered a two-step process. Firstly the dsRNA would be cleaved into 21–25 nucleotide long small interfering RNAs (siRNAs) in the cell cytoplasm by the RNaseIII enzyme, Dicer. The separation of double-strand siRNA in the second step allows the siRNA to attach to the RNA-induced silencing complex (RISC). These RISC then target sequence-complementary mRNA molecules and cleave them, inducing RNA silencing [41]. Similar mechanisms of action appear to function in PPNs as well [25], so RNAi can be used to develop RNAi against PPNs.

1.2 *In vitro* RNAi Delivery Method

Three different methods have been successfully applied to introduce dsRNA into the free-living nematodes. The three ways are microinjection [33], feeding the nematodes with bacteria which contain dsRNA of the target gene [52] and soaking of nematodes in dsRNA solution [50]. However, the sedentary PPNs are too small for microinjection. They are obligatory parasitic so that feeding with bacteria cannot be used to introduce dsRNA into these worms. The sedentary PPNs only can survive outside of their host plant when they are at the stage of eggs, second stage juveniles (J2s) and adult males. Eggs and J2s stages have been used for transmitting RNAi by soaking.

The most commonly used RNAi delivery way for J2s of PPNs is neurostimulant-mediated oral ingestion. Chemicals such as octopamine, serotonin and resorcinol are used in the solution to promote dsRNA ingestion. These chemicals successfully induce dsRNA ingestion in several species including *Heterodera schachtii* [53], *Meloidogyne javanica* [19] and *Heterodera glycines* [6]. Fluorescein isothiocyanate (FITC) in the solution or fluorescently-labelled dsRNAs have been used as a visual marker to show the uptake of dsRNA [12]. Although pharyngeal stimulant can induce ds RNA ingestion efficiently, some side effects have also been reported during incubation. Adam, Phillips, Jones, and Blok [2] found that the death rate of *Meloidogyne javanica* J2s would increase significantly if they were soaked in 0·5% or 1% resorcinol solution overnight.

Soaking eggs in dsRNA or siRNA solution is another way to induce RNAi in PPNs [10]. Fanelli, Di Vito, Jones, and De Giorgi [16] constructed the dsRNA targeting a chitin synthase which is responsible for chitin layer synthesis in the eggshell. After dsRNA construction, the eggs of *Meloidogyne artiellia* were soaked in dsRNA solution at 20°C for between 24 and 72 h. This result of a reduction in chitin transcript illustrated that the dsRNA could go through the eggshells with chitin. More recently, Dalzell et al. [10] performed a siRNA-mediated RNA silencing in

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Meloidogyne incognita. They silenced the nuclear RNase III enzyme Drosha, a key effector of miRNA production by soaking *Meloidogyne incognita* eggs in siRNA solutions. The irregular growth of eggs and embryonic lethality suggested that eggshells of *Meloidogyne incognita* are permeable to siRNA. When it comes to other species of PPN, whether the eggs are similarly susceptible to dsRNA or siRNA still not clear, because the permeability of the PPNs eggshells would change before hatching, according to [23].

As migratory parasites are not obligate biotrophic, they can be targeted by RNAi at other stages of the life-cycle. Haegeman, Vanholme, and Gheysen [21] incubated different stages of *Radopholus similis* with dsRNA with a sequence derived from the xylanase gene. Octopamine was used in this research to stimulate dsRNA ingestion. A reduction in the transcript of the xylanase gene and reduction of infection rates were observed at the end. Cheng, Dai, Xie, and Xiao [9] found that 24h of incubation was necessary for ingestion of FITC soaked *Bursaphelenchus xylophilus*, but a pharyngeal stimulant was not required in this research. L2-L3 larvae of migratory parasites also can be induced RNAi by soaking, but intestinal microinjection of dsRNA into adult female is efficient as it leads to a 46% lethality rate in the F1 generation while soaking in dsRNA solution only induced 25% lethality [36].

Generally speaking, the *in vitro* RNAi delivery method could be considered efficient to manipulate PPNs pathogenicity. However, the silencing achieved due to soaking in dsRNA solutions is often lacking stability. Rosso, Dubrana, and Cimbolini [40] accessed the duration of the silencing effect. They silenced the gene which encodes polygalacturonase (*Mi-pg-1*) in *Meloidogyne incognita* by *in vitro* RNAi technology. The suppression of *Mi-pg-1* transcription was optimal at 44 h after soaking, but this silencing effect cannot be detected for both at 68 h after soaking. Bakhetia, Urwin, and Atkinson [4] observed that the reduction in the transcript levels of β-1, 4-endoglucanase In *Heterodera glycines* after an incubation of 16 h with the corresponding dsRNA. However, after 15 days of dsRNA treatment, they reported that the transcript levels of β-1, 4-endoglucanase returned to normal. In contrast to *in vitro* RNAi technology, host delivery strategy would prevent gene suppression reversal because the transgenic plants provide continuous availability of the dsRNA to the nematode [54]. In addition, *in vitro* method only can be used in the lab for proof of RNAi principle and test constructs before transferring dsRNA into the plant. Therefore, the *in planta* RNAi method is more suitable for enhancing plants resistant to PPNs.

1.3 *In planta* RNAi Method

Host-mediated RNAi is a novel approach to confer resistance against PPNs in plants. In this technology, plants are genetically modified to express dsRNA molecules with sequence derived from the target gene. RNAi constructs are made by cloning the sense and antisense cDNA sequences of the target gene. In order to initiate the expression of the dsRNA, a tissue-specific or constitutive promoter is used during vector construction. After transcription, a loop or hairpin structure is formed by the self-complementary sense and antisense strand. Then dsRNA with a loop or hairpin structure is cleaved by the plant enzyme called Dicer into siRNA, and the siRNA is ingested by the nematodes subsequently. This dsRNA can also directly be ingested by nematodes [13].

Using the conventional methods to develop dsRNA constructs for host delivered RNAi is time- consuming, so the Gateway cloning system, which is much easier and effective, has been employed for the development of RNAi constructs [35]. The Gateway cloning system uses two different enzyme mixtures, each of which performs a different type of recombination reaction [39]. The BP clonase enzyme mix recombines the *attB* sites and the *attP* sites, generating *attL* and *attR* sites. On the contrary, the LR clonase enzyme mixes catalysis the reverse reaction (Figure 1).

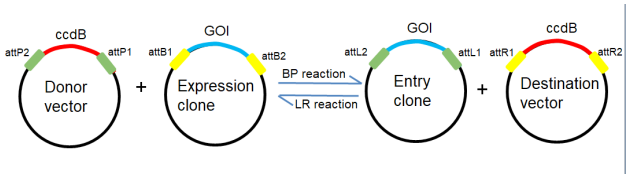

Figure 1. The mechanism of BP reaction and LR reaction

Initially, the gene of interest is amplified by PCR using long-tailed primers. The 3' part of the primers is specific for the DNA of interest, and the 5' tail of the primers containing appropriate *attB* sites. This PCR product is transferred into a Donor vector. The gene of interest in a donor vector is available for transfer into destination vectors by a Gateway LR cloning reaction. The vectors containing the gene of interest are introduced into plants through *Agrobacterium tumefaciens* mediated transformation. With the developing of experimental techniques, the Gateway cloning system becomes compatible to include RNA interference. Rual et al. [42] reported that completed *Caenorhabditis elegans* genome sequence allows application of high-throughput (HT) approaches for phenotypic analyses using RNAi, but HT-RNAi resources are limited by lack of flexibility. In order to overcome this disadvantage, they created the complete set of ORFs in genome

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(ORFeome) resources of the nematode *Caenorhabditis elegans* through the Gateway cloning system. Then they showed these ORFeome resources could be used to create alternative HT-RNAi resources with enhanced flexibility. This flexibility suggests that additional HT-RNAi libraries can be generated to perform gene knockdowns under various conditions, which increase the possibilities for phenome mapping of *Caenorhabditis elegans*.

The genes which are targeted in the *in planta* RNAi method could be classified into three categories: effector genes, housekeeping genes and genes implicated in nematode development or reproduction. The first demonstration of plant-mediated RNAi was accomplished by Yadav, Veluthambi, and Subramaniam [55]. The dsRNA they developed targets two housekeeping genes: an integrase and a splicing factor gene of *Meloidogyne incognita*. The results show a decline of more than 90% of PPN infection in transgenic tobacco as the expression of the targeted gene was repressed. Klink et al. [26] reported that four embryonic lethal genes encoding ribosomal protein 3a and 4, spliceosomal SR protein and synaptobrevin could be used for PPNs control. They silenced these four genes by using *in planta* RNAi method resulting in a restriction of the female development of *Heterodera glycines* in their host. Another example of silencing housekeeping genes is given by J. Li, Todd, and Trick [30]. The *Heterodera glycines Y25* gene encoding a β-subunit of the coatomer (COPI) complex, was used as a template to synthesise dsRNA. The transgenic soybean expressing RNAi constructs effectively suppressed *Heterodera glycines* infection and development. When splicing factor and integrase genes in *Meloidogyne incognita* were used for RNA interference in *Arabidopsis thaliana*, a dramatic decrease in the number of galls, females and egg masses was observed by Kumar et al. [27]. Based on the examples mentioned above, housekeeping genes could be considered as a good choice for RNAi targeting. Before the development of dsRNA targeting housekeeping genes, a risk assessment is always needed because most of these genes are highly conserved across different species and the dsRNA constructs could have non-specific effects on host plants or other beneficial species [46].

Some literature indicates that nematode effector genes can also confer resistance against PPNs when targeted by host-mediated RNAi. Dinh, Brown, and Elling [11] constructed dsRNA molecules which are complementary to the effector gene, *Mc16D10L*, and these dsRNA molecules were transferred into three potato cultivars. The number of *Meloidogyne chitwoodi* eggs and egg masses in transgenic potato was reduced by up to 68% compared to empty vector control plants. Recently, Niu et al. [34] found that the effector gene *MiMSP40* of *Meloidogyne incognita* could downregulate plant immunity to help parasitism. Overexpression of *MiMSP40* makes Arabidopsis more susceptible, but *in planta* RNAi targeting against this gene caused a reduction in parasitism in Arabidopsis. Similarly, Zhuo et al. [56] reported that overexpression of effector gene *MeTCTP* in *Meloidogyne enterolobii* promotes infection, but host generated RNAi aiming at *MeTCTP* resulted in less parasitism.

The genes involved in nematode development and reproduction are also used to inhibiting the growth and reproductive nematodes. In the case of cyst nematodes, silencing the major sperm protein (MSP) by RNAi leads to a 68% reduction in the number of eggs [48]. The impaired fecundity of *Heterodera glycines* carried over to the next generation of nematodes regarding the ability to reproduce declined in their progenies. Two genes from *Meloidogyne incognita*, a dual oxidase gene and signal peptidase gene, were revealed to be of relevance to the development and reproduction of root-knot nematode [8]. Silencing of these two genes led to more than 50% reduction in nematode numbers in the roots and diapause of the egg-producing female. Antonino et al. [3] found that serine protease in *Meloidogyne incognita* is relevant to root-knot nematode reproduction. In their study, they knocked-down serine protease gene *Mi-ser-1* by RNAi. Nematodes that infected modified tobacco produced fewer eggs, and the progeny of nematodes matured in these plants has a declined success in egg hatching. This result indicated that serine protease is involved in different processes during nematode development, like reproduction and embryogenesis.

2. The Problems of Enhancing the Resistance by RNAi and Their Solution

2.1 Off-target Effects and Their Solution

RNAi has emerged as a potent and successful technology for crop protection in recent years, but there remain certain limitations that need to be addressed before adopting this technology in the field. One major concern regarding the employment of RNAi-based nematode management strategy is the potential for off-target effects. Non-target genes with similar sequence to the target gene are likely to be silenced by mRNA degradation or translational repression as the basis of RNAi is sequence identity [32]. J. F. Rual, Klitgord, and Achaz [43] reported that when an mRNA shares more than 95% identity over 40 nucleotides with the dsRNA in nematode *Caenorhabditis elegans*, the off-target effects will happen.

Due to the issues of off-target effects, more emphasis should be put on avoiding the off-target effects when

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RNAi is employed as a novel method in PPNs management. Some strategies could be used to prevent off-target effects. Recently Banerjee, Gill, Jain, and Sirohi [7] reported that using a database for *in silico* homology searches to identify off-target sequences is efficient in avoiding off-target effects. Thorat [51] used nematode-induced promoter and root-specific promoter of *Arabidopsis thaliana* to transform tomato with GUS gene. Then they observed a strong expression of the GUS gene in the nematode infection sites. Therefore the root-specific promoters and nematode inducible promoters could be used to prevent RNAi in the non-target parts of the plant. In addition, sequences of the 5'-untranslated region (5'-UTR) and the 3'-untranslated region (3'-UTR) also could be used as RNAi targets to reduce off-target effects because they are less conserved than coding regions [20].

2.2 The confirmation of RNAi effectiveness

Even though several studies reported the success of RNAi phenotype detection, it is still difficult to determine if RNAi results in the phenotype or not [45]. Most commonly, detection of the RNAi phenotype is based on the number of nematodes which establish an infection successfully, the fecundity of females or the ratio of males to females in cyst nematodes. However, under some unfavourable conditions such as insufficient nutrition, the process of establishing a feeding site is slower (Lilley, Atkinson, & Urwin, 2005). RNAi targeting the genes which express in pharyngeal gland cells of *Heterodera glycines* would lead to more males [5]. Because the number of phenotypes which can be observed is easily affected by other factors, some phenotypes changes would not be appeared or overserved [31].

RT-PCR has been used to overcome the difficulties of RNAi phenotyping. This method would reveal a decline in transcript level of the target gene. Patel et al. [37] reported that the presence of dsRNA could be detected by RT-PCR of the intron or spacer region of the hairpin construct. Other studies have used RT-PCR to illustrate the decline of control gene transcripts in dsRNA expressing plants [22]. However, this method may not be the suitable for confirmation of RNAi effectiveness, because RT-PCR has not always detected the down-regulation of the target gene despite observing an RNAi phenotype in the nematodes [36].

2.3 Conclusion and Future Prospects

RNAi was used as a successful technology for enhancing crops resistance to different pathogens in recent years, but there remain some limitations that impede the commercialisation of RNAi-modified plants. Usually, RNAi-modified crops are only resistant to one nematode species because of the high specificity level of dsRNA or siRNA. However, plants are infected with multiple species of PPN in soils [17]. In the meantime, the public suspicions on biosafety aspects of RNAi-based GM plants have widespread even though the risk assessment of GM crops is obligating before releasing them to market. Many people think the risk assessment of commercialised GM plants is not suitable for RNAi-based GM plants, because the current risk assessment does not include the identification of potential off-target effects. These problems remind us that we still have a way to go in the future due to the acceptance of that technology by the general public

Hopefully, the rapid growth of RNAi research will help to increase public trust in this technology. Development genome databases of related species and the stacking of dsRNA sequences to target multiple genes would contribute to effective nematode control [28]. Use of nematode-induced promoters and plant tissue-specific promoters would limit dsRNA gene expression to specific plant tissue in response to the particular nematode [44]. More efficient algorithmic programs facilitate more reliable risk assessment of RNAi-based GM plants [38].

In conclusion, some problems and limitation have to be taken into consideration in order to ensure RNAi successful and safe application. Nevertheless, RNAi surely will become a powerful strategy to control multiple pest and pathogens as we are moving toward the goal of sustainable development.

Reference

[1] Abd-Elgawad, M. M., & Askary, T. H.. 1 Impact of Phytonematodes on Agriculture Economy. In Biocontrol Agents of Phytonematodes, CABI Wallingford, UK, 2015: 3-49.

[2] Adam, M. A. M., Phillips, M. S., Jones, J. T., & Blok, V. C.. Characterisation of the cellulose-binding protein Mj-cbp-1 of the root knot nematode, Meloidogyne javanica. Physiological and Molecular Plant Pathology, 2008, 72(1-3): 21-28. DOI: 10.1016/j.pmpp.2008.05.002

[3] Antonino, Coelho, R. R., Lourenço, I. T., da Rocha Fragoso, R., Viana, A. A. B., de Macedo, L. L. P., de Almeida-Engler, J.. Knocking-down Meloidogyne incognita proteases by plant-delivered dsRNA has negative pleiotropic effect on nematode vigor. PLoS One, 2013, 8(12): e85364.

[4] Bakhetia, Urwin, & Atkinson. qPCR analysis and RNAi define pharyngeal gland cell-expressed genes of Heterodera glycines required for initial interactions with the host. Molecular Plant-Microbe Interac-

  DOI: https://doi.org/10.30564/jrb.v2i1.1759

tions, 2007, 20(3): 306-312.

[5] Bakhetia, M., Urwin, P., & Atkinson, H. J.. qPCR analysis and RNAi define pharyngeal gland cell-expressed genes of Heterodera glycines required for initial interactions with the host. Molecular Plant-Microbe Interactions, 2007, 20(3): 306-312.

[6] Bakhetia, M., Urwin, P. E., & Atkinson, H. J.. Characterisation by RNAi of pioneer genes expressed in the dorsal pharyngeal gland cell of Heterodera glycines and the effects of combinatorial RNAi. Int J Parasitol, 2008, 38(13): 1589-1597.
DOI: 10.1016/j.ijpara.2008.05.003

[7] Banerjee, S., Gill, S. S., Jain, P. K., & Sirohi, A.. Isolation, cloning, and characterization of a cuticle collagen gene, Mi-col-5, in Meloidogyne incognita. 3 Biotech, 2017, 7(1): 64.
DOI: 10.1007/s13205-017-0665-1

[8] Charlton, W. L., Harel, H. Y., Bakhetia, M., Hibbard, J. K., Atkinson, H. J., & McPherson, M. J.. Additive effects of plant expressed double-stranded RNAs on root-knot nematode development. Int J Parasitol, 2010, 40(7): 855-864.
DOI: 10.1016/j.ijpara.2010.01.003

[9] Cheng, X.-Y., Dai, S.-M., Xie, B.-Y., & Xiao, L.. Influence of cellulase gene knockdown by dsRNA interference on the development and reproduction of the pine wood nematode, Bursaphelenchus xylophilus. Nematology, 2010, 12(2): 225-233.
DOI: 10.1163/138855409x12469541205044

[10] Dalzell, J. J., McVeigh, P., Warnock, N. D., Mitreva, M., Bird, D. M., Abad, P., . . . Maule, A. G.. RNAi effector diversity in nematodes. PLoS Negl Trop Dis, 2011, 5(6): e1176.
DOI: 10.1371/journal.pntd.0001176

[11] Dinh, P. T., Brown, C. R., & Elling, A. A.. RNA interference of effector gene Mc16D10L confers resistance against Meloidogyne chitwoodi in Arabidopsis and potato. Phytopathology, 2014, 104(10): 1098-1106.

[12] Dutta, T. K., Papolu, P. K., Banakar, P., Choudhary, D., Sirohi, A., & Rao, U.. Tomato transgenic plants expressing hairpin construct of a nematode protease gene conferred enhanced resistance to root-knot nematodes. Front Microbiol, 2015, 6(260). DOI: 10.3389/fmicb.2015.00260

[13] Dutta, T. K., Papolu, P. K., Banakar, P., Choudhary, D., Sirohi, A., & Rao, U.. Tomato transgenic plants expressing hairpin construct of a nematode protease gene conferred enhanced resistance to root-knot nematodes. Frontiers in Microbiology, 2015, 6(260).
DOI: 10.3389/fmicb.2015.00260

[14] Elling, A. A.. Major emerging problems with minor Meloidogyne species. Phytopathology, 2013, 103(11): 1092-1102.

[15] Fairbairn, D. J., Cavallaro, A. S., Bernard, M., Mahalinga-Iyer, J., Graham, M. W., & Botella, J. R.. Host-delivered RNAi: an effective strategy to silence genes in plant parasitic nematodes. Planta, 2007, 226(6): 1525-1533.

[16] Fanelli, E., Di Vito, M., Jones, J. T., & De Giorgi, C.. Analysis of chitin synthase function in a plant parasitic nematode, Meloidogyne artiellia, using RNAi. Gene, 2005, 349: 87-95.
DOI: 10.1016/j.gene.2004.11.045

[17] Ferguson, C. M., Barratt, B. I., Bell, N., Goldson, S. L., Hardwick, S., Jackson, M., Rennie, G.. Quantifying the economic cost of invertebrate pests to New Zealand's pastoral industry. New Zealand Journal of Agricultural Research, 2018: 1-61.

[18] Fire, A., Xu, S., Montgomery, M. K., Kostas, S. A., Driver, S. E., & Mello, C. C.. Potent and specific genetic interference by double-stranded RNA in Caenorhabditis elegans. nature, 1998, 391(6669): 806.

[19] Gleason, C. A., Liu, Q. L., & Williamson, V. M.. Silencing a candidate nematode effector gene corresponding to the tomato resistance gene Mi-1 leads to acquisition of virulence. Molecular Plant-Microbe Interactions, 2008, 21(5): 576-585.

[20] Gu, S., Jin, L., Zhang, F., Sarnow, P., & Kay, M. A.. Biological basis for restriction of microRNA targets to the 3′ untranslated region in mammalian mRNAs. Nature structural & molecular biology, 2009, 16(2), 144.

[21] Haegeman, A., Vanholme, B., & Gheysen, G.. Characterization of a putative endoxylanase in the migratory plant-parasitic nematode Radopholus similis. Mol Plant Pathol, 2009, 10(3): 389-401.
DOI: 10.1111/j.1364-3703.2009.00539.x

[22] Ibrahim, H. M., Alkharouf, N. W., Meyer, S. L., Aly, M. A., Gamal El-Din Ael, K., Hussein, E. H., & Matthews, B. F.. Post-transcriptional gene silencing of root-knot nematode in transformed soybean roots. Exp Parasitol, 2011, 127(1): 90-99.
DOI: 10.1016/j.exppara.2010.06.037

[23] Jones, Tylka, G., Perry, R., & Wright, D.. The Physiology and biochemistry of free-living and plant-parasitic nematodes. In: CABI Publishing London,1998.

[24] Jones, J. T., Haegeman, A., Danchin, E. G., Gaur, H. S., Helder, J., Jones, M. G., Wesemael, W. M.. Top 10 plant-parasitic nematodes in molecular plant pathology. Molecular Plant Pathology, 2013, 14(9): 946-961.

[25] Jones, L. M., Giorgi, C. D., & Urwin, P. E.. C.

elegans as a Resource for Studies on Plant Parasitic Nematodes. In J. Jones, G. Gheysen, & C. Fenoll (Eds.), Genomics and Molecular Genetics of Plant-Nematode Interactions. Dordrecht: Springer Netherlands, 2011: 175-220.

[26] Klink, V. P., Kim, K. H., Martins, V., Macdonald, M. H., Beard, H. S., Alkharouf, N. W., Matthews, B. F.. A correlation between host-mediated expression of parasite genes as tandem inverted repeats and abrogation of development of female Heterodera glycines cyst formation during infection of Glycine max. Planta, 2009, 230(1): 53-71.
DOI: 10.1007/s00425-009-0926-2

[27] Kumar, A., Kakrana, A., Sirohi, A., Subramaniam, K., Srinivasan, R., Abdin, M. Z., & Jain, P. K.. Host-delivered RNAi-mediated root-knot nematode resistance in Arabidopsis by targeting splicing factor and integrase genes. Journal of General Plant Pathology, 2017, 83(2): 91-97.
DOI: 10.1007/s10327-017-0701-3

[28] Li, J., Todd, T. C., Lee, J., & Trick, H. N.. Biotechnological application of functional genomics towards plant-parasitic nematode control. Plant biotechnology journal, 2011, 9(9): 936-944.

[29] Li, J., Todd, T. C., Oakley, T. R., Lee, J., & Trick, H. N.. Host-derived suppression of nematode reproductive and fitness genes decreases fecundity of Heterodera glycines Ichinohe. Planta, 2010, 232(3): 775-785.
DOI: 10.1007/s00425-010-1209-7

[30] Li, J., Todd, T. C., & Trick, H. N.. Rapid in planta evaluation of root expressed transgenes in chimeric soybean plants. Plant Cell Rep, 2010, 29(2): 113-123.
DOI: 10.1007/s00299-009-0803-2

[31] Lilley, C. J., Bakhetia, M., Charlton, W. L., & Urwin, P. E.. Recent progress in the development of RNA interference for plant parasitic nematodes. Mol Plant Pathol, 2007, 8(5): 701-711.
DOI: 10.1111/j.1364-3703.2007.00422.x

[32] Meister, G., & Tuschl, T.. Mechanisms of gene silencing by double-stranded RNA. nature, 2004, 431(7006): 343.

[33] Mello, C. C., & Conte Jr, D.. Revealing the world of RNA interference. nature, 2004, 431(7006): 338.

[34] Niu, J., Liu, P., Liu, Q., Chen, C., Guo, Q., Yin, J., Jian, H.. Msp40 effector of root-knot nematode manipulates plant immunity to facilitate parasitism. Sci Rep, 2016, 6: 19443.
DOI: 10.1038/srep19443

[35] Papolu, P. K., Gantasala, N. P., Kamaraju, D., Banakar, P., Sreevathsa, R., & Rao, U.. Utility of host delivered RNAi of two FMRF amide like peptides, flp-14 and flp-18, for the management of root knot nematode, Meloidogyne incognita. PLoS One, 2013, 8(11): e80603.

[36] Park, J.-E., Lee, K. Y., Lee, S.-J., Oh, W.-S., Jeong, P.-Y., Woo, T., . . . Koo, H.-S.. The efficiency of RNA interference in Bursaphelenchus xylophilus. Molecules & Cells (Springer Science & Business Media BV), 2008, 26(1).

[37] Patel, N., Hamamouch, N., Li, C., Hewezi, T., Hussey, R. S., Baum, T. J., . . . Davis, E. L.. A nematode effector protein similar to annexins in host plants. J Exp Bot, 2010, 61(1): 235-248.
DOI: 10.1093/jxb/erp293

[38] Ramon, M., Devos, Y., Lanzoni, A., Liu, Y., Gomes, A., Gennaro, A., & Waigmann, E.. RNAi-based GM plants: food for thought for risk assessors. Plant biotechnology journal, 2014, 12(9): 1271-1273.

[39] Reboul, J., Vaglio, P., Tzellas, N., Thierry-Mieg, N., Moore, T., Jackson, C., Thierry-Mieg, J.. Open-reading-frame sequence tags (OSTs) support the existence of at least 17,300 genes in C. elegans. Nature genetics, 2001, 27(3): 332.

[40] Rosso, Dubrana, & Cimbolini.. Application of RNA interference to root-knot nematode genes encoding esophageal gland proteins. Molecular Plant-Microbe Interactions, 2005, 18(7): 615-620.

[41] Rosso, M. N., Jones, J. T., & Abad, P.. RNAi and functional genomics in plant parasitic nematodes. Annu Rev Phytopathol, 2009, 47: 207-232.
DOI: 10.1146/annurev.phyto.112408.132605

[42] Rual, Ceron, J., Koreth, J., Hao, T., Nicot, A.-S., Hirozane-Kishikawa, T., . . . van den Heuvel, S.. Toward improving Caenorhabditis elegans phenome mapping with an ORFeome-based RNAi library. Genome research, 2004, 14(10b): 2162-2168.

[43] Rual, J. F., Klitgord, N., & Achaz, G.. Novel insights into RNAi off-target effects using C. elegans paralogs. BMC Genomics, 2007, 8(106).
DOI: 10.1186/1471-2164-8-106

[44] Siddique, S., Wieczorek, K., Szakasits, D., Kreil, D. P., & Bohlmann, H.. The promoter of a plant defensin gene directs specific expression in nematode-induced syncytia in Arabidopsis roots. Plant Physiology and Biochemistry, 2011, 49(10): 1100-1107.

[45] Sindhu, A. S., Maier, T. R., Mitchum, M. G., Hussey, R. S., Davis, E. L., & Baum, T. J.. Effective and specific in planta RNAi in cyst nematodes: expression interference of four parasitism genes reduces parasitic success. J Exp Bot, 2009, 60(1): 315-324.
DOI: 10.1093/jxb/ern289

[46] Singer, G. A., Lloyd, A. T., Huminiecki, L. B., &

  DOI: https://doi.org/10.30564/jrb.v2i1.1759

Wolfe, K. H.. Clusters of co-expressed genes in mammalian genomes are conserved by natural selection. Molecular biology and evolution, 2004, 22(3): 767-775.

[47] Sontheimer, E. J.. Assembly and function of RNA silencing complexes. Nat Rev Mol Cell Biol, 2005, 6(2): 127-138.
DOI: 10.1038/nrm1568

[48] Steeves, R. M., Todd, T. C., Essig, J. S., & Trick, H. N.. Transgenic soybeans expressing siRNAs specific to a major sperm protein gene suppress Heterodera glycines reproduction. Functional Plant Biology, 2006, 33(11).
DOI: 10.1071/fp06130

[49] Stram, Y., & Kuzntzova, L.. Inhibition of viruses by RNA interference. Virus Genes, 2006, 32(3): 299-306.
DOI: 10.1007/s11262-005-6914-0

[50] Tabara, H., Grishok, A., & Mello, C. C.. RNAi in C. elegans: soaking in the genome sequence. Science, 1998, 282(5388): 430-431.

[51] Thorat, Y. E.. Develop Root-Knot Nematode, Meloidogyne incognita Specific Gene Expression System in Tomato, Solanum lycopersicum L. Division of Nematology Icar-Indian Agricultural Research Institute New Delhi, 2017.

[52] Timmons, L., Court, D. L., & Fire, A.. Ingestion of bacterially expressed dsRNAs can produce specific and potent genetic interference in Caenorhabditis elegans. Gene, 2001, 263(1-2): 103-112.

[53] Vanholme, B., W, V. A. N. T., Vanhouteghem, K., J, D. E. M., Cannoot, B., & Gheysen, G.. Molecular characterization and functional importance of pectate lyase secreted by the cyst nematode Heterodera schachtii. Mol Plant Pathol, 2007, 8(3): 267-278.
DOI: 10.1111/j.1364-3703.2007.00392.x

[54] Wani, S. H., Sanghera, G. S., & Singh, N. B.. Biotechnology and plant disease control-role of RNA interference. American Journal of Plant Sciences, 2010, 1(02): 55.

[55] Yadav, B. C., Veluthambi, K., & Subramaniam, K.. Host-generated double stranded RNA induces RNAi in plant-parasitic nematodes and protects the host from infection. Mol Biochem Parasitol, 2006, 148(2): 219-222.
DOI: 10.1016/j.molbiopara.2006.03.013

[56] Zhuo, K., Chen, J., Lin, B., Wang, J., Sun, F., Hu, L., & Liao, J.. A novel Meloidogyne enterolobii effector MeTCTP promotes parasitism by suppressing programmed cell death in host plants. Mol Plant Pathol, 2017, 18(1): 45-54.
DOI: 10.1111/mpp.12374

 DOI: https://doi.org/10.30564/jrb.v2i1.1759

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REVIEW

Covid-19, Challenges and Recommendations in Agriculture

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ABSTRACT

On 11 March 2020, the World Health Organization declared Covid-19 is a pandemic disease that is spreading at different speeds in different countries of the world. Given these issues, the global economy is experiencing a different and new experience that is currently taking place in different countries. We are seeing a decrease in production, logistical problems, as well as a change in production patterns, demand and consumption. The agricultural sector has not been immune to the economic damage of the outbreak and has suffered serious damage. If the necessary measures are not taken for sustainable production in agriculture and maintaining the supply and demand cycle, health and food security will face a crisis. Given that there is always a zero point again about the prevalence and infection, social quarantine and health care are still essential. To manage the problems caused by the Corona crisis, accurate and appropriate programs, mechanisms, and evaluations with different strategies are needed, and appropriate sustainable models should be considered for spatial and temporal requirements.

1. Introduction

Coronavirus is the common name for Coronaviridae and Orthocoronavirinae, also called Coronavirinae [1]. Their structure also has a typical RNA genome. Of the 40 different species of the coronavirus family, seven have been found to have been transmitted to humans, leading to diseases such as the common cold family [2]. Sometimes some coronaviruses attack the respiratory tract, and sometimes their symptoms go away and their stomachs appear. These types cause disease in human populations and have mild to severe symptoms. The new type is Covid-19, which causes fever problems, dry coughs and sometimes respiratory problems such as shortness of breath, shortness of breath and sore throat and runny nose [3]. The first cases of the disease have been observed in Wuhan, China, which is now widespread in most parts of the world, and has spread from epidemic to global pandemic. The coronavirus pandemic has now killed more than 200,000 people. At least 177 countries have reported cases of Covid-19 [4]. It has severely affected the global economy in recent months. In addition to the sharp fall in global stock markets and the sharp and unpredictable instability of oil prices, it is also experiencing a record low [5].

There is currently no antiviral or vaccine treatment for coronavirus infections. The production of safe and stable vaccines is a major challenge, and the research and testing period is very long. Although the prevalence of a clinical threat is global, our knowledge of this new virus is very limited [6]. Therefore, the only way to deal with this disease is to reduce traffic and travel restrictions, and

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finally to cut off the transmission chain, which is now being implemented in most countries. Banning traffic and shutting down businesses may be a good way to maintain public health, but it will also have adverse consequences. The risk of Covid-19 is such that it can be considered the beginning of a recession in the global economy. Among the global effects of the Corona outbreak are rising unemployment, a severe shock to the economy and damage to foreign trade. The sharp decline in trade, declining consumption and rising unemployment have been the consequences of a lash to some industries. The decline in manufacturing activity in the world is obvious. Injured jobs from the lowest to the highest damage are respectively, education, agriculture, construction, art, factories, and, ultimately, retail and wholesale trade [7]. Given the globalization of the virus, there are concerns about the future at all levels, and it is necessary to consider the consequences on different scales in the form of different scenarios so that, God willing, this serious crisis can be managed and overcome. Agriculture and food security may be harmed, and there is a need to minimize the prevalence and pollution in agricultural communities and farms with guidelines and recommendations.

2. Materials and methods

Numbers of reports of news agencies, national and international sites and some articles have been used to write this article [1-9].

3. Results and Discussion

The food supply chain is a complex network, and agriculture is one of its most fundamental components in countries. One of the main services of the agricultural sector is food production, providing raw materials for other sectors, employment, income generation, and expanding non-oil exports [8]. The agricultural sector is one of the most important and influential sectors in the country's food security. Corona crisis, its effects on these categories should be identified and studied and practical solutions should be provided for relevant organizations and officials.

The food supply chain includes various phases of production, processing and processing of goods (factories) and, transportation (logistics), storage (and warehousing), retail and goods services.

Although there was no or less supply shock today due to the availability of food, due to its upward role, the vulnerabilities need to be identified and reduced. In each case, there is a need for guidelines to reduce the risk of disease. In the production section, we are faced with the following groups: (1) farmers (owners and tenants) and workers (indigenous, seasonal and permanent), (2) agricultural experts, (3) tools, equipment and mechanization, (4) agricultural inputs including fuel, seeds, seedlings, fertilizers, pesticides, etc, basic resources including soil, water, plants and livestock...

There are different things in the supply sector, but what is important is to shorten the production and supply route, and to deliver it to all consumers in a healthy way.

Hypotheses, some suggestions and recommendations include the following. In order to reduce the speed or stop the spread of corona, the possibility of contamination of various components including human, soil, water, plants, livestock, tools and equipment should be considered and health strategies and special instructions should be considered. Some coronaviruses that infect animals are able to infect humans and then spread to other individuals, although they have become rare. Acute Respiratory Syndrome (SARS) and Middle East respiratory syndrome (MERS) (MERS) are examples of diseases caused by coronaviruses that originate in animals and are common in humans [9]. It is recommended that veterinary specialists in particular have a solution in this regard. On the sites, I saw that due to the contamination of people in the water pool in China, the virus survives in the water and there is a possibility of contamination of water resources. In general, one should be vigilant and plan in various fields.

In general, in the supply or production sector, less production is possible, although it may not be significant. Work and effort should not be stopped, especially in the field of production, and the ability to do the work must be provided and double incentive policies must be provided.

If the crisis continues, food supply chains are likely to be disrupted in the coming months. The workforce will shrink, and food production and processing will be disrupted, and intensive agricultural production will be affected. The livestock sector will be affected, and due to logistical constraints and labor shortages, we will have no access to livestock feed and slaughterhouse capacity reduced.

Restrictions on transportation and quarantine measures are likely to prevent farmers and consumers from accessing the entry and exit markets, leading to a loss of income, a loss of production and an impact on future cultivation. Obstruction of transport routes, especially for food supply chains, may lead to an increase in the level of food losses and wastes.

On the demand side, as the disease spreads, we will see a significant increase in demand over time. Food demand is generally unhealthy, although dietary patterns may change.

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Fear of contamination will reduce the risk of double risk by reducing visits to food markets. Serious monitoring of the distribution of goods and prices is needed more than ever.

Purchasing methods will change. Restaurant traffic will decrease, e-commerce will increase, and production and consumption at home will increase.

There may be a problem with imported and exported products.

In general, vulnerable populations and their immediate needs in agriculture and the production chain should be identified, quickly met, and supportive and encouraging assistance packages should be considered. Plans to purchase agricultural products, especially from small farmers, and to shorten the production to consumption route should be pursued and implemented to reduce the risk of contamination as the cycle shortens. Free donations to poor people who have lost their income should be considered alongside financial assistance to restart production.

The Ministry of Agriculture and the Faculties of Agriculture should work closely with the Ministry of Health and other departments as part of the response to Covid-19.

All necessary precautionary measures should be taken to protect employees and clients in accordance with health recommendations.

Agricultural-related assemblies should work as much as possible during the current epidemic so that manufacturers and processors can continue to operate effectively and keep supply lines open.

Operation of food and other processing equipment, ensuring the continuation of payment and commercial activities that are necessary to protect farm income, and that employees and farmers can continue their activities and put them on the market are important. In many cases, these activities depend on the ability to perform monitoring and inspection tasks.

Collections must reassure people that they are doing their best in all their activities.

Important Note: It is necessary to there are several ways to help farmers look for their needs in relation to Covid-19 restrictions. A wide range of online features should be available.

As always, farmers need to remember to follow basic environmental safety protocols and safety rules when working on the farm.

Production of food and proper functioning of the food supply chain during this difficult period is essential. It is very important that farmers, gardeners, ranchers, and those involved in processing, retail, and distribution continue to do what is important to ensure that the food supply chain is active and that progress is being made.

There is currently no report that Covid-19 can be transmitted through food or food packaging. However, it is always necessary to observe hygienic tips when using or preparing food (for example: washing hands and surfaces and packages, separating raw meat from other foods, cooking at the right temperature, etc.). Be more careful when receiving food products from countries that have approved Covid-19. There is no evidence so far to show that food produced can transmit Covid-19. Because the virus that causes Covid-19survives at different times, depending on the levels or objects. For this reason, it is very important to follow the 4 main steps of food safety, cleaning with the recommended methods, separation, cooking and cooling. Public health and safety experts, and workers working in food processing plants that have work activities, if they are suspicious of Covid-19, should be exempted from work and activity and stayed in home quarantine or considered hospitals for care and treatment. Food establishments, like other workplaces, need to comply with the protocols set by the Ministry of Health.

Make the necessary arrangements with the officials of urban, nomadic and rural health for all jobs, so that timely and accurate information can guide appropriate responses to agricultural-related collections wherever their operations are located.

In working environments, maintaining the social distance, the distance between 180 and 200 cm, is important in preventing the spread of this virus.

4. Conclusion

The prerequisite for success in combating the disease is, first and foremost, social distance and maximum testing of the disease, followed by isolation of patients. World chances of contracting the coronaviruses are high by the end of 2020. There are ways to get out of the recession and the crisis that make it easier to move forward with a smart economy. These strategies include flexibility of working hours, division of labor presence during the week, regulation of the regulatory system on corona credits, and maintenance of supply and demand cycle, and serious management and supervision. Attention to models and consideration of strategies, solutions and recommendation should be considered, and readiness and action at all levels should be appropriate to the place and time.

References

[1] Anonymous. ICTV Taxonomy history: Orthocoro-

DOI: https://doi.org/10.30564/jrb.v2i1.1841

navirinae. International Committee on Taxonomy of Viruses (ICTV), 2020.
https://talk.ictvonline.org//taxonomy/p/taxonomy-history?taxnode_id=201851847

[2] Fielding B. A brief history of the coronavirus family – including one pandemic we might have missed, 2020.
https://theconversation.com/a-brief-history-of-the-coronavirus-family-including-one-pandemic-we-might-have-missed-134556

[3] Seladi-Schulman J.. Signs and symptoms of coronavirus (COVID-19), reviewed by Meredith Goodwin, Healthline Media a Red Ventures Company, 2020.
https://www.healthline.com/health/coronavirus-symptoms

[4] The New York Times. 2020.
https://www.nytimes.com/2020/04/26/world/coronavirus-ews.html

[5] Donyaeh Eghtesad. 2020.
https://www.donya-e-eqtesad.com/fa/tiny/news-3642232

[6] Farnush. Gholamreza et al.. Recognition of new cronavirus-2019 and Covid-19 based on available evidence-review study, Journal of Military Medicine, 1399, 22(1).

[7] Eghtesadnews. 2020.
www.eghtesadnews.com/fa/tiny/news-332286

[8] Pragyandeepa. Role of agriculture in the economic development of a country. 2020.
http://www.economicsdiscussion.net/economic-development/role-of-agriculture-in-the-economic-development-of-a-country/4652

[9] National Institute of Allergy and Infectious Diseases, Covid-19, MERS & SARS,
https://www.niaid.nih.gov/diseases-conditions/covid-19

 DOI: https://doi.org/10.30564/jrb.v2i1.1841

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REVIEW

Effects of GMO Agricultural Products on Living Things

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ABSTRACT

By obtaining changes on gene sequences of living things with the applied biotechnological methods; The idea of "Genetically Modified Organisms (GMO)", which aims to bring the living creature in question the original gene combinations with the desired characteristics, came to life in the late twentieth century. Despite the high probability that hunger problems may increase with the increasing world population; It is thought that plant breeding with classical farming methods will be insufficient in solving these problems. With various GMO applications developed all over the world, it aims to produce solutions to these problems. With the presence of GMO, it was possible to increase the shelf life of qualitative and quantitative values of the existing foods. In addition, decreases in agricultural use of pesticides used in agricultural struggle and threatening human health with GMO production are noteworthy. However, some concerns about anomalies that may occur in living things fed GMO products remain on the agenda. Because, in the long term, there is no clear and precise information that GMO will not have negative effects on living things; There are many recorded incidents showing their negative effects.

1. Introduction

This review study prepared by evaluating the data obtained as a result of article scans; In addition to the negative effects that can be seen or seen on living things, GMO agricultural products, which are frequently discussed today, are; It is aimed to reveal the existence of positive sides, which may be beneficial and possible for humanity.

The nutritional deficiency problem brought by the increasing world population; it has made scientists obliged to work on the solution of problems by developing new techniques and technologies in this field. Because it is estimated that trying to solve the problem by increasing the areas where agriculture can be done will not be sufficient due to population growth. Biotechnological methods, developed to prevent the ever-increasing hunger problem and to meet the standard nutritional needs, have had wide repercussions and found serious support all over the world. According to Tecer, it seems inevitable to use new technologies in plant breeding studies, considering that it has come to the limits of using classical plant breeding methods for increasing yield in nutrients [1]. The ideas that were introduced towards the end of the twentieth century, by interfering with the genetic structures of foods, that qualitative and quantitative increases in food production could be made caused the birth of the "Genetically Modified Organisms (GMO)" technology. This important

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discovery that excites the world of science; The public was found to be equivalent to the invention of the printing press, the discovery of fire, and the disintegration of the atom [2]. Organisms obtained by changing the gene sequences of living creatures by using biotechnological methods, changing their properties or gaining new properties to them are called "GMOs" [3,4].

In GMO technology, it is aimed to increase the resistance of plants against all pests in addition to increasing the yield in the production of plants. In addition, agricultural products have extra features that will increase their shelf life in the market with GMO technique. As stated by Çetiner regarding this; The most common trait transferred to plants is herbicide resistance genes, which significantly reduces the production costs of farmers. BT, which is the Bacillus thuringiensis endotoxin gene that provides resistance to lipidoters, is also effective against caterpillars that are harmful for corn and cotton. In this way, drug consumption decreases in the agricultural struggle and both the cost decreases and the negative effects that may occur for the environment and human health are reduced [5].

In addition to numerous scientific studies showing the benefits of GMO agricultural products to the creatures fed with them; The number of studies showing that it threatens health is also at a considerable level. In this review study, the results of the literature review were made; The positive and harmful effects of transgenic plants on living things have been studied.

2. GMOs in Agricultural Field

Like the production of vaccines and antibiotics that are considered as milestones in human history, GMO production is also considered a major revolution. This biotechnological invention, discovered towards the end of the twentieth century, has entered our lives at a very high level today. So much so that agricultural products such as potatoes, corn, soy, tomatoes, which are frequently consumed today, appear as GMO products. These biotechnological interventions to increase efficiency and quality in such food products; The fact that the properties that are basically not in the plant are taken from another creature and transferred to the plants brought along some question marks and concerns. For example, when you isolate the cold resistance gene that we want to see in tomato by transferring it from tomato to tomato, you can make the tomato resistant to cold. However, not being able to predict what kind of abnormal reactions this new genetic code, which is not found in the gene pool of tomato, can cause in living things that consume that tomato, causes concern. Moreover, there is no clear information about the effects that GMO products may cause in the long term.

Some authorities have expressed concerns about the potential hazards that may arise due to the out-of-control spread of genes, which are created during the transfer of genes. As a result of this situation, it is concluded that the natural fauna and flora, where new wild species may emerge in nature, may be negatively affected and the balance between the species may be disturbed [6].

Transgenic plants, which were first commercially produced in 1996, continue to be cultivated in more varieties and areas today. GD plant planting area, which started in 1996 in an area of approximately 2 million hectares in the world, has exceeded 185 million hectares according to 2016 data (Table 1). In addition to the increasing world population, the gradually decreasing areas to be used for agriculture encourages GMO production.

Table 1. GM plant cultivated area amounts in the world, 1996 - 2016 [7,8]

Years	Cultivated Area (Million Hectares)	Index(1996=100)
1996	1,7	100
1997	11	647
1998	27,8	1.635
1999	39,9	2.347
2000	44,2	2.600
2001	52,6	3.094
2002	58,7	3.452
2003	67,7	3.982
2004	81	4.764
2005	90	5.294
2006	102	6.000
2007	114,3	6.723
2008	125	7.352
2009	134	7.882
2010	148	8.705
2011	160	9.411
2012	170,3	10.017
2013	175,2	10.305
2014	181,5	10.676
2015	179,7	10.570
2016	185,1	10.888
Toplam	2.149,7	-

3. Positive and Negative Effects of Agricultural Sourced GMOs

3.1 Benefits of GMOs

Special industry members, scientists, food technology and food experts, food processors, distributors, retailers,

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American farmers, regulatory agencies, advocates of poor and hungry people and supporters of gren revolution in the world; With the facilitated genetic engineering, they think that the food and medicine required due to the increasing world population can be produced in large quantities [2].

Russert Burbank potatoes, whose starch content was developed more than usual by Monsanto Company, produced potatoes that reduced less cooking time, reduced cooking time and cost during the frying process [9].

To increase the carbohydrate content of GMOs, tomatoes can be used in products such as ketchup and tomato sauce to be more intense [2].

Fats that are responsible for the production of cholesterol in the body are high in saturated fat. Fats that are low in saturated fat and high in unsaturated fat, which are important for our health; It is used in frying and other processes and is resistant to high temperatures. The genetics of these plants can be changed to further increase the level of unsaturated fatty acids in existing oils of plants such as canola, soy, sunflower and peanut, which are frequently used to obtain oil [2].

In addition to improving the nutritional quality of food products, GMO products are produced to increase their contribution to health. With GMO technology, the levels of anti-oxidant vitamins (vitamins A, C and E), carotenoids, flavonoids and minarets, which increase the heart disease, some cancers and some natural compounds that cause blindness and slow down or prevent biological oxidation, is also increased [2].

When the allergen proteins in foods such as hazelnuts, peanuts and wheat, which can cause allergic reactions in humans, are extracted with GMO technology, it is possible to completely or partially get rid of the possible allergen reactions.

3.2 Losses of GMO

The expression and genetic function of the transgene introduced into genetically modified organisms can lead to unpredictable changes in living things. Thus, the protein product of the transgene can cause unpredictable reactions and the emergence of potential toxins [10].

The results of the study on mice fed transgenic potato against genetically modified production pests showed that; cancer cells develop in rats; brain, liver, and testicle development are prevented; Some of the liver is blunted and histological differences appear in the pancreas and intestines [6].

4. Results and Discussion

As it turns out, reactions about the health-threatening effects of GMO products in the short and long term will continue to keep the world agenda busy and react.

The lack of complete and clear information on the long-term effects of GMO products on health suggests that labeling transgenic products should inform consumers and ensure their right to choose [11]. Research results in five states in the US have shown that consumers feel that GMOs do not have adequate protection against “unknown” and “unpredictable” health risks [12].

If GMO products threaten genetic diversity, an irreversible process will also be entered. For this reason, such products should be offered for consumption after sufficient scientific researches and their use should be constantly checked within the legal framework [13]. International conventions on this should also be implemented meticulously.

In order for the Biosafety Law in force to serve the effective and safe use of GMOs, the public should be made conscious of scientific resources [14].

GMO agricultural products are like double-edged knives; In addition to the benefits it provides, it is necessary to take measures by knowing the existence of possible harm to living things. Scientific studies to increase the nutritional quality and quantity in agricultural products should also be supported in a controlled manner.

References

[1] Atsan, T., Kaya, T. E., Genetiği Değiştirilmiş Organizmaların (GDO) Effects on Agriculture and Human Health, Journal of Faculty of Agriculture, 2008, 22(2): 1-6. (In Turkish)

[2] Uzogara, S. G., The Impact of Genetic Modification of human Foods in The 21st Century, Biotechnology Advances, 2000, 18: 179-206. (In Turkish)

[3] Kulaç, İ., Ağırdil, Y., Yakın, M., Sweet Trouble in Our Table, Genetically Modified Organisms and Their Effects on Public Health, Turkish Journal of Biochemistry, 2006, 31(3): 151-5. (In Turkish)

[4] Beyatlı, y., Biotechnology Lecture Notes, Gazi University, Faculty of Arts and Sciences, Department of Biology, Ankara, 2000, 146. (In Turkish)

[5] Çetiner, S., Turkey and the World Agricultural Biotechnology and Food Security: Issues and Recommendations, food Cooperation Platform, 2008. (In Turkish)
http://students.sabanciuniv.edu/~sedakaya/index.php?option=com_content&task=view&id=61&Itemid=76

[6] Cebirbay, M. A., Aktaş, N., Genetiği Değiştirilmiş Organizmalar (GDO) and Its Effects, Billur Publishing, 2018, 309-325. (In Turkish)

DOI: https://doi.org/10.30564/jrb.v2i1.1764

[7] Hall C, Knight B, Ringrose S, Knox OGG. What have been The Farm-level Economic Impacts of the Global Cultivation of GM Crops?. Collaboration for Environmental Evidence, 2013, 11: 1-45.

[8] ISAAA. Global Status of Commercialized Biotech/ GM Crops: 2016, Brief 52. http://www.isaaa.org/resources/publications/ briefs/52/download/isaaa-brief-52-2016.pdf.

[9] Whitney, S.L., et al. This Food May Contain. What Nurses Should Know About Genetically Engineered Foods, Nursing Outlook, 2004, 52(5): 262-266.

[10] Fagan, J.B.. Genetically Engineered Food-A Serious Health Risk, 2005. http://www.netlink.de/gen/fagan.html

[11] Topal, Ş.. Genetic Modification Procedures and Biosafety, Wheat, 2004, 26. (In Turkish) http://www.bugday.org.

[12] Zimmerman, L., Kendall, P., Stone, M., Hoban, T.. Consumer Knowledge and Concern About Biotechnology and Food Safety, Food Technology, 1994, 73-77.

[13] Çelik, V., Turgut-Balık, D.. Genetiği Değiştirilmiş Organizmalar (GDO), Erciyes University, Journal of the Institute of Science, 23(1-2): 13-23. (In Turkish)

[14] Özdemir, O., Duran M.. Biotechnological Applications and Genetiği Değiştirilmiş Organizmalara (GDO) Related Consumer Behavior, Akademik Gıda, 2010, 8(5): 20-28. (In Turkish)

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