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PRODUCTION OF GENETICALLY MODIFIED RICE

[1]D.S.R.S. Prakash, and [2]B.Preethi Chandrakala
[1]Department of Biotechnology, Adikavi Nannaya University, Rajahmundry, Andhra Pradesh, India
[2]Department of Microbiology, Rajiv Gandhi Institute of Management and Science, Kakinada, Andhra Pradesh, India

Abstract:

Transgenic crops are defined as the term refers to the plants whose DNA is modified through genetic engineering . The genes from one species or different species are taken and combined and introduced with the genetic material of the plant which changes in their traits of the original genome. These are also known as genetically modified or GM crops. A transgenic crop plant contains genes that are inserted through artificially instead of pollination. Genetic engineering spreads up the process and increases the variety of genes which can be inserted into a particular plant. The main aim is to introduce a new trait to the plant which does not occur naturally in the species. The main advantage is to improve combinations in the plant, is to make it as useful and productive as possible. Transgenic crops provides advantages like improving shelf life, higher yield ,improved quality ,pest resistance, tolerant to heat, cold and drought resistance against a variety of biotic and abiotic stresses. The first transgenic plants were reported in 1983. GM technology has been improved many varieties of crops to date. As population increases the food sufficiency can also be increased, in order to overcome this problem, the GM crops is used to improve production yield, lowering transportation costs and enhancing the nutritional content. The goal of GM crops is to engineer lines that expresses novel traits that cannot be introduced into the crop by conventional breeding.

Keywords: Production of genetically modified rice, through genetic engineering, sustainable development in crop production. East Godavari District , Coastal Andhra Pradesh

INTRODUCTION:

The efforts of the bioengineering builds a target gene conferring to desired trait with suitable regulatory elements .There are many agronomic species of plants including tobacco, corn, tomato, potato, banana, alfalfa and canola. (32)(33). The nucleus of the plant cell is the target for the new transgenic DNA. In many cases, the inserted DNA has to be modified slightly in order to correct and efficient express in the host organism. Consumers are also concerned about the commercial release of GM crops. There are many crops that are genetically modified, out of which rice (Oryza sativa) is the most important cereal crops that provides nutrition for nearly half of the global population. The genetic modification plays an important role in the recent decades to meet specific requirements, and a number of agronomically traits that are resistant to insects and diseases , tolerance to herbicides and quality improvement ,nutritional value that are introduced into rice. The first transgenic plant was obtained by Toriyama et al, in 1988. The two insect rice varieties of Huahui 1 (TT51-1) and Bt shanyou 63 in august 2009 obtained security certificates allowing their production. The heavy crops are obtained in China by use of heavy pesticides and herbicides. The use of GM crops reduces the use of chemicals for the protection of crops(1) Cotton (Gossypium hirsutum L.) is a dual-purpose crop, globally cultivated for its high-quality fiber and oil.Around 33.1 million hectares of cotton are cultivated around the world. (16)

NUTRITIONAL BENEFITS :

Vitamin A deficiency causes half a million children to become partially or totally blind each year. The transgenic crops containing a high concentration of vitamin A. Researchers have introduced three genes into rice, two from daffodils and one from a microorganism. The transgenic rice exhibits an increased production of beta carotene as a precursor to vitamin A and the seed is yellow in colour . such yellow, or golden , rice may be a useful tool to treat the problem of vitamin A deficiency in young children.Another crop is papaya (Carica papaya L.) a tropical fruit that contains high nutritive, medicinal

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value and good sources of vitamins A, C, and proteolytic enzyme papain and chymopapain. The major cultivators are India, Brazil, Mexico, Nigeria, Indonesia, China, Peru, Thailand, and Philippines. The crop is affected by many diseases especially Papaya ringspot virus. The rapid development un biotechnology by the genetic transformation of plants made possible to introduce selected genes into plants to control pests and plant diseases. By using genetic transformation the transgenic papaya from somatic embryos after cocultivation with Agrobacterium tumefaciens strain C58-Z707. The coat protein which is expressed by papaya shows resistance to papaya ringspot virus.(34)(35)



The main cause of blindness and mortality to infectious diseases is vitamin A deficiency(VAD). Beta carotene has the more provitamin A activity that is accumulates in plant foods such as carrots, apricots, and peaches and do not present in the staples such as rice, wheat, maize, cassava, potatoes. In order to overcome the deficiency of provitamin in the staples , the beta carotene content in maize and cassava provides biofortification by conventional breeding , provides nutritional levels of beta carotene for rice, wheat, and potato by GM. Potatoes are the source of starch, fibre, protein, vitamin c, thiamine, and folate and low amounts of provitamin A and vitamins B6, B12, D and E. so the efforts to improve the provitamin A content of potato by the beta accumulation by expressing bacterial gene or over expressing the cauliflower OR gene. The provitamin content is increased by transforming the Desiree cultivar, consists of light yellow fleshed tuber phenotype and low carotenoid concentrations with three genes encoding the phytoene synthase (crtB), phytoene desaturase/isomerase (crtI) and lycopene beta- cyclase (crtY) enzymes from Erwinia herbicola. The final content results in the GOLDEN tubers appears a deep yellow to orange fleshed tuber phenotype, due to the accumulation of beta carotene. Proteins are the structural and functional units of biological systems. Biocatalyst are dependent on coenzymes and cofactors such as iron, zinc, vitamin A. The staple (rice, cassava, banana) food which are rich in potassium and energy and poor content of iron. India is the largest producer of banana in the world. Hence there is a strategy to achieve both increased iron content and tolerance to oxidative stress is desirable. Ferritin plays plays a key role in improving the iron status of the plants as it contains high amounts of iron storage protein family which are present in plant species such as legumes, grasses, and in dicot weed Arabidopsis. The over expression of native ferritin (MusaFer1) in transgenic banana accumulated iron and improved tolerance to oxidative stress compared to untransformed control plants.(35)

SALT AND DROUGHT STRESS RESISTANT PLANTS :

One of the major important environmental factors are soil salinization and desertification. Most of the agricultural areas are affected by salt and drought stress and causes great problem to the agricultural production worldwide. It not only decreases the crop growth but also yield, and quality due to the increasing of osmotic and oxidative stress. The population increases the economic crisis to meet food and energy requirements in the future is also increases so, we need to develop crops with good excellent environmental stress tolerance.(5)

For example, alfalfa (Medicago sativa L.) which is cultivated throughout the world for its economic value and excellent agricultural traits. It is the high yield crop and has more nutritional quality, with high levels of protein, minerals, amino acids, vitamins and contain nitrogen fixing ability which has the ability to reduce the amount of energy required for production and soil structure with addition to this it also contain deeper root system that can able to adapt all environmental conditions including drought, soil erosion and salt stress. The studies regarding to these adaptability is based on activation of cascades with stress response, carotenoids, which contain capacity in antioxidant activity, roles in light collection,

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protection of photosynthetic systems and abscisic acid (ABA) synthesis. (48) The accumulation of carotenoids in tobacco and Arabidopsis plants results in the tolerant to abiotic stress such as UV irradiation , high light ,and salt stress. The gene which is responsible for carotenoid accumulation(Orange gene (Or)) shares a high level of homology in many crops such as cauliflower, rice, tomato, Arabidopsis thaliana. The content of carotenoid is increased by cauliflower Or gene by the formation of chromoplasts in transgenic potato tubers. The Or gene from orange fleshed sweet potato involves in carotenoid accumulation and response to abiotic stresses by increasing nutrient contents. The promoter known as sweet potato peroxidase anionic 2(SWPA2)induces high levels of exogenous gene expression than the 35S promoter from the Cauliflower mosaic virus (caMV35S promoter) response to various stress treatments, and applied to transgenic plants such as potato and rice.(48)(49)(50)

DISEASE RESISTANT CROPS :

Rice is the main food source for more than half of the world's population. Bacterial leaf blight and blast are the most important diseases in rice crops. The diffusion of these crops is very fast and becomes difficult to control over the crop. The most used method is to spray the pesticides. The continuous and more use of pesticides cause long term exposure of the environment leads to contamination. The micro-organisms can acquire resistance to chemical pesticides. The best way is to cultivate the resistant plants which is eco friendly and control the leaf blight and blast diseases. The modern breeding for transgenic resistant varieties requires complex system source of donor genes, time and cost of breeding , stability of resistance and location tests. The Antimicrobial peptides (AMPs) are components of the defense system of animals and plants against pathogens ,usually less than 50 amino acids/peptide. (42)

This present study was undertaken to develop GENETICALLY MODIFIED TRANSGENIC crop in RICE plants . It was intended To develop disease resistant, stress, drought, pest resistant crop.Transfer of genes to produce genetically modified crops. Increase in productivity of plants in short period.Production of nutrient rich rice crop.

Probably eat Genetically Modified crops (GMO's) everyday and even we don't realize that what we are eating. Then what is a genetically modified crops- The genes from one plant or species that is taken and inserted into another crop plant in order to get the modified crops which are resistant to the pest, stress, drought, salt, herbicide. More often they provide more nutrition the normal crop. Most people think about the food they buy being modified from their natural state.

Most of the population is probably unaware of the use of GM crops that are in food today. GM crops are relatively new and scientists have not yet determined the affects related to the health. By doing analysis on this study, the benefits and bad effects of GM crops are known. GM foods changed the world's economy for producing the more crop in lesser time. There is a fear that without GM crops there will be less food for consumption. Because of producing GM crops, there is enough feed to everyone. They are often not recognized as genetically modified crops when they are bought and consumed. GM crops refer to plants scientifically created for animal or human consumption using the most recent molecular biology techniques. These crops are modified in the laboratory to improve biology techniques. For example, the gene related to the particular tolerance can be cut off and inserted into the specific plant can tolerant to the particular resistant conditions



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Rice is one of the most important food crops in the world. The genetically modified crops are used in order to get the resistance over the herbicide tolerance and pathogen or insect resistance. China largely invests heavily on genetically modified rice. By the end of 2014 over 250 varieties of transgenic rice lines are developed in china. To detect the presence of GM rice in food and feed, we collected information on foreign elements from 250 transgenic rice lines and found 5 elements, by the agrobacterium tumefaciens nopaline synthase terminator (T-NOS). the primers, amplicons, reaction mixture and pcr program were developed based on standards. Rice (Oryzae sativa) belongs to the grass family Oryzeae, and is one of the top most crop in the world. Rice is the second most cultivated crop after wheat. Rice is known as a semi aquatic, annual grass plant and grows in a wide range of soil and water regimes, irrigated, rain fed, lowland, upland and flood prone. It is found in a wide range of areas, from deeply flooded to dry flat fields or hilly terraced or non-terraced slopes. Irrigated rice is defined as rice produced when water is added to supplement that supplied by natural processes such as rainfall.(57)(58)

Rice Types and Products:

There are two main types of rice, Japonica and Indica, differentiated by the area where they are grown and by their traits when cooked. Japonica types grow best in temperate environments and have kernels that are either medium or short in length and are sticky (glutinous) when cooked. Indica types have long kernels that are mostly not sticky (non -glutinous) when cooked and are produced in southern Asia and the Americas. There are three grain types based on kernel traits and cooking qualities which include kernel dimensions of length and width after milling and sizing . Within each kernel type is a brown-rice and white-rice type, depending on how much it has been milled.

Cooking quality is of primary importance to millers and processors. These traits depend on the chemical composition of the rice grain. Long-grain rice is usually dry and fluffy (non-sticky) when cooked and is used in parboiled, quick-cooked or processed-rice products. There are also aromatic long-grain types that have a distinct taste and aroma when cooked.(55)

Medium-grain rice is usually moist, sticky and used for dry cereals, soups, baby food and brewing purposes while short grain rice is very sticky. Sake, or rice wine, and beer are made from non-waxy rice. Other non-grain rice products include rice bran, rice straw, rice milk, etc. Low-amylose rice is used for baby food, cereals, and rice bread, while intermediate-amylose rice is used in fermented rice cakes. High-amylose rice is the basis for extruded-rice noodles. Rice bran is a feed concentrate for livestock and fish. Bran oil is used for human consumption and in pharmaceutical products. The bran itself, though containing protein, has limited use. Traditionally, in many places in Asia, the bran was fed to swine and piggeries, which were often located in the direct of the mills. Rice straw has been used to make coarse paper, as a cellulose source for ruminant livestock, composting and building materials, and as an ultra-pure source of silica. Other uses for rice plants include footwear and headwear, and rice glue from boiling ground rice. One unique use of rice is called rice marble, where rice is placed on book covers during manufacturing for a decorative effect. (54)(58)

RICE VARIETIES :

Depending on the information source, there are between 40,000 and 80,000 rice varieties made up of old traditional varieties, semi-dwarf varieties, and hybrids. Traditional rice varieties have changed through the years from the land races that were tall, slender in stature with droopy leaves, photoperiod sensitive along with a strong grain dormancy to plants with a semi-dwarf stature, erect leaves, photoperiod insensitive, lodging resistant, a greater harvest index as well as a greater responsiveness to fertilizers – particularly nitrogen fertilizers. Through the years traditional varieties have been collected, catalogued, increased, and distributed by private and public institutions such as the IRRI (International Rice Research Institute, Philippines) and the USDA-ARS (United States Department of Agriculture- Agricultural Research Stations) Small Grains Lab. (59)

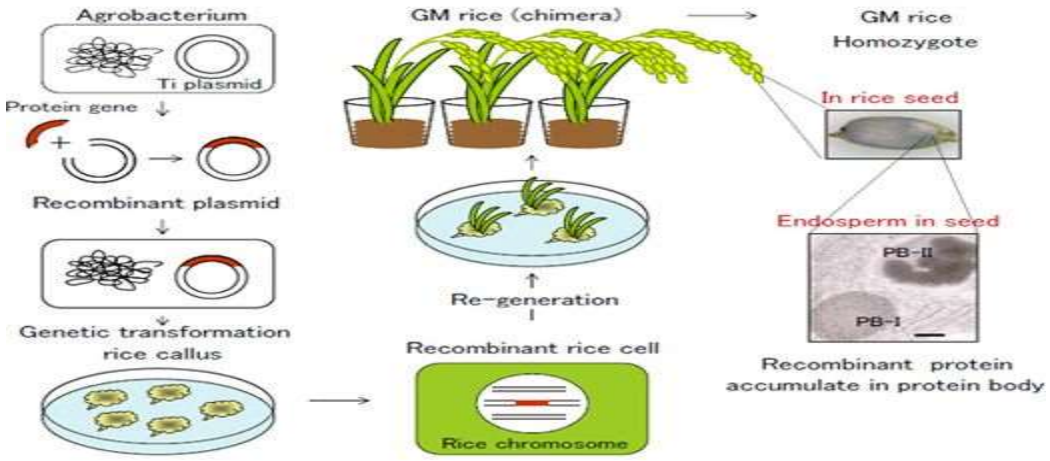
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Newer conventional varieties from public breeding programs are now adapted to shorter growing seasons, tolerant to a-biotic factors such as saline, acidic or alkaline soils, drought, deep water or flooded soils, variations in ambient temperatures, either high or low, and biotic stress factors due to disease or insects, as well as varieties adapted to specialty-use markets; the latter situation is mainly true for irrigated rice, and less for other rice ecosystems where water control is much more a problem. Hybrid rice varieties from private and public breeding programs have been on the increase as breeders seek to match the best traits of two parents in a short time as well as increase yields to meet the consumer demands. The amazing experience of the Chinese public research institutes is a good example of this.
In recent years transgenic / biotech rice varieties have been developed to meet world food needs as a result of increasing food demand of a growing world population, and to incorporate specialty traits Examples of these are: improved nutritional content of rice by adding genes to increase nutrients such as pro-vitamin A (such as in the GMO Golden Rice program), increased iron content, and phytic acid. The incorporation of other traits such as soil acidity tolerance, disease and pest resistance like Bt rice from China, early maturity, heavier grains, longer grain filling period, and sturdier stems, or even a new plant type, all allow for increased production in areas previously limited in their potential.

The primary goal of plant breeding in past years has been to increase yield, improve milling quality, and to introduce good agronomic traits and some level of pest tolerance, primarily against rice diseases. Morphological traits such as semi-dwarf varieties have been added while trying to hold the grain quality constant. Other agronomic traits of importance to breeders include lodging, days to heading, maturity date and straw strength.



Magnaporthe oryzae (M. oryzae) is a plant-pathogenic fungus that causes rice blast, a destructive rice plant diseases around the world. These traditional fungicides are used to control blast. This approach increases the resistance of M. oryzae to fungicides and has adverse effects on human health and the environment and strategy for generating disease resistant, drought resistant and high quality rice varieties.(45)(46)(47)



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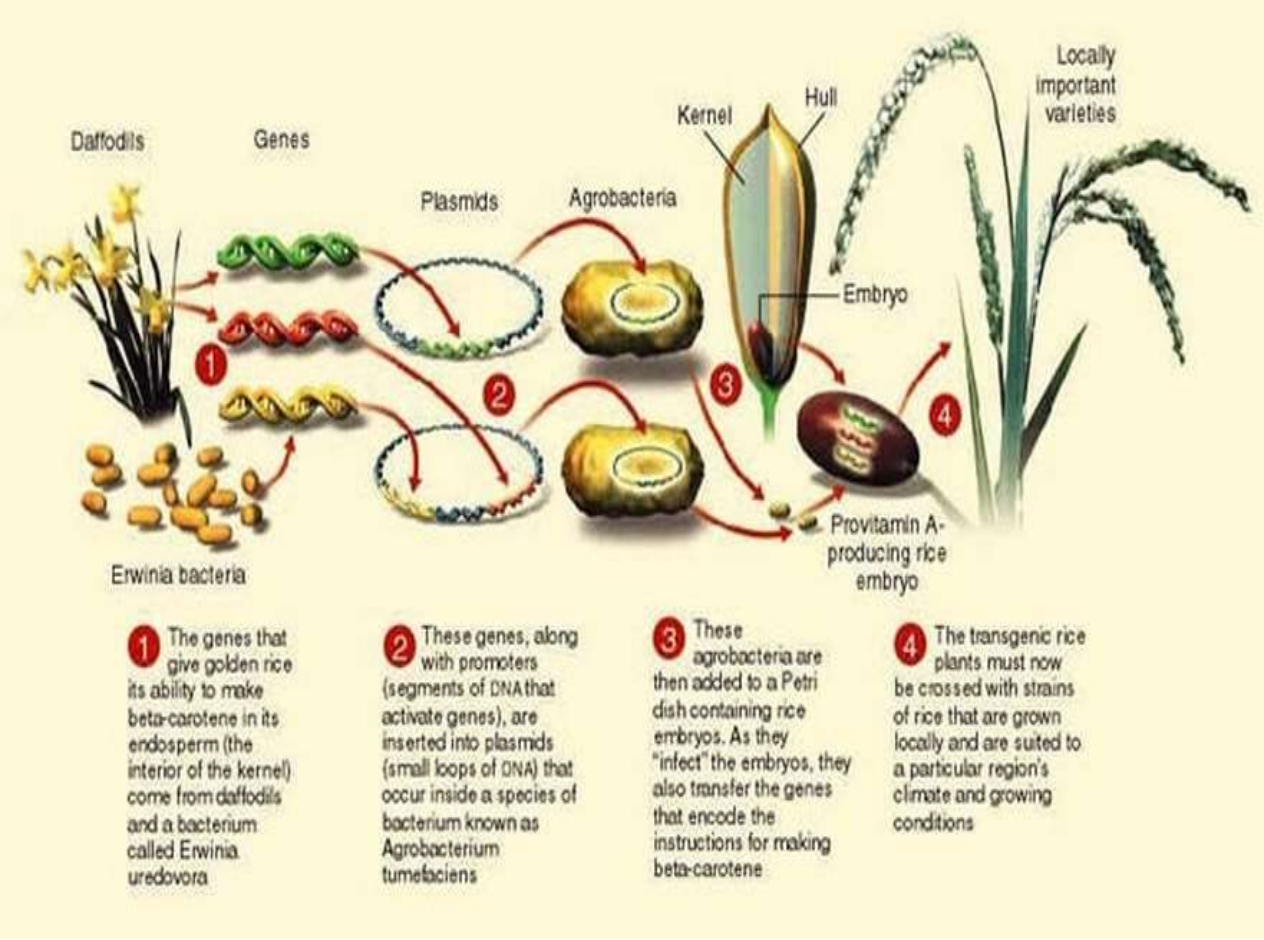
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GOLDEN RICE :

Golden rice is rich in vitamin A. Rice kernals are deficient of vitamin A, so people who obtain most other calories from rice are deficient of vitamin A as rice does not contain it. German and Swiss researchers have engineered rice to produce beta carotene which is a precursor to vitamin a in the rice kernel, The beta carotene turns processed white rice to gold colour hence the name is given as golden rice. Hence people who consume golden rice can obtain vitamin A which contains beta carotene. (55). The Golden Rice Project was first introduced in 1999, when two professors Ingo Potrykus and Peter Beyer, proposed their project to Rockefeller Foundation to genetically engineer rice to increase its nutrients. Rockefeller Foundation supported their goal to provide a sustainable biofortification approach to combat vitamin A deficiencies in developing countries. Vitamin A deficiency (VAD) is prevalent in developing countries whose diets are dependent on rice or other micronutrient-poor carbohydrate foods, which do not contain vitamin A. The World Health Organization estimates that about 250 million preschool children are affected by VAD and about 2.7 million children die because of the deficiency. VAD can have numerous negative health effects such as dryness of the eye that can lead to blindness if untreated; reduced immune system response, and an increase in the severity and mortality risk of infections. VAD is one of the main causes of preventable blindness of young children from developing countries.

The Golden Rice Project began as a proposed solution for VAD; however, despite the establishment of a Humanitarian Board and abiding by national and international regulations governing GMOs, opposition to the project has blocked the roll-out of the Golden Rice Project in developing countries. (44)



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DETECTION OF GM CROPS AND PRODUCTS:

GM crops and their products can be identified by detecting either the inserted genetic material at DNA level, the mRNA transcribed from the newly introduced gene, the resulting protein, metabolite or phenotype. The analytical tests are generally carried out with the polymerase chain reaction (PCR method) detecting the inserted DNA, immunological assays detecting the resulting protein, or using bioassays to detect the resultant phenotype. Although much progress has been achieved in the development of genetic analysis methods, such as those based on the use of PCR, several other analytical technologies that can provide solutions to current technical issues in GM sample analysis are emerging. These methods include mass spectrometry, chromatography, near infrared spectroscopy, micro fabricated devices and, in particular, DNA chip technology (microarrays). So far only PCR has found broad application in GMO detection as a generally accepted method for regulatory purposes. In general the procedure consists of three distinct steps: (39)(40)(41)

Detection: The objective is to determine whether a product is GM or not. For this purpose, a general screening method can be used. The result is a positive/negative statement. The screening methods are usually based on the PCR, immunoassays or bioassays. Analytical methods for detection must be sensitive and reliable enough to obtain accurate and precise results.

Identification: The purpose of identification is to find out which GM crop or product are present and whether they are authorized or not in the country.

Quantification: If a crop or its product has been shown to contain GM varieties, then it became necessary to assess compliance with the threshold regulation by the determination of the amount of each of the GM variety present. Normally, quantification is performed using Real time PCR. GM detection methods are available to monitor and verify the presence and distribution of traits which are accurate, fast, inexpensive and methods that are developed to analyze the GMOs in food products using PCR, ELISA (Enzyme Linked Immunosorbent Assay),microarrays and electrophoresis. The most commonly used method for determination of GMOs in sample is PCR, that is well chosen ,specific transgenic DNA segments have been demonstrated for qualitative and quantification of transgenic crops. PCR is very effective and reliable method for nucleic acid detection ,high specificity and sensitivity. It is tested according to the targets and divided into four types :

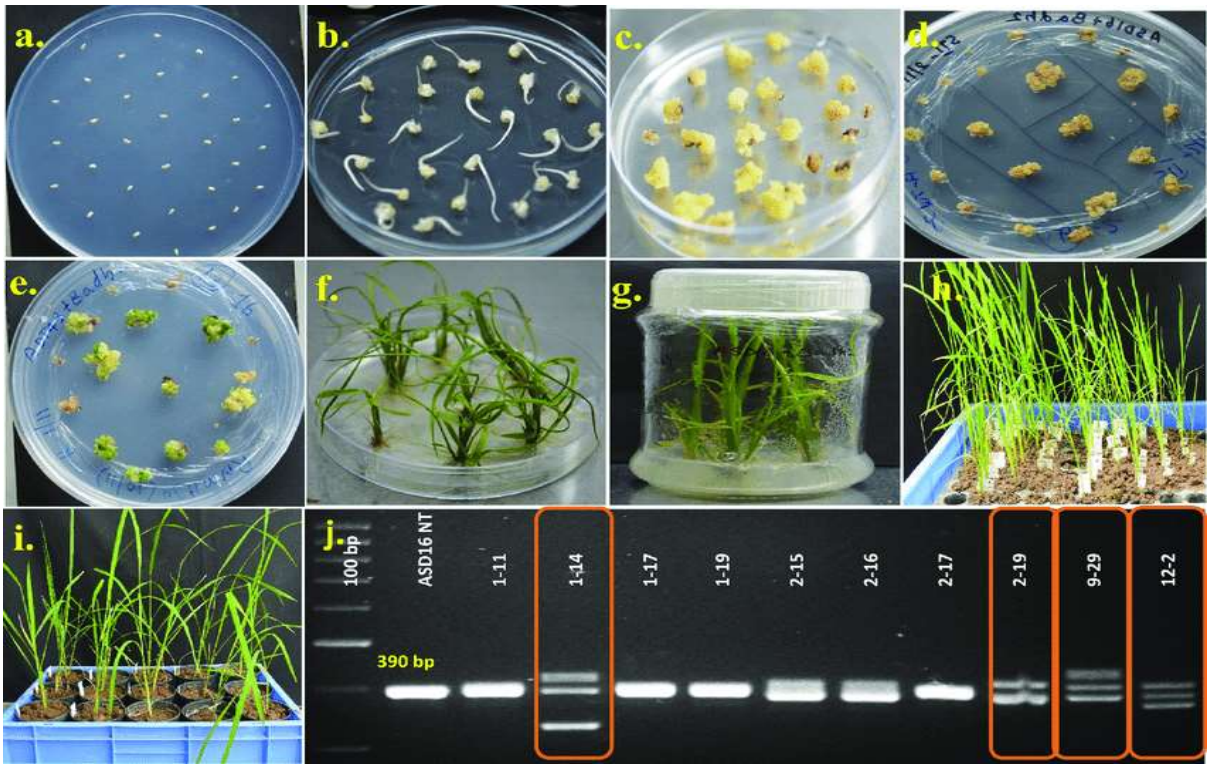
1.Screening tests, the targets of which are the specific promoter and terminator.
2. Gene specific detection, the targets of exogenous genes.
3.Structure specific detection, which are structures of transgenic elements.
4. Event specific detection the targets of which sequence between the exogenous DNA and plant genome.

It depends upon materials which are used for calibration and quality control of GMO detection. plasmids have been demonstrated to be good alternative to reference materials. They may be single or multiple target plasmids, containing either one GM specific or endogenous sequence or several specific GM elements from one species. The transformation involves many genes from various sources than a crossable plant. one of the approach is cisgenesis ,the origin of the gene for transfer is an identical copy of the endogenous gene including the promoter, introns and the terminator, which is produce apple resistant to scab and to fire blight. Cisgenic barley is produced with improved phytase activity .The another approach is intragenes which can have different elements of different genes from the same crossable plant. The another transformation is Agrobacterium mediated, it is differ from one species to another species from one cultivar to another. It has tremendous advantages over direct transformation since it reduces the copy number of transgene.

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DNA-BASED METHODS :

DNA based methods are based on detection of the specific genes, or DNA genetically engineered into the crop. Although, there are several DNA based methodologies, the most commercial testing is conducted using PCR technology. The PCR technique is based on multiplying a specific target DNA allowing the million or billion fold amplification by two synthetic oligonucleotide primers. In PCR, the first step in a cycle involves separation of the two strands of the original DNA molecule. The second step involves binding of the two primers to their oligo nucleotide primers. The third step involves making two perfect copies of the original double stranded DNA molecule by adding the right nucleotides to the end of each primer, using the strands as templates. Once the cycle is completed, it can be repeated, and for each cycle the number of copies is doubled, resulting in an exponential amplification. The amplified fragment can be detected by gel electrophoresis or hybridization techniques. The process consists of extraction and purification of DNA, amplification of the inserted DNA by PCR and confirmation of the amplified PCR product. In principle, PCR can detect a single target molecule in a complex DNA mixture.

QUALITATIVE PCR ANALYSIS:

The most critical parameter for successful PCR is the design of primers. A poorly designed primer can result in little or no product due to non-specific amplification . It is essential that care should be taken in the design of primers for PCR. Several parameters. The selection of primers is the most important component for detection of GM crop by PCR and it depends upon the choice of target gene. The general screening PCR detects the presence of GMO, which then need to be identified.

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MULTIPLEX PCR- BASED DETECTION METHODS:

With multiplex PCR-based methods, several target DNA sequences can be screened and detected in a single reaction. The advantage of multiplex methods is evidently that fewer reactions are needed to test a sample for potential presence of GMO-derived DNA. Development of multiplex assays requires careful testing and validation. After the PCR the resulting pool of amplified fragments needs to be further analysed to distinguish between the various amplicons.

QUANTITATIVE PCR :

In principle, PCR based quantitation can be performed either after completion of the PCR (end-point analysis), or during the PCR (real-time analysis). Quantification using conventional PCR: Conventional PCR measures the products of the PCR reaction at the end point in the reaction profile. End-point analyses are based on comparison of the final amount of amplified DNA of two DNA. The competitor has the same binding sites for the same primer pair but is different in size. This is called competitive quantitative PCR, and the two DNA targets are amplified with equal efficiency. A dilution series of the DNA to be analysed is prepared, and a constant amount of the competitor is added. After completion of the PCR the resulting amplification products are visualized through gel electrophoresis and when both DNA targets yield the same amount of product it is assumed that the starting amount was also the same. By setting up two competitive PCRs, one for the GM crop (e.g. Bt Corn) and one for the species of interest (e.g. maize), and including competitors in both, the quantity of GM crop relative to the species can be estimated by extrapolation from the degree of dilution and concentration of the competitors. The competitive PCR methods are semi-quantitative. Quantification using Real-time PCR: Real-time PCR is a system based on the continuous monitoring of PCR products. This is done via fluorometric measurement of an internal probe during the reaction. In real-time analyses the amount of product synthesized during PCR is estimated directly by measurement of fluorescence in the PCR reaction. Several types of hybridisation probes are available that will emit fluorescent light corresponding to the amount of synthesized DNA. However, the amount of synthesised product can also be estimated with fluorescent dyes, e.g. SYBR Green I that intercalates double-stranded DNA. With the latter, it is not possible to distinguish between the specific product and non-specific products, and consequently the use of specific hybridisation probes is normally preferred. The quantitative estimate is based on extrapolation by comparison of the GM crop sequence relative to the reference of interest (e.g. gene sequence from Roundup Ready soybean and lectin gene from soybean). The idea is that with the use of fluorescence it becomes possible to measure exactly the number of cycles that are needed to produce a certain amount of PCR product. (39)

SOUTHERN BLOTTING :

The molecular characterization of a single copy gene in the genome of a transformed sample or GM crop is performed by Southern blot hybridization (Southern 1975). SB is the most commonly used technique for the nucleic acid (DNA)-based detection of successful gene integration, copy number insertion, and incorporation of sequences from the transformation plasmid backbone. SB is a low density screening method that employs a combination of few restriction enzymes and probes . PCR-based detection Molecular detection methods based on PCR are used to determine the presence of small quantities of foreign DNA/transgene inserts in genetically modified crops.

PROTEIN BASED METHODS :

Immunoassay is the current method for detection and quantification of new (foreign) proteins introduced through genetic transformation of plants. Immunoassay is based on the specific binding between an antigen and an antibody. Immunoassays can be highly specific and samples often need only a simple preparation before being analysed. Moreover, immunoassays can be used qualitatively or quantitatively over a wide range of concentrations. Western blot, ELISA (Enzyme-Linked Immunosorbent Assay) and lateral flow sticks are typical protein-based test methods.

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ELISA (Enzyme Linked Immunosorbent Assay) :

In ELISA the antigen-antibody reaction takes place on a solid phase (microtiter plates). Antigen and antibody react and produce a stable complex, which can be visualised by addition of a second antibody linked to an enzyme. Addition of a substrate for that enzyme results in a colour formation, which can be measured photometrically or recognised by naked eye. ELISAs have been designed to detect a novel GM protein or trait. There are the reports that some ELISA detected the novel protein such as the CP4 EPSPS protein from A. tumefaciens expressed in Tripathi 1477 CP4 EPSPS protein from A. tumefaciens expressed in Roundup Ready Soybeans.

Lateral flow sticks :

The lateral flow test (dipstick format) uses a membrane based detection system. The membrane contains two capture zones, one captures the bound GM protein, the other captures color reagent. Paper strips or plastic paddles are used as support for the capture antibody that is immobilized onto a test strip in specific zone. Most tests are provided usually in kit form. The lateral flow test strip is dipped into the prepared sample in extraction solution and the sample migrates up the strip by capillary action. As the sample flows through the detection antibody strip and the capture antibody strip, the protein of interest will accumulate and thus give a high intensity band, but the volume is not as well controlled. These tests generally provide qualitative or semi-quantitative results using antibodies and colour reagents incorporated into a lateral flow strip. Lateral flow techniques are qualitative or semi quantitative.

PHENOTYPIC CHARACTERISATION (HERBICIDE BIOASSAYS) :

Phenotypic characterisation allows detection of the presence or absence of a specific trait. So far only tests for traits as herbicides tolerance are available. Such methods can be used to test for presence or absence of herbicide resistant GM varieties and is termed herbicide bioassays. They consist of conducting germination tests on solid germination media in the presence of a specific herbicide, where non-GM and GM seeds show distinct characteristics. The detection level is dependent on germination of the seed and the germination methods should ensure that all viable seeds of the tested sample germinate. Seeds tested positive should be exposed to subsequent tests for confirmation. The herbicide bioassay tests are claimed to be accurate, inexpensive, and useful as a preventative test primarily for seed companies. Companies are using the herbicide bioassays to check individual shipments as a quality assurance program. Negative trait and positive trait seeds should be included as controls with every sample testing. At the moment herbicide bioassays are available for Roundup Ready soybean, maize, cotton and oilseed rape, and Liberty Link maize. In the future bioassays for insect-resistant or other GM varieties may be developed.

ADVANTAGES OF GENETICALLY MODIFIED CROPS :

Genetic modification can provide improved resistance against diseases and pests, hence the cost incurred in crop cultivation will decrease. It can provide production for more nutritious staple crops which will provide micronutrients in diet of poor people. The crops will be able to cope with problems such as droughts or salty soils. It can also increase the productivity of crops thus helping in growing of the population. Various studies on GM crops have found that quantity of pesticides and insecticides used for GM crops is much decreased (about 39% -50%) than that used in other crops. can solve food shortages. Richer in nutrients. Better in taste and quality: with changes to other genes, the genes of the crops can also be altered to have a better flavor and nutritional value. cheaper and more efficient. quick growing time. Development of flood resistant crops. Improved nutritional quality. Reduced herbicide use is better for the environment.

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RISKS INVOLVED IN THE GM CROPS CULTIVATION AND CONSUMPTION :

The effects of GM crops on human health have not been sufficiently examined, so critics argue that these should not be used until there is a guarantee that it will not affect human health. There can be irreversible effects on biodiversity as the modified genes can get transferred to other plants and organisms, and lead to widespread destruction. There is a fear that the benefits of GM crops will not reach small scale farmers and make them poorer . As the result of commercialization, the private firms might sell their findings to other countries and real masses will not be benefitted. As India has passed the Food Security Act, the govt. might approve for certain crops to meet the increasing burden on agriculture , this might cause damage to the whole ecosystem. production of toxins, allergens, antinutrients and diseases. Not totally safe to eat : food allergies has been risen significantly. Decreased antibiotic efficacy. cross pollination : the pollen from the GM plants is also contaminated

New diseases will arise. Food supply at risk. Leakage of GM proteins into soil. Disruption of traditional practices and economic in less developed countries.

CHALLENGES :

Harm to wildlife. Harm to soil. Indirect harm to human health: pesticide use. Direct harm to human health : consuming GE foods, Antibiotic Resistance. Lack of labelling and transparency.

FUTURE PREDICTIONS:

As the topic of genetically modified crops becomes more popular and public awareness is raised there will be a continuation of concern and research regarding the new technology. Clearly as more information is discovered there will be opposition to the new technology and support at the same time. As far as India is concerned, their agriculture will become dependent upon the GM crops and it will help the farmers of the regions that are currently producing very low average cereal crop yields. The trait that prevents disease, protect in water limited areas and allow vitamin enriched crops will be taking advantage of fully. This will feed more of the starving population. Of course, there is always a downside to every solution; the problem with the new technology is that government policy must be implemented or there will be limited success of the GM crops. As long as there is support to provide GM seeds and transportation to move the crops for sales the farmers will benefit from the new agriculture technology. Since so many people are and will be affected by the GM crops scientists will continue research of the health impacts of modified foods. There will be further discoveries of benefits and costs of consumption of GM foods.

FRAMEWORK :

Overall, use and research of GM crops have only just begun and they will only increase. They are most beneficial in India at the time being but, things may change and the technology may be altered to help benefit the agriculture of regions around the world.

Although the number of people in danger of malnutrition worldwide has decreased in the past 30 years, thanks in part to the Green Revolution of the 20th century, an estimated 800 million people still lack adequate access to food. The second revolution is agricultural revolution , the so-called Gene Revolution, in which modern biotechnology can enable the production of genetically modified (GM) crops tailored to address chronic agricultural problems in specific regions of the world. Genetically Modified crops has huge benefits to the society by increase in crop production and cultivation.

India’s policy on GM crops has undergone various shifts. India set up a regulatory structure to screen GM crops and showed interest in agricultural biotechnology as early as the 1980s. The first application for the commercialization of a GM crop

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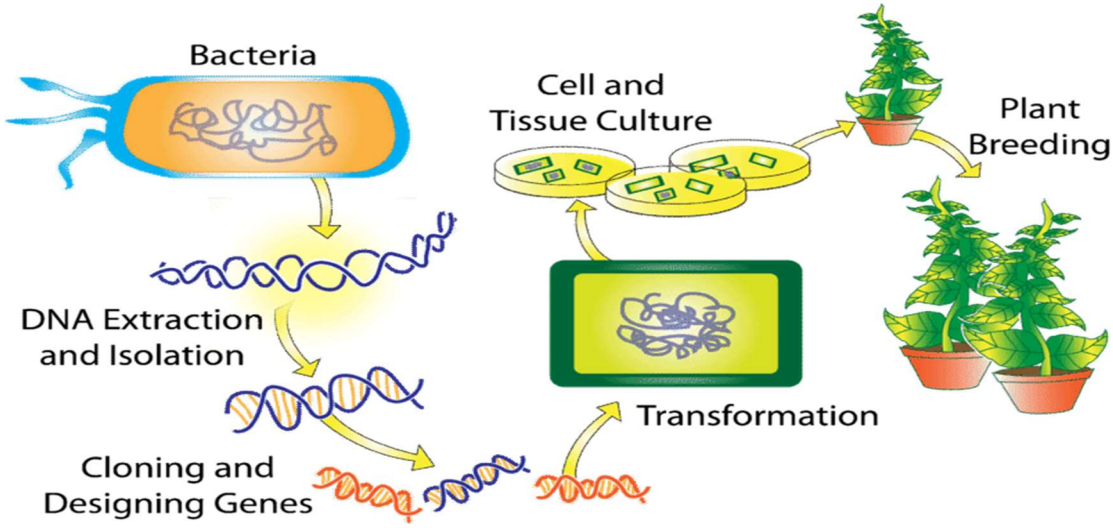
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was accepted in 1996, but it was only 8 years later 2002 that the first GM crop, Bt cotton was introduced , after being rejected just a year earlier in 2001. In 2005, more application for Bt cotton were cleared ,but no other GM crop has been approved.

DEVELOPMENT OF CROPS
PROCESS OF DEVELOPING GENETICALLY MODIFIED CROPS :

The main steps involved in the development of GM crops are :



1.ISOLATION OF GENES OF INTEREST :
Existing knowledge about the structure, function or location on chromosomes is used to identify the genes that is responsible for the desired trait in an organism for example, drought tolerance or insect resistance.

2.INSERTION OF THE GENE INTO A TRANSFER VECTOR :
The most commonly used gene transfer tools for plants is a circular molecule of DNA (plasmid) from the naturally occurring soil bacterium, Agrobacterium tumefaciens. The gene of interest is inserted into the plasmid using recombinant DNA (rDNA) techniques.

3.PLANT TRANSFORMATION :
The modified A. tumefaciens cells containing the plasmid with the new gene are mixed with plant cells or cut pieces of plants such as leaves or stems. Some of the cells take up a piece of the plasmid known as T- DNA to transfer DNA is particle bombardment method where the small particles are coated with DNA molecules are bombarded into the cell.

4.SELECTION OF THE MODIFIED PLANT CELLS :
After the transformation, various methods are used to differentiate between the modified plants and the majority of the cells that are not incorporated the desired genes. The selectable marker genes that confer antibiotic or herbicide resistance are used to favor growth of the transformed cells relative to the non transformed cells. For this method, genes responsible for resistance are inserted into vector and transferred along the genes conferring desired traits to the plant cells. When the cells are exposed to the antibiotic or herbicide, only the transformed cells(containing and expressing the selectable marker gene) will survive. The transformed cells are then regenerated to form whole plants using tissue culture methods.

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5.REGENERATION INTO WHOLE PLANTS VIA TISSUE CULTURE :
It involves placing the explants (plant parts/ cells) onto media containing nutrients that induce development of the cells into various plant parts to form whole plantlets. Once the platelets are rooted they are transferred to pots and kept under controlled environmental conditions.

6.VERIFICATION OF TRANSFORMATION AND CHARACTERIZATION OF THE INSERTED DNA FRAGMENT:
Verification of plant transformation involves demonstrating that the gene has been inserted and is inherited normally. Tests are done to determine the number of copies inserted. Testing of gene expression is done to make sure that the gene is functional.

7.TESTING OF PLANT PERFOMANCE :
It is determined whether the modified plant has the desired new trait and does not have any new unwanted characteristics. Those that perform well are planted into the field for further testing. In the field, the plants are first grown in confined field trials to test whether the technology works in the open environment . If the technology works then the plant are tested in multi-location field trials to establish whether the crop performs well in different environmental conditions, if the GM crops passes all the tests, it may then be conducted for commercial production.

8.SAFETY ASSESSMENT :
Food and environmental safety assessment are carried out in conjunction with testing of plant performance. Description of safety testing are described in the Food Safety Assessment and Environmental Safety Assessment.(51)(52)(53)

MATERIALS AND METHODS :
A SIMPLE AND ACCURATE PCR METHOD FOR DETECTION OF GENETICALLY MODIFIED RICE
Cauliflower mosaic virus (CaMV) 35S promoter (P-35S) and nopaline synthase terminator (T-nos) from Agrobacterium tumefaciens are the most common transgenic elements that can be targeted for GMO screening . These genetic elements are frequently used to indicate whether the analysed sample contains GM ingredient or not. In addition, the proper Certified Reference Material (CRM) is necessary as a positive control for validation of GMO detection method.(17)(18)(19)

A.SAMPLE PREPARATION :
Two certified reference materials (CRMs) were obtained from the Institute Of Reference Materials and Measurements (IRMM, Geel, Belgium) in the E.U. these two referenceswere two available GM varieties (Bt 11 maize 5% and Roundup Ready soy 5%), which GM target sequences are present in both, thus have been used as the positive controls in present study. 81 rice seed samples each consists of 500g were purchased randomly from various local markets in Tehran, during 2018. (20)(21)
All the samples were homogenized using an electric homogenizer and stored at −20°c before DNA extraction.

B.DNA EXTRACTION AND QUALIFICATION :
The seed samples which are taken and references were grounded with an electric grinder. From all the samples the Genomic DNA was extracted by using DNA extraction kit from plant materials (MBST, IRAN) according to the instruction , some adjustments are also done by improve the quality of DNA.(22). 100milligrams of the powder (grinded seeds) are transferred into the clean Eppendorf tube containing 300µl lysis buffer. 20µl proteinase k was added to the mixture and incubated for 15minutes at 60 °c. After the completion of incubation period 580µl of binding buffer was added to the tube and these are mixed by vortexing and incubated for 10 minutes at 70 °c, and then centrifuged for 1 minute by 8000xg and now transfer the supernanent into the clean 1.5ml Eppendorf tube. Place a spin column (A) in a 1.5 ml Eppendorf tube and then supernatant applied to the spin column (A) and centrifuged at 8000xg for 1 minute, removed the column(A), then 440µl ethanol (100%) added into the solution and then transfer into new spin column B and centrifuged at 8000xg for 1 minute. After the process of centrifugation, 500µl wash buffer added to column B and centrifuged at 8000xg for 3 minutes to

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remove the ethanol completely. The tube containing infiltrate can be discarded. Now place the spin column B in a clean Eppendorf tube and then add 35-50µl elution buffer preheated to 70°c to the column and incubated at room temperature for 3 minutes, then centrifuged at 8000xg for 1 minute. The solution obtained, stored at −20°c prior to screening. The purity and quality of isolated DNA is the main step to the efficiency of the PCR. The concentration and purity of extracted DNA was evaluated by ultraviolet (UV) absorption at a wavelength of 260 nm and 260/280nm using Nanodrop spectrophotometer.

C.OLIGONUCLEOTIDE PRIMERS : (23)(24)(27):

4 primer pairs are used for detection of GM rice's. Moreover, the sucrose phosphate synthase (SPS), soy Lectin and maize invertase genes were used as rice specific, soy specific and maize specific endogenous reference genes respectively. Therefore, the three primer pairs including SPS-F/R, Lectin -F/R and Invertase -F/R were used for verification of the presence and quality of extracted DNA from rice samples, soy CRM, and maize CRM, respectively. The DNA quality determination of certified reference materials (CRMs) is required to detect the GM rice samples accurately. These materials are presented as control group in this study. The primer pairs p-35S and T-nos were prepared according to the International Standard Organisation. The sequences of the SPS, lectin and Invertase genes for primer designing were obtained. The target sequences were also analysed using NCBI primer BLAST search to verify the specificity of the primers. The information of primers used are as follows :

Primer name	Sequence (5'-3')	Target	Lenth
SPS-F	TTG CGC CTG AAC GGA TAT	SPS	277
SPS-R	GGA GAA GCA CTG GAC GAG G		123
P35S-cf3	CCA CGT CTT CAA AGC AAG TGG	P-35S	123
P35S-cr4	TCC TCT CCA AAT GAA ATG AAC TTC C	P-35S	
HA-nos-118f	GCA TGA CGT TAT TTA TGA GAT GGG	T-NOS	
GM03	GCC CTC TAC TCC ACC CCC ATC C	Lectin	118
GM04	GCC CAT CTG CAA GCC TTT TTG TG		118
IVR1-F	CCG CTG TAT CAC AAG GGC TGG TAC C	invertase	226
IVR1-R	GGA GCC CGT GTA GAG CAT GAC GAT C		

D. PCR CONDITIONS :

1.The analysis of PCR were carried out in a thermal cycler. The amplification reaction contains 2µl of genomic DNA and PCR mixture. 2. the reaction mixture including 12µl ready to use PCR master mix 2x (The composition: TrisHCL pH 8.5, (NH4)2SO4, 3 mM Mgcl2, 0.2% Tween 20, 0.4 mM dNTPs, 0.2 units/µl Amplicon Taq DNA polymerase, Inert red dye and stabilizer), 1µl of each primer, and 9µl sterile free ions distilled water. 3. The concentration of primers for all target gene was 0.1µl.Finally, PCR assays were performed in a volume of 25µl. 4. The reaction conditions of PCR were as follows: **A**. for SPS: initial denaturation at 94 °C for 5 min, amplification at 94 °C for 30 s, annealing at 58 °C for 45 s, extension at 72 °C for 75 s, and a final elongation for 8 min at 72 °C.

B. For GM03/GM04: initial denaturation at 94 °C for 5 min, amplification at 94 °C for 1 min, annealing at 60 °C for 40 s, and a final elongation for 8 min at 72 °C. **C.** For IVR1-F/IVR1-R: initial denaturation at 94 °C for 5 min, amplification at 94 °C for 1 min, annealing at 64 °C for 40 s, and a final elongation for 8 min at 72 °C. **D.** For P35S-cf3/P35S-cf4 and HA-nos118f/ HA-nos-118r: initial denaturation at 94 °C for 5 min, amplification at 94 °C for 1 min, annealing at 60 °C for 40 s, and a final elongation for 8 min at 72 °C.

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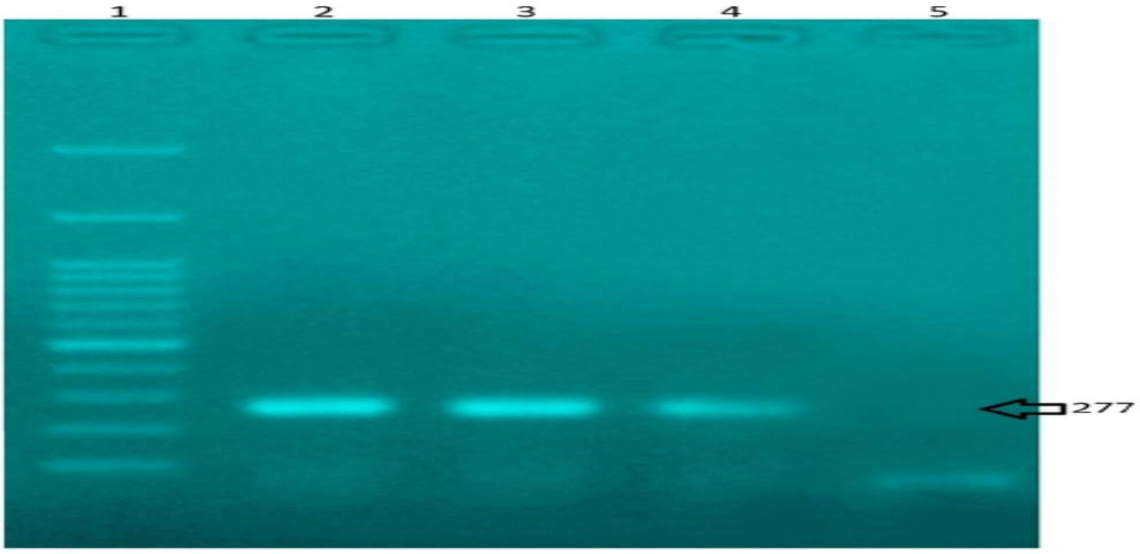
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E. AGAROSE GEL ELECTROPHORESIS :

8µl of PCR products (including PCR amplification products, positive and negative controls) were electrophoresed on 2% agarose gel containing 2µl DNA safe stain at 80 Voltage in 1× TBE running buffer (containing 600 ml dH2O, 48.4 g Tris base, 11.42 ml glacial acetic acid and 40 ml EDTA (0.5 M), PH 8.0, then dilute with dH2O to obtain a final volume of 1 L) for 60 min. 4µl of 100 bp DNA ladder was used as a reference marker. DNA fragments were separated through a gel based on size and then visualized using UV transilluminator.

RESULTS AND DISCUSSION :

The present study about 40µl DNA solution was obtained from rice seeds and reference materials using DNA extraction kit (MBST, IRAN). (25) . The range of absorbance ratio to quality control and concentration of DNA extracted for both sample types was between 1.7 and 1.9. But in some cases, for achieving to this range we repeated attempts.(26)(28). The internal positive control such as SPS endogenous PCR products was amplified to confirm the presence of a sufficient amount of DNA from rice samples are as follows :



Agarose gel electrophoresis of PCR products from rice samples;
1. 100 bp DNA ladder 2. Standard rice sample 3. Rice sample 1, 4. Rice sample 2,
5. sterile water

Moreover, to reduce false positive results during the extraction of DNA or PCR analysis, sterile water was used in parallel to the sample preparation and analysis.

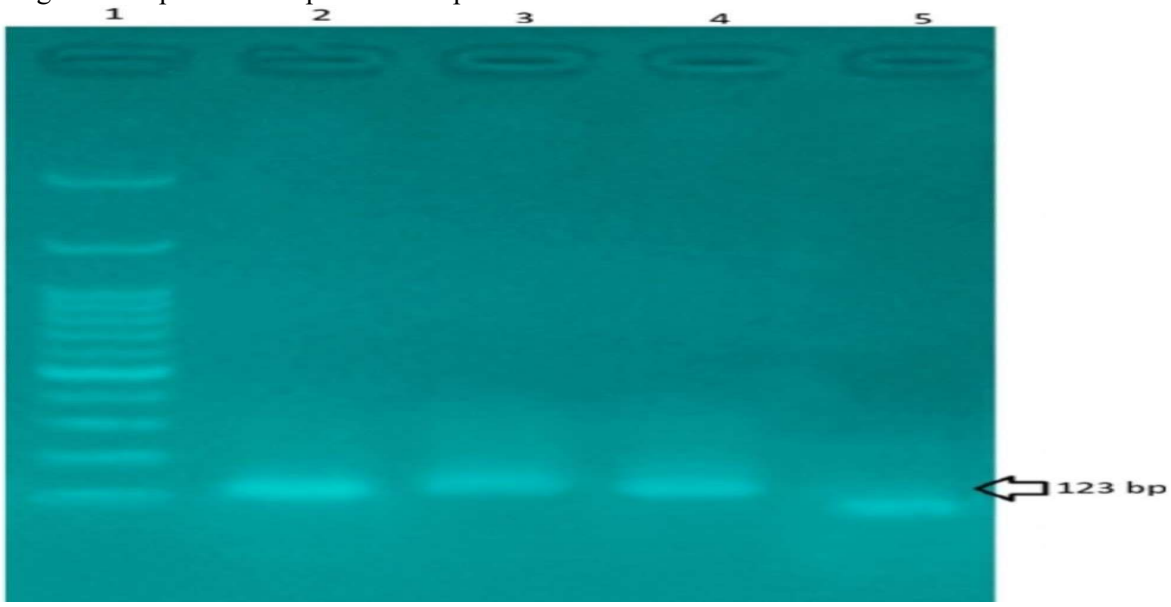
All 81 positive sample rice screened for SPS gene were analysed for detection of the CaMV 35S promoter and NOS terminator which indicates the presence of GMO targets. The control of these samples was performed with extracted DNA from GM soy. The CaMV 35S promoter sequences was observed only in two DNA rice samples in a size of 123 bp using a primer pair P35S-cf3/P35S-cf4. While none of the analysed samples were positive for the presence of NOS terminator using primer pair HA-nos-118f/ HA-nos-118r at the size of 118 bp. (30)(31)

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The results of gel electrophoresis for positive samples are as follows:



Agarose gel electrophoresis of PCR products from rice samples :
1.100 bp DNA ladder 2. positive control 3. Rice sample 1, 4. Rice sample 2, 5. Negative control

The results of sequencing, were analysed with BLAST search of the NCBI and revealed that 2 of the 81 samples contained 35S promoter. In our present study, out of 81, 2 rice samples show positive results for the primer pair P35S-cf3/p35Scr4, are to be genetically modified while none of them has been labelled. However, because of the assessment of Food and Drug Administration, the positive results might be due to illegal entry of this product to Iran or their infected by the CaMV virus.(29)(30).

CONCLUSION :

Genetically modified crop technology may possibly be helpful to increase productivity in agriculture and in reducing seasonal fluctuations against ever increasing demand for food and other agricultural products, global climatic changes, scarcities in natural resources etc. GM crops may reduce the environmental and health related problems by reducing the use of chemical pesticides and insecticides. Despite the current uncertainty over GM crops, one thing remains clear. This technology, with its potential to create economically important crop varieties, is simply too valuable to ignore. There are, however some valid concerns. If these issues are to be resolved, decisions must be based on information. Finally, given the importance people place on the food they eat, policies regarding GM crops will have to be based on an open and honest debate involving a wide cross section of society. There is no single solution for solving all the problems of developing countries but GM crops can contribute in the reduction of food scarcity and sustainable growth in agricultural sector. So there is a need to support GM crops for more development . Hence , more and more scientific investigations are necessary to achieve the significant trust from the general people and thus can ensure the true facts of this technology in the path of sustainable development in crop production.

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