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MicroRNA Transformed Genetically Modified Crops and their Effect on Human Health.

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Research Article

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Abstract

GM crops or Genetically Modified crops are attracted a wide range of media attention in recent years and continues to do so. Media given awareness about the genetically modified crops to public. They reported the uses and drawbacks of the GM crops. The technique offers with regards to the range of advantages of the use of genetically modified crops. In the Pioneer stage of the production of GM crops, two different sectors of concern have been evolved, which includes impact on the agriculture and dangerous to human life. Safety of the eatables have a vital role in the world. The issue can be reduced by enhancing the productivity and quality of the crop. Genetic manipulation technology depends on the MiRNA, it is one of the main problem-solving methods, which influence the environmental product formation through improving major rules used for miRNAs modification and its objectives in GM plants, it contains constitutive, induction to stress, or specific tissue expression of micro RNAs or their aim, RNA gene silencing mechanism, micro-RNA-resistant target and gene expressions. Genetically Modified Organisms is one of the major focuses in biomedical research from 1980s. Since, Genetically Modified models with animal enable researchers for treatment of human genetic diseases. Genetically Modified microorganisms, crops, and animals are used for the production of drugs that are complex by which helps the generation to vaccines that are cheaper. However, this article+ more focused on the human health associated with the genetically modified foods and role of miRNAs in respected to GM food products.

1. Introduction

200,000 natural substances are produced and accumulated by plant crops [1]. That can be used as eatables, flavours, medicines, colouring dyes etc. Some of the compounds such as alkaloids, terpenoids, steroids, polyketides, phenylpropanoids and flavonoids were obtained from plant variety which is economically important. GM crop methods involves the transfer of genes of interest from one plant to another or from one plant kingdom to another target plant. Crops are very important part of the environment. Which utilised by humas for shelter, pharmaceuticals and also for eatables. The major impact on our ecosystem caused by humas. Human exercises incorporate abiotic stresses like dry season, soil harmfulness, environmental alteration, and biotic dangers like creepy crawlies, herbivores, microbial microorganisms, and so on the expansion in the populace on the planet with the associative expansion popular authorization of plant items an expansion in harvest capitulate. One such strategy includes decreasing the yield punishments by planning and receiving earth cordial harvest insurance measures [2–5]. These riboregulatory can hence fill in as particles that can be controlled to support promoting plant efficiency. This audit gives a short portrayal of micro-RNA formation and revelation. It additionally examines the job of microRNAs as potential quality controllers in grains, vegetables, tubers, natural products, sources of biofuel, refreshments, and crops with fibre dependent on the latest distributions. The procedures depend on miRNA utilized for enhancing financially significant plants are additionally depicted.

In recent studies shows that microRNAs plays an important role in life of plant, especially environmental stress response, development and architecture of plant using microarray and high-throughput sequencing methods [6–9].

2. Genetically Modified Plants : An Introduction

Verity of methods exists for the manufacturing of Genetically Modified crops. Two methods are commonly suggested for the production of GM crops. The ‘gene gun’, which transfer the DNA coated on a microparticle in to a cell of a crop. DNA should be transferred into target plant cell that will propagate to produce GM plants by tissue culture mechanism [10]. The main concepts of the protocol are as follows

- The transformed cells are finding the help of selectable markers.
- Delivery of desired DNA into the crops genetic material
- The chances of high mutations in Genetically Modified crops compared to non- Genetically Modified crops are used in rearrangement and synthesis of DNA along with addition of new genes.

To enhance the delivery of desired DNA into plant cell genome, a selectable marker gene was provided. e.g., Antibiotic resistance, is transfer of desired DNA to allow discrimination of genetically modified tissue and development of genetically modified crops. There will be a problem due to consumption of genetically modified plant. When it consumed by human that will affect the microflora in the human gut and the microorganisms that are resistant to antibiotic in the soil. There by the antibiotic resistance gene in the bacteria will spread in the bacterial colonies. As a result of production of GM crops, that will have large mutation than the original plant [11]. Tissue culture techniques that can cause mutations in plants, causes soma clonal variation and rearrangements of DNA may occur in transgene, where the plants can produce toxins in theoretical aspects [11]

2.1 Genetically Modified plants act as a resource of foods

Collection and generation of eatable yields, fibers and another product is one of the methods used in past days. The plants that is genetically manipulated plants can be used as a food, and which is also helps in the production of oils (extractable) or can give proteins system expression. There are multiple problems to solve when transfer from studies to wet lab work [12]. Direct application, shows the effects and market for the particular character of the target and it will helps for a better knowledge of metabolism for gene regulation to new product that is applied. The information regarding expression system in particular tissue or organ or organelle, in various stages of progression can be known, for getting largest level of accumulation. For confirming the safety measures of new product consumption there must be a constant or predictable accumulation level is required for nutraceuticals [13, 14]. Golden Rice, is the second generation of genetically modified foods, gives a better research study for individual concerns.[15].

2.2 Future of Genetically Modified plants

Acceptance of genetically modified plants by public is the main hurdles, commercially. Because of the safety related to primary generation of modified plants, it will take much time for getting public acceptance [16]. Metabolomics is one of the feasible solutions for analysis of comprehensive metabolite [17, 18]. Well known cells like; transgenic animals, yeast, bacteria, and mammalian cell culture were included in the recombinant protein expression systems. The main factors implicated for commercial applications are, the expenses during operation and culture[19]. While generating recombinant proteins such as antibodies and vaccines, folding of proteins and manipulation of post-translational product that is glycosylated, are the main problems. It shows that plants are better systems for producing protein products that are effective and also active [19]

3. For Improving Plant, New Sources Of Transgene Candidates: Mirnas

MiRNAs of plant is a part of 20–22 nucleotides in length of small regulatory RNAs. The miRNAs are coded by endogenous microRNA genes and then transcripted to primarily produced micro RNAs or pri-miRNA by an enzyme called RNA polymerase II [20]. An uncommon partially double stranded stem-loop structures of primary miRNAs transfer to pre-miRNAs, mediated under Dicer-like 1 and assisted by HYPONASTIC LEAVES and proteins of SERRATE [20]. Plant miRNAs have slicing mode much frequent than animal miRNA [21]. After insertion process, directed to a protein called Argonaute, which is a RNA-induced silencing complex component. So, the matured micro-RNA direct RISC to bind the aimed transcript by better match of sequencing, and it will break a single phosphodiester bond in the target molecule of mRNA [20–22, 7]. Multiple researches reported that miRNA plays an important role in almost all of the metabolic and biological processes [22, 23, 7]. MicroRNAs are regulators and act as a core of regulatory networks of gene [20]. Those challenging traits can be unraveled by the regulated networks of miRNA or advances in miRNAs functional analysis [7]. miRNAs can represent themselves as a gene reservoir, which can act as a new candidate for manipulation of challenging traits [7, 8, 24]. The elucidation of miRNA directed interactions will leads to the detection of much useful transcription factors and well known of the mechanisms of several processes in which transcription factors are implicated.

3.1 MiRNAs and its activity for improving crop yield

The research shows that microRNAs act a crucial effect in responses to different stresses including infections of bacteria and fungus, deficiency of nutrients, salt, drought, cold and temperature. The possibility of the important activity recognized by the detection of multiple miRNAs that response to stress produced by various tools of bioinformatics; analysis and database sequence prediction, analysis of microarray [6–9, 22, 26, 27]. So, confusions about molecular level mechanism, influence of microRNA in accordance to plant stress, detection of regulation network of gene via miRNA and characterization of particular miRNA were cleared utilizing the research information. For developing new varieties of stress resistant having good activity during various environmental stresses, some transgenic methods are used in crop species for over expressing or knock out/down genes of particular miRNAs. So, these modification

of micro-RNA in transgenic plants leads to make better results in biomass and seed yield, it agricultural productivity of agriculture. The review is separated into 5 parts, which includes regulation networks of gene via miRNA, and progress of the study about the modification of miRNAs expression in transgenic plants for improving agricultural production.

3.2 MiRNAs for Genetically Modified target traits—resistance to stress

Multiple micro RNAs are associated with responses to biotic stress of plant (virus, bacteria, fungi and nematodes)[28–43]. So, the studies about this topic mainly target the detection of micro-RNA, which response to biotic stress, using bioinformatics tools(deep sequencing or data mining computationally). The remaining investigation have to be done for the characterization of specific miRNAs functionally.

3.3 miRNA Target Screening and Prediction Methods

Identification of target genes of miRNAs of plants, utilizing tools of bioinformatics by experimental methods [44–46]. With the help of complementarity scoring and analysis of secondary structure are: psRNATarget; TAPIR, miRTour and miRTarBase, the experimentally validated aimed relation of miRNA database were utilized for target identification of miRNA [48, 46, 47].

Real-time PCR is used for evaluating target mRNAs expression level, the cutting site of target can be structured with the help of 5'- Rapid Amplification of cDNA Ends or RACE. Degradome sequencing method was progressed, recently. So, this one is the manipulated version of 5'-Rapid Amplification of cDNA Ends utilizing a high-throughput, deep sequencing method [49, 50, 45].

3.4 Functional Roles of miRNAs

Arabidopsis plant and others are shows that micro RNAs are implicated in multiple procedures like growth and development, genome integrity maintenance, transduction of signal, signalling pathways of hormone, homeostasis of hormone, innate immunity, and abiotic and biotic stress response [36, 7, 51, 47]. Recent study reported that micro RNAs can be used to reschedule metabolism of cells at the time of plant attacked by pathogen. It leads to changes in dynamic to the micro transcriptome connected with regulation of differential transcription with the help of immunity and basal resistance [53]. Proteins or enzymes were coded by gene transcript in metabolic process.

4. Micro Rnas Functions In Cereal Crops

4.1 Rice (Oryza sativa)

In world, half of the populations are using rice as their first resource of food. There are some factors that affect growth and yield of the crops, those are drought, salinity, ice cold, heat and deficiency in nutrients. From the library of cDNA of rice, 20 miRNAs were primarily recognized in 2014 and their forecasted genes of target were concerned in transfer, resistance to disease, transcription, metabolism etc. [54]. In another

research, 35 rice miRNAs were recognized in the upcoming year, from that 14 of them are new and target genes were included in various physiological procedures in rice were also forecasted [55]. 592 miRNAs sequences contained in version 21 were recognized in miRNAs of multiple rice. High tillering and early blossoming are the two phenotypes which are important for productivity and it was showed by OsmiR393, plants that is overexpressing[56]. For responding to heat stress and adaptation of rice by L-ascorbate oxidase (*OsLAC*) expression, which is controlled by OsmiR397, reported as a responsive miRNA for high-temperature [57, 58].

4.2 Wheat *(*Triticum aestivum *L.)*

High nutritional qualities and good contribution for security leads wheat to be the most important cereal crops. For miRNAs investigation at the sub genomic levels there are lots of efforts in wheat, utilizing process of sequencing which leads to the finding miRNAs of 58 wheat including families of 43 miRNA; conservation of 20 families such as miRNA156/157, miR159, miR160, miR164, miR165/166, miR167, miR168, miR169, miR170/171, miR172, miR319, miR390, miR393, miR396, miR397, miR399, and miR408, and in which 23 of them are noval[59]. The following proteins like squamosa enhancer binding proteins, scarecrow-like blue copper proteins are included for the growth of wheat crop as well as its various physiological procedures, so these are directed by the above miRNAs[59]. In miRbase version 21 containing 116 miRNAs sequences have identified more miRNAs of wheat. Characterization of tissues of wheat and 323 new miRNAs included in 276 families were conducted a wide survey with their related target genes very recently. The above miRNAs plays a significant part in progress of grain and also it will expressed in grain[60].

4.3 Maize (Zea mays)

In world, maize is secondary crucial crop, that is utilized for feed as well as forage resource for fuel production in ethanol. Maize will use as a model plant in studies due its agricultural and economic importance[61]. miR156, miR160, miR166, miR167 and miR169 are the families of miRNAs included in development of maize, growth, and biotic stress responsive were primarily explained and property differentiation with objective genes[62].

In maize, 321 miRNAs present in miRbase version of 21 plays an important function to environmental stresses in growth, development and crop responses. Negative regulation of ZmNAC1 which is a plant specific transcription factor family included in regulation of stress and development[63].

4.4 Barley *(*Hordeum vulgare *L.)*

It is other crop better in cultivation. For human consumption and livestock feeding, barley grain is utilized [64]. It is a experimental model utilizing in studies in genomics and breeding [65]. Identification of 100 miRNAs in barley crop using sequencing of which 56 orthologs expressed in wheat or rice, while up to 44 appear expressed in barley. Utilizing short RNAs Solexa sequencing by high-throughput technique, 133 new, and 126 barley miRNAs, that is highly conserved were detected from libraries [66, 67]. The novel

miRNAs detected barley miRNAs which responsive to new salt in and their aimed genes which enhance tolerance to salinity.

5. Fruit Crops

5.1 vine of Grape *(Vitis vinifera L.)*

Grapevine having best nutritional and processing qualities, so it can be cultivated in worldwide and it is also economically important fruit crop. Genome sequencing of grapevine was completed, so scientists were much interested to miRNAs investigation[68, 69]. To miRbase 21, 349 miRNAs of grapevine are settled. For understanding the functions of already detected miRNAs, there is a prediction of known miRNAs 112 aimed genes and novel grapevine-specific miRNAs having 44 target genes. In one of the researches, 25 grapevine miRNA families have to aim total 134 target genes. So, these genes will encode transcription factors that are included in growth of plant, development of plant, and its phase alters from vegetative to reproductive. ATP sulfurylase/APS kinase and ATP synthase were encoded by target genes which plays an important role in various procedures of metabolism. Those target genes involved in resistance to disease, response to immunity, response to stress, and signaling transduction [70]. Grape vine implicated in biotic and abiotic stresses responses; a study suggested that cold-inducible grapevine miRNAs play a major role in response to cold stress [71].

5.2 Apple *(Malus domestica Borkh)*

It is another economically important tree of fruit, and consumed widely. It contains flavonoids and compounds of phenol, that plays an important function in decreasing chronic disease risk in humans [72]. From apple, identification and characterization of 413 miRNAs in miRBase version 21. Identification of 146 microRNAs from *M. domestica* (cv. Golden Delicious) utilizing bioinformatic analysis and Ribonucleic Acid library sequencing methods. The research suggested that new miRNA of apple, Md-miRLn11, controls the NBS–LRR protein expression at the time of infection of pathogen which leading to plant bacterium resistance [73]. Some of the miRNAs are implicated in fire blight resistance, caused by *Erwinia amylovora*, bacterial disease.

5.3 Tomato *(Solanum lycopersicum L.)*

Tomato is widely consuming and commonly using vegetable and also it can utilize as a experimental plant model for the research of ripening and senescence which attracted by scientists for detecting function of miRNA at the time of development of fruit by aiming genes implicated in pathway of ethylene and ripening of fruit [74]. Small Ribonucleic Acids from fruit and leaf of tomato were detected, majority of them plays a crucial role in development of fruit. miR1917, a key negative regulator of ethylene responses, acts on ripening of tomato [75]. In a research, studied about the defense mechanism of microRNAs in tomato against *Fusarium oxysporum*. It demonstrates that miR482/2118 inhibits genes of nucleotide-binding site, which cause resistance to *F. oxysporum* [76]. Before, it was suggested that

miR159/319 and miR172 might contributes its role in pathogenesis of viruses in tomato against the curl virus of Tomato leaf, chilling and freezing like Cold stresses.

6. Genetically Modified Foods: Benefits

6.1 Resistance to pest

The plants or crops that are modified using genetic engineering techniques has modified DNA that show resistance to pest and agents that are specific and harmful to plants which ensures the growth improvement of crops and helps farmers for good yield. Mutagenesis and GE varies as the exposure to chemicals and radiation contribute mutagenesis that cause stable changes that are not specific [77] Other methods in breeding such as Selective plant or animal breeding or soma clonal variation are also used by human to for modification of food, mainly by addition of a new trait that is not present naturally which shows characteristics of pest resistance, worse diseases and also certain nutrient or drug agent that are beneficial. Genetically modified foods such as Bt. Corn doesn't need the addition of pesticides as it modified in such a way which is cost effective during the time to harvest [78]

6.2 Tolerance to Herbicide and Resistance to Disease

The environmental hazards caused by herbicides can be controlled by plants or crops that are engineered to resist herbicide. [79–81] Genetically-engineered crops designed for herbicide resistance provide a positive environmental aspect with the limited use of herbicides [79–81], in turn reducing its production expense and limiting the leaching of agricultural waste. A number of plant diseases are triggered by the infection of various viruses, fungi and bacteria. Plant scientists points out the fact that genetically modification likely tends to have least unintended changes than the conventional bred crops, which may be inferred from the results of a detailed crop composition profile. In advancement, genetically engineered plants which resist the specific plant infections are being designed by plant biotechnologists [82].

6.3 Cold, Drought and Salinity Tolerance

A recent report shows that plants such as tobacco and potato has been successfully introduced with an antifreeze gene from a cold-water fish, resulting them to withstand the cold temperatures, and generally killing unmodified seedlings [82].The rise in global population has resulted the increase in demands for land in housing rather than food cultivation, leading farmers to cultivate in previously unsuitable localities for crops. The modified plants aimed to survive periods of drought, cold or high salinity in soil and groundwater would enable to cultivate on such uncongenial dwellings [83].

6.4 Nutrition Properties

Undernourishment is commonly prevalent in the third world countries, as the poor rely on a single crop such as rice, as their major staple diet. Unfortunately, rice does contain all the essential nutrients in adequate amounts to tackle malnutrition. So, if rice could be modified to incorporate all the additional vitamins and minerals with adequate nutraceutical significance, nutrient limitations could be satisfied. A study conducted at the Swiss Federal Institute of Technology for Plant Sciences lead to the development

of ‘golden rice’, incorporating a high content of beta- carotene(Vitamin A) [84, 85].Further improvements on the golden rice are also studied, having increased iron content.

6.5 Pharmaceutical Characteristics

The production of medicines and vaccines are often costly, and certain times require specific storage condition. Working on this specific line of action led to the way in developing certain edible vaccines in potatoes and tomatoes[86],probably compromising with easier way to ship, store and administer compared to others[87].

6.6 Phytoremediation

Certain plants such as safflower and poplar trees[88] have been genetically altered, to upgrade their physiological and biochemical functioning, as a result clean up the heavy metal contaminated soil in vicinity. Phytoremediation is the biotechnological plant application in detoxifying pollutants, and is a innovative technique for environmental clean-up. The unique features of plants such as genetic, biochemical and physiological characters are significant agents for remediation of soil and water. The quantification of the tolerance capacity of the plant is categorized by tolerant, non-tolerant or partial in responses to their coefficient parameters. These parameters are used for estimation of accumulation of toxins in roots, seeds and mature leaves. These studies provide [89, 90] new vision to products which is extracted with little concentration of toxins in the aerial regions of plants

7. Human Nutrition And Gm Animals

Huge movement in creation and treatment of transgenic plants has revived investigations in animals. Comparable in plants, microinjection and equal strategies are utilized considering alien gene (DNA) into the core of prepared egg-cell if there should arise an occurrence of animas. Whenever ovum is created to blastula then moved to the uterus in the animal is where life form develops transgenic ally. Hereditary linkage maps for cows, pigs and sheep explaining chromosomal zones for financially significant characteristics will observably pay to improved worth and amounts of meat. Quality innovation is wealthy in livestock creation and in progress of value and amount attributes [91]. Genetic technology persuades the creations, more nutrient supplement and animals’ prosperity. These singularities can be updated straightforwardly by quality exchange or utilizing development hormones, immunizations, antibodies, resistance energizers and antiallergy DNA made by technology associated with genetics. Quality exchange is foreseen to propel those creation characteristics in animals, which are ailing inherited, for instance of forestalled piglets [92] depicted transgenic crops, that delivered immunizations in which animals overwhelmed by rummage, are framed. The quality of encounter enables rearing of animals impenetrable to infections. Immunization for invulnerable maiming of animals, easy in male animals and debilitates decisiveness while female animals are liberated from negative impacts of oestrus, unquestionably influences the monetarily critical quality remains adaptation. All things considered; transgenic milk can be utilized as: (a) Food for wide use; (b) crude materials for milk items; (c) nourishment for babies; (d) wellspring of organically dynamic substances for drug industry. Indeed,

even non-protein mixes of human milk, similar to oligosaccharides, are incredibly esteemed in milk of transgenic animals. Caseins and lactoglobulins are created distinctly during lactation period. Qualities from referenced mixes are utilized for transgenic milk creation, being used for cheese creation and for assistant to human milk with the end goal of baby sustenance depicted on wide utilization of ox-like development hormone (somatotropin) in steers to raising creation of both, meat and milk [93]. Today there is still a lot to learn about science just as hereditary designing consider with human milk, and thus there is a lot to chip away at the best arrangement of baby formulae taking into account overhauling the human milk's quality utilizing an ever-increasing number of exact works on with respect to hereditary change.

Conclusion And Future Perspectives

The research concluded that, currently miRNAs are taken as the most crucial regulators of gene. Until now, there is a significant progress has been made plant miRNAs analysis and characterization, with large amount of research conclusions in miRNA's important function in crops. The review summarizes that several findings that contribute miRNA and its agricultural and economic importance [7]. The small nucleic acids regulate different environmental stresses also influences the growth of plants, the signaling of hormones, reproductive and vegetative phase changes along with the responses in homeostasis and signal transduction pathways. A few studies concluded that miRNA of crops shows in molecular regulations of defense and immune responses of plant. For the enhancement in the agricultural properties of plants and crops, the genetic engineering targets the modification of miRNA [35, 27].

The manipulation of miRNA and its expression levels focus on the responses of plants against pathogens, parasites and environmental stress which enhances it growth, for this purpose several transgenic approaches such as over expression, expression specific to tissues, stress-induced, artificially target mimic etc., are focused on important miRNAs [47].

In such cases the miRNA act as positive regulators of stress that suppress the corresponding messenger RNA which leads to the improvement of plants which causes an undesirable effect in the native target genes. In cases when the target genes show a desirable effect then the miRNA acts as negative stress regulators which leads to over expression or artificial target mimics of the target genes [94]. Even though its feels like successful the practical application of the miRNA crops is challenging in agronomics as the complexity of the multiple genetic traits requires tuning of different genes in various stages of the development of plants. For the future crop improvement, the above-mentioned methodologies and a detailed analysis on the miRNA-based regulation of target in crops and plants will benefit the future strategies and design. The over expression of the miRNA and the knockout of important genes leads to same phenotypes and can lead to the reduction of the miRNA is another major [95].

The miRNA activity regulates and act as a efficient tool to relate the activities if microRNA families and engineering of plants gene expression and this experiment offer a chance that can alter the sequence of

decoy sites of miRNA that enhances the inactivation of miRNA and its targets also affects the production of plant phenotypes. But the major challenges faced is of false positive outcome by off- type effects of due to inactivation of miRNA, the action and communication between the miRNA and its decoy are very complex and that can also lead to plants losing its stability [96].

The latest invasion of biochemistry and physiology has a special focus in GE and transgenic technology has several uses in production of microorganism which includes plants, microorganism and animals. The augmentation via genetic alteration has influenced several crops. The foods that are genetically modified have much benefits such as tolerance towards salinity, resistance towards insects and high harvest. Genetically modified foods have numerous acts on health on human, these foods have both good and bad effects, which can be direct effect on the human that is consuming it or can be wider impacts such as in food chain that includes several other organisms.

For instance, if the expression of a miRNA or the targeted gene is changed, it may result in unacceptable pleiotropic changes in the developmental and morphological characteristics of that particular plant species. Hence, it is important to realize the miRNA regulation method in growth and development of plant or its reaction to different stresses. This will ease the pattern of procedures which are satisfying and thus results in the wish for traits but with limited trade-offs when it comes to modifies crops.

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