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Biotechnology and breeding approaches to increase disease resistances in cabbage

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Abstract
Cabbage is an important vegetable crop of the family Brassicaceae and is grown in many countries of the globe. There are different types of cabbage depending upon shape, size, and color. It is a highly cross-pollinated crop where heterosis in F1 hybrid progeny has been exploited so largely in developing countries too. Due to high yield, strong disease resistance, wide adaptability, good quality, and uniform economic characters. The rate of conversion from open pollinated varieties to hybrid varieties and seed replacement ratio is very high in this crop. There are different temperature requirements for fresh vegetable and seed production purposes. The self-incompatibility and male sterility systems are present in the crop, which facilitates easy and cheaper hybrid seed production. For the successful seed production program we need suitable production area, congenial environment, and stable parents. The present review deals with the molecular approaches and biotechnology tools for the production of new plant varieties. Plants resistant to biotic and biotic stress tolerant to drought or other harsh environmental conditions had produced. A number of techniques including tissue culture mutagenesis, transformation have been used. Advance functional genomics studies give better understanding of plant genome and help in modifying it. RNA interference, next generation sequencing and nanotechnology have become a new promising technique for improving crop according to future need.

Keywords: cabbage, breeding methods, hybrid variety, biotechnology approaches and resistance

Introduction

The value of Vegetables as an important article of daily human diet is well known since time immemorial as they supply all main components of human diet. They are commonly called "protected food" because of their protective effects against degenerative diseases. Although main breeding objective will continue to be increasing yield to meet the food requirement of ever increasing population, but in order to ensure health security, it is imperative that nutrition rich varieties are breed. Conventional breeding in conjunction with molecular biology has bright prospects of developing vegetable varieties with high nutraceuticals and bio active compounds suitable for fresh market. India is the second largest producer of vegetables in the world but current production is not sufficient to meet the demand of ever increasing population. The improvement of vegetable crops has until recently, been largely confined to conventional breeding approaches and such programmes rely as interspecific sexual hybridization of plants which have desirable heritable characteristics and on naturally or artificially induced random mutations [1]. The introduction of new genetic information can result in increased resistance to insect pest, diseases tolerance to environmental condition, improved quality etc. India is bestowed with varied agro-climatic conditions which allow growing of all types of vegetables in one part of the country or other. Export potential of vegetables to generate valuable foreign exchange required to be fully tapped. If we grow greens or planning to grow them, here are the common diseases of leaf vegetables, their causes, prevention, control and treatment. In this age of technology, biotechnology has opened up new horizons in the field of science. It is a viable option, which can provide improved genotypes that can survive under changing climate. Advancements in fields of genomics, stress biology and bioinformatics can help in development of stress tolerant crops [2]. There are multiple approaches like transformation, mutagenesis and proteome profiling in practice to adopt better traits of agronomic importance. We will focus on molecular biology applications for crop improvement like allele mining, gene pyramiding, linkage and association mapping, genetic engineering (GE) or recombinant DNA technology, Molecular Breeding (MB), Marker assisted back cross (MABC) and Marker assisted recurrent selection (MARS), Genome wide selection (GWS) and Next Generation Sequencing (NGS) [3].

A. Methods of breeding in cabbage

Conventional Breeding methods: In conventional breeding, progeny inherit genes for both desirable and undesirable traits from both parents. Breeders conserve desired characteristics and suppress undesirable ones by repeatedly selecting meritorious individuals from each generation to be the parents of the next.

Non-conventional Breeding methods: In non-conventional breeding, encompasses essential all cell and tissue culture technique that assist in propagating studying and manupulating the plant gene without use of sexual cycle.

Breeding methods for cross pollinated vegetable crops

Cabbage is a highly cross pollinated crops. Following breeding methods are used for cabbage [8]:

1. Introduction: Introduced materials may be valuable in selecting desirable plants. In case of non-uniform introductions the desirable plants can be selected, their progeny increased, purified and later tested against the standard or local varieties for selecting the most promising lines. The chances of an introduction depends to a great extent upon the relationship between the agro climates, particularly temperature and day length of the donor and receptor areas.

2. Mass selection: In this method the best individual plants are selected in the population and their seeds are composited for following generation. Since the mass selection is made exclusively based on the phenotype of the plants without any progeny testing, the success of selection depends upon the heritability of the characters under selection.

3. Family breeding: There is elaborate testing of progenies not only in F1 generation but also in later 2 or 3 generations and usually more than one cycle of selection. This method is practised in beet and can also be adopted in radish, carrot and cauliflower.

4. Recurrent selection: The source population of recurrent selection is heterozygous. It may be an open pollinated variety, single cross or double cross F1 hybrid, intercrossed progenies of selected inbred lines, a synthetic variety or a composite. There are four types of recurrent selection viz. simple recurrent selection, recurrent selection for general combining ability and recurrent selection for specific combining ability, recurrent selection for general and specific combining ability and reciprocal recurrent selection.

5. Heterosis breeding: The hybrid varieties are developed by exploiting the dominance variance in heterosis breeding. Hybrids are available for commercial cultivation in all the cross pollinated crops like cole crops and cucurbits etc. For the last two decades, vegetable breeders in India diverted their attention toward the development of hybrids and disease resistant varieties/hybrids. Vegetables have great potential for export and after value addition these can become an important commodity of agricultural export out of India.

B. Biotechnology approaches in cabbage

1. Plant tissue culture in crop improvement

Major things required for the plant tissue culture are the plant tissues (explants), medium containing organic and inorganic compounds on which the plant could grow and develop further and a high amount of growth hormones particularly Auxin and cytokine. Crops used to produce from this technology facilitate the interspecific and intergeneric crosses to overcome physiological based self-incompatibility [11, 18]. A vast variety of crops has been recovered through IVF via pollination of pistils and self and cross pollination of ovules. Agricultural crops like tobacco, clover, corn, canola, Cole, poppy, cotton etc. the use of delayed pollination, distant hybridization, pollination with abortive and irradiated pollen and physical and chemical treatment of host ovary have been used to implied haploidy. Embryo culture is another kind used to make crops valuable. These technologies could easily simplify breeding programs and overcome some important economical and agronomic traits that would never be produced from conventional ways of plant breeding and plant improvement.

2. Crop improvement by genetic engineering

Gene transfer between unrelated species of plants has been playing a very crucial role in Cole crop improvement. By transforming genes many useful traits like resistance to insects, stress and disease has been transferred to many crop varieties from non-cultivated plants. Recombinant DNA methods and many other methods are in use for transformation of genetic information. Genetic engineering is a DNA recombination technique that has made possible gene transfer between dissimilar genera or species [12,116]. Genetic engineering is an exceptional way of breeding as compared to conventional breeding. It is a way of extending genetic base. Secondly, as it avoids the problem of linkage drag associated with the conventional breeding it is more effective and it is less time consuming. Till now, many genetic engineered crops have been developed and commercialized that result in improved production efficiency, increased market focus, and enhanced environmental conservation. Such crops include longer post-harvest storage tomatoes, insect resistant cotton, cabbage and maize, virus resistant potato, herbicide resistant soybean and canola.

Gene transfer through hybridization

Plant breeding and intraspecific gene transfer

In 19th century plant breeding began with discoveries of how plant traits are inherited. Plant breeding could be carried out by selection of plants with attributes of interest and manipulating into cross fertilization. Improved variety with desired characteristics is formed when a cultivated variety is back crossed with a wild variety [19].

Interspecific gene transfer

In 20th century, plant breeders used inter-specie hybridization for gene transfer from a non-cultivated plant species other convertible crop species. For example cabbage, oat and sugar beet has been transformed and resulted in increased yields 25-30% and sugar beet nematode resistance respectively [19].

Gene transfer by non-sexual methods

As plant cells, tissues and organs can be cultured *in vitro* so transfer of genes between plants is possible by non-sexual methods. Non-sexual gene transfer methods depend on ability to produce in certain plant species fully differentiated plants from non-sexual organs and tissues. Stems, pieces of leaves and different undifferentiated clumps of cells in culture can be used as starting material for regeneration. In some species, even a single somatic cell can be used. Cell fusion methods and recombinant DNA techniques for gene transfer have been used from many years. Here we will discuss some gene transfer techniques that are used for crop improvement [14].

Gene transfer by manipulating DNA directly

In 1940s, methods for transferring DNA directly from one organism to another organism developed as DNA established as a chemical base of genetic inheritance. Non-sexual DNA transfer techniques make possible manipulations that are outside the repertory of breeding and cell fusion techniques. Genes can be obtained from plant, animal, bacterial and viral sources and injected in crops. Tissue specificity, timing and level of gene expression is under control and it can be modified by gene modification into new host [6]. These methods provide the source of diversity and allow controlling the expression of genes.

***Agrobacterium-mediated* gene transfer**

Agrobacterium tumefaciensis is a plant-pathogenic bacterium that holds ability to transfer some part of its own genetic material into other plant species by a simple process called transformation. The genes encoded in a region of Ti plasmid called T-DNA. This causes tumorous growth called "crown gall" disease in plants. This bacterium is modified in lab and it transfers gene of interest into plants without causing symptoms of disease [5].

By using this method, genes for insect and disease resistance has been transferred in many Cole crops. This is the most suitable method of non-sexual gene transfer and there are many useful crops that are tested and are good candidates for agriculture use. By recombinant DNA technique many plant and bacterial genes that encodes enzymes has been engineered that makes plant crops tolerant to broad spectrum and environmentally safer herbicide. For this bacterial gene is engineered in such a way that its enzyme is insensitive to herbicide and then transfer it to plant [19].

3. Mutagenesis and crop improvement

Mutational breeding is powerful tool for raising plant varieties with desired traits with equally beneficial to food crop as well horticulture. About 2,000 plant varieties with induced mutation have been cultivated commercially. The main purpose of mutation breeding technology is the development of new and desired variation(s) through breeding program for crop improvement. Induced mutations can play an important role in the conservation and preservation of crop biodiversity [12]. Induced mutations and related advance technologies are important not only for increasing the genetic diversity of crops but also are an important source of additional biodiversity enhancement of neglected and local crops. In this approach, mutants with desired traits were selected in the M1 or M2 generation after treatment with mutagens and then released as new variety for cultivation after evaluation and trials [8]. According to the FAO, there are 1,357crop species which are officially released mutant cultivars, 490 mutant varieties of ornamental and decorative plants were mainly developed in seed propagated plant species (1,284 entries), whereas vegetative propagated crops are represented by only 73 varieties. Among the cereals (869 mutant varieties), rice (333) ranks first, followed by barley (261), bread wheat (147), maize (49), durum wheat (25), and others (54). Most of the rice mutant varieties (67.6 %) were released as 'direct mutants'.

4. RNA Interference

RNA interference is an emerging tool in biotechnology for crop improvement. It has been widely used for increasing crop yield, resistance against biotic and Abiotic stresses and enriched nutrient fruits [5]. RNAi includes the sequence specific gene silencing at post transcription level. Two major player of RNA interference are (endogenous) microRNA and exogenous, such as transgene, small interfering RNA (SiRNA). They are produced by the breakdown of dsRNA by the ribonuclease enzyme DICER or DICER like enzymes (DCL). Then a RNA induced silencing complex (RISC) is activated by the incorporation of these single stranded RNAs. Activated RISC- RNA (antisense strand) than bind to target sequence specifically by complementary base pairing and degrade the mRNA. siRNAs can also regulate gene expression at transcription level by regulating the chromatin siRNA maintain the transcription rate at minimal level by controlling histone modification including the cytosine methyl transferase Chromomethylase3 (CMT3) which keep the DNA into transcriptional inactive state [13, 15].

5. RNAi for Male Sterility

RNAi has also been used for generating sterility in seeds and producing hybrid seed. Genes that involved in pollen production can be targeted by RNAi. A male sterile tobacco line has been developed by targeting the expression of TA29, a gene necessary for pollen development. Male sterility is also generated by RNAi by controlling the Msh1 gene expression in tobacco and tomato that result rearrangements in the mitochondrial DNA that is associated with natural cytoplasm male sterility [11, 15].

6. Next generation sequencing

which The term NGS is applied to detail all the latest sequencing technologies other than Sanger hold potential to sequence human genome at the cost of thousand dollars. Next-generation sequencing (NGS) technology is the cutting-edge technology for genome sequencing of several species. It has been proved an essential gadget for development of novel or atypical molecular markers and determining genes of agricultural importance [11]. Long drawn out and tedious clone-by-clone process has been replaced by NGS, this previous method was used for genome sequencing with the strategy of identifying the least redundant super-imposed clones, a physical genetic map of the crop to be sequenced is the prerequisite for carrying out these time-taking experiments. GS-FLX and Illumina HiSeq, are leading NGS methods for utilizing the whole genome shotgun (WGS) approach for sequencing of several crops as massive amounts of data is being generated in lesser time using these platforms [18]. There are a number of companies that have made third generation sequencing technologies available to the market [16].

Approaches like Marker-assisted Breeding(MAS), designing of molecular markers for MARS, de-novo sequencing, resequencing of previously characterized crops, metagenomics, SNP haplotyping and epigenetic modifications have been taken to next level by using next generation sequencing approaches [4, 9].

7. Bioinformatics tools in crop improvement

Bioinformatics resources in addition to different web databases are providing vast information about the genomic data that is largely required for the research purpose. Crop improvement through bioinformatics tools are more promising these days. With the passage of time the technology has been enhanced to surprising level [17]. The bioinformatics is providing crucial information about the genomic data of crops and the sequence of many genes are being explored by this technology [5, 9]. This could possibly help us to sequence the corps which is economically important and the traits that are more beneficial. Whole genome comparisons are accelerating the rate of competent research [10].

8. Nanotechnology in Crop Improvement

Nanotechnology is a novel, explanatory, vast scientific technology that involves designing, development and application of materials at molecular level in nanometer scale. It is a broad spectrum emerging field of science which has examples in all field of science and agriculture is no exception. There are many reports which have shown the involvement of Nano-particles or nanotechnology in crop improvement. Mostly used or studied Nano-particles are carbon and metal-oxide based particles [7]. The positive effects observed by using these Nano-particles include enhanced germination, enhanced length of roots and shoots, and increased vegetative biomass of seedlings in many crops. In many crops including soybean, spinach and peanut enhancement in many physiological parameters have been observed such as photosynthetic activity and nitrogen metabolism. In 2009 it was reported that the germination of seed of tomato plant was enhanced by penetrance of carbon nanotubes (CNTs). The seed germination in this case was enhanced due to water uptake ability of CNTs.Ti02 Nano-particles have been known to enhance the growth of Cabbage [10, 13].

Conclusions

India is the second largest producer of vegetables next to china, yet less than one percent of the produce is exported. The reason can be found in lack of quality, poor post-harvest, management and lack of market intelligence. So as to remain competitive, the quality has to be maintained which is of prime importance in horticultural crops like vegetables. The quality being most important for export, the variety chosen has to be acceptable internationally/nationally and the improvement in which it is produced should be conductive for the development of inherent characters of the variety to the best. However in India no systematic breeding work has been done to produce varieties/hybrids acceptable in the international market. Among vegetables, onion is the major item of export. Exports of onion from india is primarily directed to Malaysia, Singapore, Srilanka and Gulf countries. Light red and dark red varieties are in demand in Gulf countries, Srilanka and for East while small onions are in demand in Malaysia and Srilanka. Arka bindu and yellow onion evolved by IIHR, Bangalore are suitable for export. Arka Bindu has good demand in Middle East countries. Similarly Co-4 variety of multiplier onion developed by TNAU, Coimbatore is suitable for export to South East Asian countries. In respect of fresh vegetable export, nearly 60 per cent of trade centres around okra. Varieties Arka Abhay, Varsha and Uphar, which are resistant to YVMV are suitable for export. The export demand centres on fruits having 7-9 cm length. In tomato fresh fruits can be exported to Middle East. Varieties Arka Alok and Arka Shakti from IIHR have been bred to be resistant to wilt and can be grown in sick soil in Kerala, parts of Karnatka, Maharashtar and Odisha for export. From first domestication to modern breeding, the vegetable has been continuously subjected to human selection for a wide array of applications in both science and commerce. Current efforts in vegetable breeding are focused on discovering and exploiting genes for the most important traits in vegetable germplasm. In the future, breeders will design cultivars by a process named 22 'breeding by design' based on the combination of science and technologies from the genomic era as well as their practical skills Crop improvement is the prime element of agricultural advancements and there are still many areas to be worked on in the field of crop improvement. When talking about gene transfer or transfer of desirable traits to the target plant, in future there might be an option of complete chromosome transfer via microinjection and it can confer multigenic traits. NGS technology has made access to genomic resources of multiple plants and also to those lesser studied orphan crops. It will also facilitate the identification and confirmation of introgression lines for desirable traits. Crosses between distant relatives are promoted by novel embryo rescue techniques. Isozyme technology is emerging as a rational tool for various aspects of plant breeding. Innovation in agricultural technologies is leading Molecular Farming into a new landscape but private sector companies and establishments are supposed to invest more resources to make it a successful idea that can provide higher productivity with lesser use of herbicides, insecticides and chemical fertilizers. These unforeseeable notions of future scientists will shift crops much toward the natural essence.

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