A Comprehensive Review on Crop Modification Techniques Using Traditional and Modern Biotechnological Approaches

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ABSTRACT

Crop is a plant or plant product that can be grown and harvested for beneficial purposes and profit. Food crops are the major source of food in our daily life and are harvested by human consumption. Today, in the growing world, crop modification is taking rule rapidly. Crop modification refers to the alteration of the genetic makeup of plants to achieve specific traits which are desired. Over time, repeated breeding and selection lead to the production of new plant varieties having desired significant characteristics. Modern biotechnological approaches in crop modification involve advanced techniques that allowed scientists to precisely and selectively modify the genetic makeup of crops. In the current time, Clustered Regularly Interspaced Short Palindromic Repeats can be used to modify targeted genes of the plants for desirable traits with greater efficiency and accuracy. In forthcoming, CRISPR technology will be used at a large scale for the crop modification by reducing chances of false genome sequencing and inventing economical protocols for regeneration of various plant varieties. To conclude the whole, it is reviewed that science is emerging rapidly in the field of crop modification.

Keywords: Crops, Conventional Breeding, CRISPR, Mutagenesis, TALEN, ZFN.

INTRODUCTION

Crop cultivation is rudimentary aspect of agriculture and plays significant role in providing sustainable resources [1]. When the same types of plant are grown at one place on a large scale, it is called as crop. These crops are grown by the process of agriculture by using water, seeds, and influenced by abiotic and biotic factors. Human life is not imaginable without agriculture

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because this provides the important foods to live a sound life [2]. Food crops are the major source of food in our daily life and are harvested for human consumption. The crops which are used for food purposes are known as staple crops. Wheat, rice, maize and beans are widely recognised as basic food crops. These crops are commonly used in underdeveloped nations such as Pakistan and India [3]. These are harvested as food for humans or fodder for livestock. Swift growth of population and urbanization have created immense pressures on the agronomy and increased the demands. Crops are classified on the basis of motive and are majorly cultivated [4]. Every living organism needs food to survive on this planet but because of the rapid increase in the population, there is a need of producing more crops to fulfill the requirements of all the organisms [5].

Crops cultivation is a time consuming and laborious process and it may result in good yield or destroy due to environmental factors such as storm, drought and other pandemics that will make whole work of the farmers in vain. For this purpose there is a need of creating techniques that can produce crops easily and in less time. This lead to the modern biotechnological approaches that can be used for the modification of plant [6]. There is a slight resemblance of conventional and modern techniques of crop modification as well as great difference due to occasionally increase in technology [7]. One can differentiate between the conventional and modern methods by comparing them as conventional methods are time taking, no guarantee of modification, and undesirable changes also occurred. On the other hand, modern methods are less time consuming, and desirable changes can be made [8,9].

Classification of food crops

Food crops are those plants that are cultivated especially for the parts which are used by humans as food or used for food products processed with these plants [10]. Food crops can be classified on the basis of their season, the place where these crops originated, crop plant botany, and important uses of that crop in our daily life. On the basis of classification of crops, major types include cereal, oil seed crop, fibre crops, sugar crops, medicinal crops, pulses, roots and tuber crops, vegetable and garden crops, etc. All these are essential part of our life due to their variety of usages therefore; there is a need to grow these with good yield and quality [11,12].

Cereal crops

Cereal crops are the grain crops that are used for edible purposes and contribute to the requirements of nutrients and energy for the population which is living around the world [13]. These crops belong to the family Poaceae. Cereals are highly significant due to their extensive cultivation and widespread use as a primary food source worldwide [14]. The cereal grains (staple food crop) are used for the food purpose on a large scale in many countries like Africa, America and New Zealand for example, wheat, rice, etc [11]. These cereal crops are needed to be improved to fulfill the daily needs of the human beings [15,16]. Some of the cereals have deficiency of lysine which is the essential amino acid. This is the reason that some people who are vegetarian use legumes with their cereals. Cereals can be mashed up to make flour which is the main ingredient of bread and is a staple food for many areas. The cereals' high starch content renders them suitable for alcohol production. During the brewing process, the fermentation of starch leads to the formation of ethanol.

Oil Seed crops

For millennia, Olive oil has been a section of human culture. Evidence showed that, olives were converted into oil by 6000 BC and 4500 BC. These crops are majorly grown for oil purpose and this oil is extracted from the seeds of these crops. Plant seeds can be used to extract seed oils. Vegetable oils have been utilized for illuminating lamps and for preparing meals. Oils have a higher heat capacity than water, which means they can be heated to higher temperatures before reaching their boiling point. This is why oils are commonly used for frying foods. Well-known oil seed crops are Brassica, castor bean, soybean and sunflower for the prevention of heart diseases and cooking purposes [11]. Oil seeds can be used for the medication purposes for the diseases like cardiovascular disorders and blood pressure. Scientists are working on improvement of soybean crop production with the help of new genome editing techniques affecting the oil content, height of plant and seed size [17].

Fiber Crops

Fibrous crops serve as the predominant source of raw material in the fiber industry. Examples of materials in this category include cotton, jute, and flax, which are used in the manufacturing of clothing, rugs, and ropes. Due to its extensive use in textile manufacture, cotton has surpassed all other fiber crops in terms of global production, making it the most important crop in the fibre crop category. Fiber in the diet is also very important for humans. In contrast to trees, which take several years to mature, they can be harvested

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after only one growing season.

Sugar Crops

Sugar crops are used for manufacturing sugar by extraction of the sugarcane juice. Sugarcane, sugar beet and sweet sorghum are included in this group of sugar crops [18]. Sugarcane is used for manufacturing of various industrial products, so are also called as industrial crops. Moreover, these are essential for the production of sugar which is used in household cooking [11,19]. The remaining of sugarcane which is known as sugarcane molasses is also considered as a key product in the production of ethanol.

Medicinal Crops

Medicinal crops include those plants which are used to make drugs and are involved in medicinal purposes. For example aloe vera, neem, tulsi, peppermint etc are known as medicinal crops. These major plants are very effective for medication and making specific drugs related to any disease like malaria, Stomach-ache, dysentery and Diarrhea etc [11].

Pulses

After the cultivation of cereals, legumes were produced. According to the archaeological findings, pulses were first consumed by the human beings before 10,000 years BCE. Pulses originated from the tropical areas. Edible seeds and grains of pulses are the reason for cultivation of these legumes. They are the part of family Leguminosae [20]. These are grown agriculturally for the human consumption. Cow pea, pigeon pea, mash bean, lentil and chickpea are included in this group [21,22]. The pulses like mung bean, chick pea are also used for the feed of cattle on domestic level [11]. It is considered as the source of resistant starch that can be used for food energy by the intestinal cells. Many of the legumes contain Rhizobia which is the symbiotic bacteria that have specific ability for the fixation of nitrogen from atmospheric molecular nitrogen into ammonia.

Roots and Tuber Crops

Underground parts of these crops are mainly used which are considered as roots and tuber crops. Rhizome, corns, bulbs, roots and tubers are used as edible food sources. For example, potato, ginger, turnip, garlic and many others belongs to roots and tuber crops [23]. Ginger and garlic are used for cooking purposes in many dishes. As the requirements of the food is increasing so there is the need to grow more roots and tuber crops.

Vegetable and Garden Crops

First, vegetables were collected by hunter gatherers from the wild and then grown in world during the period of 10,000 BC. Vegetables are the part of plants that are considered as food for the consumption of humans and animals. Vegetables can also be eaten raw or cooked to meal. Vegetables are considered high in vitamins and minerals. It is recommended by the nutritionist to consume plenty of vegetables. Vegetable crops are grown as portions like leaves, flowers, or fruits [24]. Cultivation of spinach, cucumber and other vegetables is called olericulture which is the branch of horticulture [11].

Crop Modification

Crop modification means alteration of the genetic makeup of plants to achieve specific advantageous characteristics. This process allowed insertion, deletion, or any other modification in the genetic makeup within the DNA of plants which helped to enhance its characteristics to increase yield, provide resistance against many pests and diseases, improvement of quality, enhancement of nutritional values and other beneficial modifications. The crop modification was done for many years but the scientific era of crop modification started in around 1900.

Conventional crop modification

Agricultural practices that alter plant genetics for human advantage date back to the dawn of crop modification. Before times, some 10,000 years ago, farmers selected the plants having desirable traits such as plants that had better yield, bore largest fruits andeasily available for harvesting. A large amount of food we eat today, were originally produced using a combination of conventional methods [25]. At that time, two techniques had been used to improve the crops which were selective breeding and cross breeding [26,27].

Selective breeding

Conventional methods that were used for the modification selecting and breeding plants with desired traits over successive generations. Selective breeding was done by choosing parents with particular characteristics to breed together and produce offspring with further desirable characteristics. Earliest farmers selected those plants that had beneficial traits, such as those with good yield and were easy to grow and harvest [28]. With the basic understanding,

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these traits passes down from one generation to the next generation, they used those plants and seeds with best traits for the crops of next year for better results to get beneficial crops. By isolation and selection of particular plants, early farmers effectively influenced the cross pollination of the plants [29]. This process was time taking and required a lot of efforts to grow and harvest crops. Moreover, there was no guarantee of desired trait as an outcome.

Cross Breeding

Cross breeding is generally a process of developing and increasing diversity and variety of plants. These cross bred plants have improved quality and quantity of crops as well as they are somehow resistant to the pests, diseases and stresses which occur due to the environment. In this method, farmers selected two plants to produce offspring having desired characteristics of both plants by crossing them [30] (Figure 1). As, plant breeders select plants to go on to the next generation that have advantageous traits they are looking for. For millennia, conventional cross breeding had been the backbone of improvement of the genetics of our crops. Crossbreeding, if they were in same species, only make use of desirable traits. The desired properties included a very good yield, resistance to pests and good quality. This created a filial generation which included all the positive characteristics of the both parents. Some of the examples of plants that were developed using cross breeding included broccolini which is a vegetable crop that resulted by cross breeding broccoli and Chinese kale, sunflower which is used in seed crops, is a hybrid flower that was the result of crossing different species of sun flowers.

An example of plant cross breeding is given (Figure 1) in which two plants having different traits were cross bred which after one year resulted in 50% F1 generation that had both the plants' trait 50% then in second F2 generation, the ratio became 75% . After that, plants having both traits were cultivated again and again until the resulting plant got desired traits and characteristics of both plants that were initially used. This technique is considered to be most time taking and risk containing because of less chance of getting the desirable traits. This process took over 12-15 years to emancipate the variety.

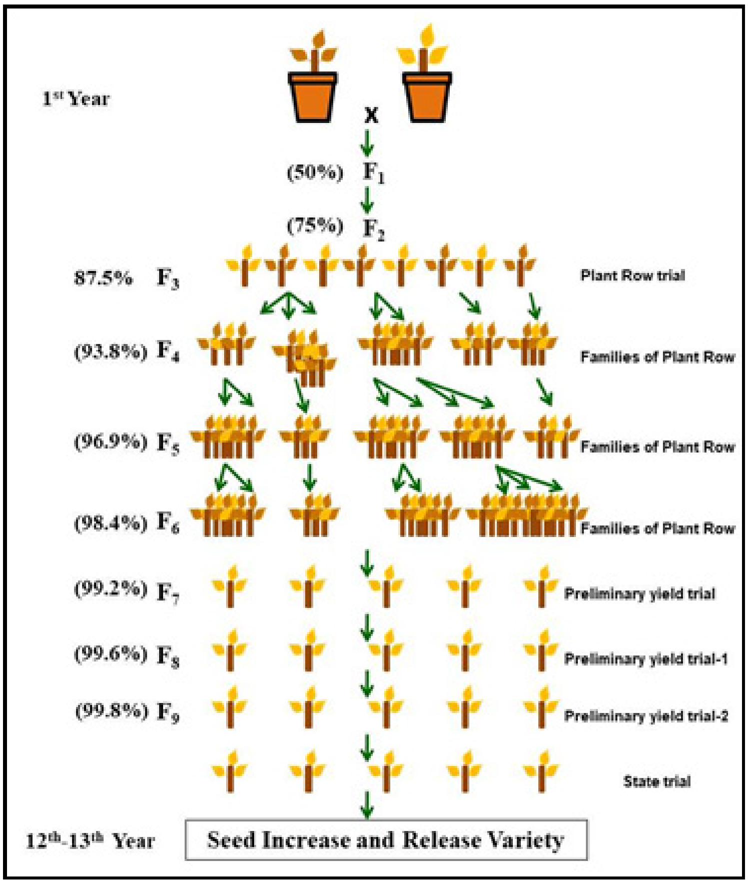

Figure 1. Conventional plant breeding method, known as cross breeding and is used to enhance traits of crop since long time. Finally, best progeny with desired trait having variety was selected.

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This method also required back crossing, rigorous selection cycle, careful planning and was complicated process [31]. Time consuming, less efficient and contained many other limitations as well thus; new techniques were required to overcome the issues [32].

Modern biotechnological approaches for crop modification

Modern biotechnological approaches in crop modification involve advanced and new techniques that allowed scientists to precisely modify the genetic makeup of the crops [33]. There was a need of less time consuming techniques to be used for the crop modification to overcome the requirements of the world food strategies. These approaches included mutagenesis, polyploidy, protoplast fusion, transgenesis and genome editing.

Mutagenesis

The heritable change in the genetic makeup of the plant is called mutagenesis [34]. About 2000 plant varieties had been cultivated commercially with induced mutation [35]. The first reported case of artificial inducing of mutation was creation of genomic lesions in wild types in 1920s with work on maize and barley [36]. Since these evolutionary activities, this type of mutagenesis had become widespread in the biological sciences, and used as a tool for functional genomics. Mutagenesis is convenient as compared to the conventional methods but still this process take time of about 6 to 7 years. In plants, mutations were induced by exposure of seeds, tissues and organs to physical and chemical agents with mutagenic properties [37].

Alkylating agents and azides were chemical mutagens. These chemical agents such as ethyl methane sulfonate and ethidium bromide caused a myriad of genome lesions and ultimately cause gross chromosomal damage. These agents brought about alteration of the DNA sequence and thus altered the characteristics of the treated plant and its appearance. Alkylating agents are very reactive, even when they react with water. Hydrolysis produces those compounds that will be no longer mutagenic but they will be toxic and harmful for the biological tissues. For this reason, mutagen solution must be assembled just before and never stored.

Physical mutagens comprised electromagnetic radiations and particle radiations for the process of mutagenesis [36,38]. In some instances, the entire plants were also exposed to induced mutation. These induced mutations caused phenotypic and genotypic changes in the mutant plants. Induced mutations were important not only for increasing the genetic diversity of the crops but also an important source of additional biodiversity enhancement of neglected and local crops.

In this approach, mutants with required traits were selected in the M1 or M2 generation after treating it with mutagens and then released as new variation for cultivation aftertrials. The increase in dose of M1 plants showed that there is a reduction in survival of plant, its germination and fertility. In mutagenized seeds delayed germination may occur as compared to the normal. Effective dosage is not indicated by seed germination. At the stage of active cell division mutation effects show clearly. In the segregation of M2 or in the M3, the selection starts only on row basis.Rare or dominant mutations ere already selected from the M1 generation because they will be totally different from the others. One of the major limitations of this process is the mutation that occurred randomly in genome, which was hard to predict [26].

Polyploidy

Polyploidy in crop modification is used for beneficial purposes, provided genome buffering, increased allelic diversity and permitted novel phenotypic variations to be generated because of duplicated genes that acquired new properties. Polyploidy played an essential role in plant evolution. There were two major types of polyploidy have long been recognized i.e. multiplication of one set of chromosome and other that resulted from merging of structurally different chromosomes sets. These types were name as autopolyploidy and allopolyploidy, respectively. Moreover, autopolyploids were formed by specific diploid population hybridization and are thought to be very rare in plants natural population while allopolyploids were made up of interspecific hybridization [39].In the agricultural sectors, plants like alfalfa and potato are grown by autopolyploidy and wheat and coffee showed allopolyploidy. It has been recognized that polyploidy is a major evolutionary factor in crops, but determining the actual frequency of this process in different plant lineages has proven challenging, despite numerous efforts made over the past 70 years to estimate it [40]. This technique also had limitation that it caused errors in the cell and nucleus. These can create problems in completion of normal mitosis and meiosis. A potential change in the gene expression was also a disadvantage of poly ploidy.

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Protoplast Fusion

Protoplast fusion had been the means of developing unique hybrid plants which cannot be produced by conventional hybridization. Including most crop species, protoplast was produced for many plants to improve the yield and enhance the quality of food. In this technique, protoplasts were isolated which are plant cells without cell walls. Scientists cultured and modified these protoplasts [41]. Eventually, fusion techniques were employed, bringing protoplast from variety of plants together. Following fusion, regenerated hybrid cells were grown to encourage the growth of fused protoplasts. The resulted hybrid plant had a combination of characteristics from the parent species, revealing the potential for crop modification.

This technique of fusing protoplast was used for same specie or different species. Researchers had success of fusing different species with the help of protoplast but closely related species that are hybrid protoplast regenerated from induction till now from culture. The major disadvantage of this fusion was sometimes instability that it brought in the genetic makeup and lacked gene splicing precision [42]. It can be divided into two classes, i.e. spontaneous and induced fusion. Still the process of protoplast fusion is not known fully, for this reason several understandings are put together for the explanation. When the protoplast is induced, the electrostatic potential results in the fusion of the membrane. When it is fused, the surface potential returns to its initial state and membrane stabilizes.

Transgenesis

Transgenesis has been recognized as the transfer of one or more genes from foreign plant into the genome of plants. These genes from foreign plant are artificially inserted [43]. Because of the use of transgenesis, plants were produced with desired traits and even increased yields [44]. These crops had ability to last longer and withstand with pests and diseases. Transgenic plants were produced using Agrobacterium mediated transformation and by direct transfer methods of DNA to increase quality and yield. Since the beginning of the 20th century, Agrobacterium tumefaciens and related Agrobacterium species were known as pathogens of plants. Since the early 1980s, when initialreports came up about the use of Agrobacterium for creating transgenic plants, scientist had been working to enhance the natural genetic engineering for the advancement of the biotechnological approaches [45]. Some modifications resulted in increased range in host of the bacterium to economically essential species of the crop [46]. Agrobacterium mediated plant transformation included attachment of Agrobacterium to the plant cells then sensing signals of plants and regulation of virulence gene in bacteria. After that, the T-DNA and virulence proteins were transported to plant cells from the bacterial cells then it was integrated and expressed in the plant genome [47].

On the other hand, direct transfer of DNA is done with the help of the protoplast for the production of transgenic plant having desired characteristics in it. This method utilized the systematic uptake of DNA by protoplast [48].

This process had toxicity risks, so human safety risk also occurred. Moreover, proteins that resulted from transgenic plant were not able to consume as foods [49].

Genome Editing

Modification of crops with gene editing provided a range of options, by changing only few nucleotides from billions found in the genome, altering the full allele or by addition of a new gene in a targeted portion of the genome [50,51]. Mega-nucleases were the first tool which was used to modify the Double stranded DNA and are generally considered as molecular DNA scissors. Mega-nucleases are an efficient tool used in genome editing for the modification of crops like maize and cotton. These mega-nucleases are the enzymes that can be naturally found in yeast and in Chlamydomonas. These enzymes because of having same binding site as well as restriction site and having little recognition site, make it difficult to modify and manipulate the DNA binding site.

Genome editing helps breeders of plant to make very careful changes in DNA [52]. Due to this, genome editing was considered more precise as compared to the other conventional methods and standard genetic engineering methods [53,54]. Genome editing involved three fundamental techniques which are Zinc Finger Nucleases (ZFN), transcription activator-like effector nuclease (TALEN) and Clustered regularly interspaced palindromic repeats (CRISPR) [55]. ZFN and TALE nucleases were considered as first generation genome editing tools [56].

ZFN-based gene editing was a technology which is important tool discovered for gene editing [57]. ZFN-based gene editing technique consisted of zinc finger (ZF) protein and Fok1 nucleic acid endonuclease domain for DNA cleavage [58]. Domains of zinc finger are made up of a chain of 4 to 6 amino acid domains that can hold together trinucleotide sequences.

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It is designed to identify specific sequences of DNA which enabled targeted cleavage [59]. Different strategies were potentially used to improve the genome of the plant species when using ZFNs [60]. ZFN expression can lead to site-specific mutagenesis, gene stacking or gene replacement, depending on the presence and structure of the donor DNA and the plant donor DNA machinery. Zinc finger nucleases were the first the first enzymes to be used in targeted genome engineering. These are created when restriction endonuclease fused with zinc finger.

TALE nucleases (TALEN) were rapidly applied for genome editing in crops [61]. Transcription activator-like effector nucleases are designed synthetic DNA-binding proteins. They combine a transcription activator-like effector (TALE) domain with the FokI endonuclease catalytic domain. The interaction of TALE with DNA is facilitated by a central region of the TALE protein, which features up to 30 tandem repeats of a sequence motif of 33-35 amino acids. Each repeat has the ability to bind to a single base pair in the target DNA, enabling precise and programmable DNA recognition and modification.The use of TALEs and TALENs for pinpoint genome modifications of plants is common practice now [62]. Many crop plants have been modified using TALENs such as tomato, potato, maize and barley for better yield and improved quality as well as development of the biotic and abiotic stress-resistant plants [63]. TALEs are proteins which are naturally occurring from the plant pathogenic bacteria genus Xanthomonas which contain DNA-binding domains. Transcription activator-like effectors offer a potential solution towards the effects of climatic changes taking place in any plant. In addition to this, this technique can contribute in efficiently enhancing the defense mechanism of plant making it able to produce resistance against pest, diseases and harsh condition of the environment [64]. However ZFN had high ratio of off-target breakdown and cleavage. TALENs are limited to simple mutation [65]. These techniques were expensive and time taking as they associated steps that required protein engineering [66].

Current Study

Today, Clustered Regularly Interspaced Palindromic Repeats (CRISPR-Cas9) technique is working gradually on the crop modification [67]. Unlike genome editing tools of first generation, CRISPR-Cas9 contains simple cloning and designing with same Cas9 which is potentially available for use with various sgRNAs targeting in the genome at different sites [66]. This single guided RNA guides the Cas9 to cleave the portion which is required to be edited [68]. The simple steps which are involved in CRISPR mediated genome editing (CMGE) allows even a small laboratory to carry out genome editing projects to improve crops with a fundamental plant transformation set up [66,69].

First, genomic target is selected then single guided RNA is designed. This sgRNA assemble with Cas9 protein and is delivered to the plant [70]. After delivering to the plant, it is regenerated and screened by various techniques such as Next Generation sequencing, and using surveyor assays [71,72] (Figure 2). CRISPR has a broad range of uses which include functional studies of gene and agricultural development [73,74]. This technique has become increasingly famous in the recent years. In the last 5 years, it is being applied in plants for various functional studies and to improve the agronomic characteristics [75]. There are various means of CRISPR-Cas9 system for causing gene mutations in plants [76,77]. Although, CRISPR technology requires further improvement to increase theon-target efficiency [78].

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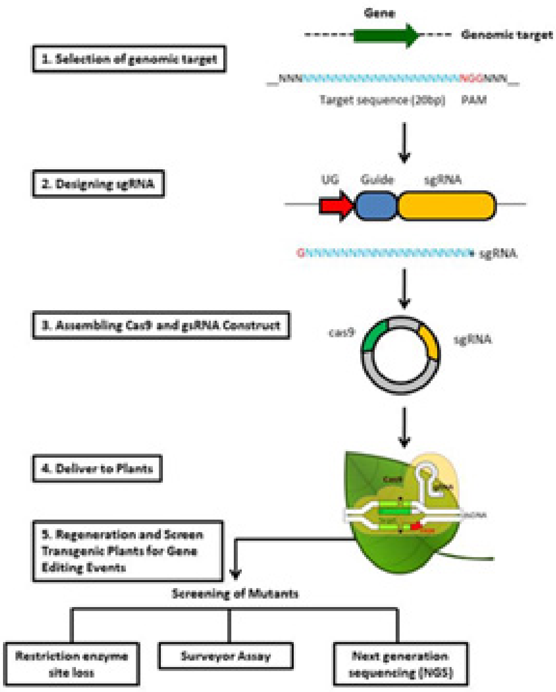

Figure 2. Steps involved in clustered regularly interspaced palindromic repeats (CRISPR) Technology of genome editing.

At present, CRISPR is considered time saving and labor saving as compared to the other conventional breeding and biotechnological approaches [79]. CRISPR also boosts up the quality and yield of the crops by altering genome of any plant [80]. For direct changing the genetic sequences in cells, CRISPR is most easy, quick, and efficient method till now [81]. Moreover, CRISPR-Cas9 is advantageous over ZFNs and TALENs because of its easy designing for its any genomic target can easily predict the off-target sites and have possibility of modifying various genomic sites. In addition, it is useful for cloning as compared of traditional homologous designing repair the recognization sequence of the sgRNA is small (20bp).

Future prospective

In future scientists will use online tools for the development of the single guided RNA sequences. Moreover, a new program, for genome wide design and evaluation of single guided RNA sequences for CRISPR experiments has recently been proposed which is called as CROSPSR [82]. This program will be useful for experiments with polyploidy genomes as well as gene regions. Thus, there are prospects for faster production of improved varieties of crops for horticulture.

CONCLUSION

To conclude the whole discussion, it is reviewed that genome editing has revolutionized the crop modification and it is taking rule over the conventional methods of crop modification. This has allowed many researchers to find new ways for modification of the plants for better yield, good quality and crops which will be resistant to pests and various diseases. Latest technique, CRISPR is becoming popular tool due to its versatility in crop modification. In future, these techniques will be used to overcome the needs of the food in the growing population of the world.

DECLARATION

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable

CONSENT FOR PUBLICATION

Not applicable

AVAILABILITY OF DATA AND MATERIALS

Availability of data and materials on request by the corresponding author

COMPETING INTERESTS

The authors have no competing interest

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