

Recent advances in molecular tactics for crop improvements

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Abstract. With the increasing volume of world population there is relative increased demand of food. Conventional breeding methods are no longer viable to overcome this situation. The fields of biotechnology and molecular biology have revolutionized agriculture and farming methods. To improve the current agronomic practice, the conventional plant breeding techniques are being integrated with the novel molecular methods in a very impetus manner. In this review we discuss some molecular approaches and biotechnology tools for the production of diverse and better yielding plant varieties. Plants resistant to biotic and abiotic stress, tolerant to drought or other harsh environmental conditions has been produced. The genetic architecture of target plants can be altered and improved by using transgenes. Advanced functional genomics studies provide better understanding of plant genome and help in modifying it. RNA interference, next generation sequencing (NGS) and nanotechnology have become promising techniques for improving crop according to future needs.

Keywords: Tissue culture, mutagenesis, transformation, RNA interference, next generation sequencing, nanotechnology.

INTRODUCTION

Man discovered agriculture almost 10000 years ago. Agriculture is domestication of wild plants for personal use of humans. Food is the necessity for sustainability of human life on this planet. Various crops have been harvested since thousands of years. However, it was not possible to cope with the demand of food by using conventional methods of cultivation. There was need to develop new approaches to improve the quality and quantity of yield. As the global world, population is rapidly and alarmingly increasing, new methods have been introduced for better production, improved nutrient content and disease resistant crops. Since start, man has

been trying to manipulate different ideas and techniques to save the crops from different diseases by using conventional methods. Unfortunately, conventional methods do not meet the current needs. Although in the past five decades global food grain production has been growing with increasing world population, still 1 billion persons of the world are malnourished because of food insecurity.

Hazell and Wood (2008) estimated that worldwide food production must be increased by 70% by the year 2050 to fulfill the need of expanding population and growing consumption of food (Godfray *et al.*, 2010). 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. There are multiple approaches like transformation, mutagenesis and proteome profiling in practice to adopt better traits of agronomic importance. In this review 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).

PLANT TISSUE CULTURE IN CROP IMPROVEMENT

Plant tissue culture is an enabling *in vitro* technology from which many novel techniques have been developed to assist plant breeders. Changes induced by plant tissue culture are known as somaclonal variations. Pieces of plant tissues will slowly divide and develop into colorless mass of the cells called Callus. Callus is the first step in the formation of new plant from the plant tissues (Jain, 2001).

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 cytokinin. Sterile conditions are mandatory to make tissue culture successful. Different techniques in plant tissue culture like micropropagation may offer certain advantages over traditional plant breeding techniques. This tool enables us to understand the vast abilities of plants such as totipotent cells (Mroginski, 1984). Plant tissue culture has been exploited to create genetic variability from which crop plants can be improved. Tissue culture in association with molecular techniques have been used to transfer desirable commercially and genetically traits. For ornamental, clonally propagated crop industries have been working tirelessly to dramatically increase the crop cultivars. As a result, chromosomal variations induced by tissue culture are observed in many crops. Molecular and transposable variations are also present. A number of serious attempts are being made to produce crops by introducing somaclonal variations. Hence, a large amount of cytoplasmic and nuclear genetic alterations are made to bring about phenotypic variations. A new type of hybrid plants and clones are being made with the improved traits. This is considered the safest technique to produce plants with desired traits. A large number of clones are required to produce desired results on large scale.

Major advantages or impacts of plant tissue culture on

crops are being mentioned in detail below. Crops produced from this technology facilitate the interspecific and the intergeneric crosses to overcome physiological based self-incompatibility (Brown, 1995). A vast variety of crops has been recovered through IVF via pollination of pistils and self and cross pollination of ovules. In 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 contributed to haploidy. Embryo culture has also been used to make crops valuable. Orchids, roses, bananas are being formed by embryo culture. *In-vitro* selection for salt tolerance is commonly occurring as temporary adaptation. Cells are being able to store extra salts in the vacuoles and survive by adjusting the osmotic pressure. This results in the production of salt tolerant halophytes, which are well-adapted to high salt environment and are unable to grow without salt. Tobacco salt-tolerant cell lines have been produced. Different varieties like stress, drought, and heat-tolerant varieties have also been successfully developed. *In vitro* propagation via meristem, cell tissue and organ culture, organogenesis and somatic embryogenesis have been presented (Filippis, 2014). These technologies could easily simplify breeding programs and overcome some important economical and agronomic traits that would never have been produced using conventional techniques of plant breeding and plant improvement (Filippis, 2014). Different growth conditions and media requirements are required for different crops. Biopharming of plants with notable advantages in plant cost and safety is regarded as the platform for the production of a vast variety of recombinant proteins and a number of potentially crucial drugs (Brown, 1989). The method of plant tissue culture plays a dominant role in the second green revolution in which plant biotechnology is considered to make desirable crops. The applications of various tissue culture approaches to crop improvement are:

- i) Breeding and biotechnology
- ii) Wide hybridization
- iii) Haploidy
- iv) Somaclonal variation
- v) Micro propagation
- vi) Synthetic seed
- vii) Pathogen eradication
- viii) Germplasm preservation

Plants are being grown *in-vitro* to produce bio-fuel. This is the most astonishing advancement in crop improvement. Yield and quality of the crops have been massively increased by using this technology. However, increased nutrition and food safety should be taken into consideration when implementing tissue culture techniques.

CROP IMPROVEMENTS BY GENETIC ENGINEERING

For many decades, gene transfer between unrelated species of plants has been playing a crucial role in crop improvement. By transforming genes many useful traits like resistance to insects, stress and disease have been transferred to different crop varieties from non-cultivated plants. Recombinant DNA techniques and many other methods have been used for the transformation of genetic information.

Genetic engineering is a DNA recombination technique that has made it possible to transfer genetic materials between dissimilar genera and/or species. Genetic engineering is an exceptional way of breeding as compared to conventional breeding. It is a way of extending genetic base. Secondly, since 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 leading to improved production efficiency, increased market focus, and enhanced environmental conservation. Such crops include longer post-harvest storage tomatoes, insect-resistant cotton and maize, virus-resistant potato, herbicide-resistant soybean and canola, and many other (Dunwell, 2000). To improve crops through genetic engineering, an efficient transformation system is required. Currently there are different approaches that are used to transform different crops.

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 was carried out by selection of plants with attributes of interest and manipulation by cross fertilization. Improved variety with desired characteristics is formed when a cultivated variety is back crossed with a wild variety (Goodman *et al.*, 1987).

Interspecific gene transfer

In 20th century, plant breeders used inter-species hybridization for gene transfer from a non-cultivated plant species to a crop species. For example *Avina sativa* (oat) and *Beta vulgaris* (sugar beet) has been transformed and resulted in increased yields of 25 to 30% and sugar beet nematode resistance respectively (Sharma and Gill, 1983).

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 for many years. Here we will discuss some gene transfer techniques that are used for crop improvement (Goodman *et al.*, 1987).

Cell fusion/ protoplast fusion

Methods to prepare large number of single plant cells without their cell walls (protoplast) were developed in 1960s. Fusion could be induced among protoplasts of various plant cells by using electroporation technique and certain chemicals and liposomes. Callus tissue produced from somatic hybrid when grown *in vitro*. In certain species a whole plant can be regenerated from this callus tissue. Sexually incompatible species could have their chromosomes combined by the use of cell fusion method. This method is of little importance for commercial use in agriculture because of its limitations.

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. These methods provide the source of diversity and allow controlling the expression of genes.

Agrobacterium-mediated gene transfer

Agrobacterium tumefaciens 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. The Agrobacterium system is appealing because of the easy protocol that is associated with minimum cost in terms of equipment and also the resulting transgenic

plants have simple copy insertion (Hansen and Wright, 1999). Many very efficient vectors have been designed that contain extra copies of virulence genes and are mutated that increases the level of expression of virulence genes (Hamilton *et al.*, 1996). For successful results we should test many parameters like feeder cells, infiltration of bacteria, agrobacterium strains, etc (Hansen and Wright, 1999).

By using this method, genes for insect and disease resistance have been transferred. 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 use in agriculture. By recombinant DNA technique, many plant and bacterial genes that encode enzymes have 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 transferred into to a plant. This can also be done by engineering plants so that they express genes that detoxify herbicide. Genes obtained from *Bacillus thuringiensis* have been engineered and transfer to plants that act as insecticides.

Biolistic transformation

Biolistic transformation is the process of delivery of micro-projectiles that are of tungsten or gold coated with DNA and push them into the target cells by acceleration. Acceleration provided by electric charge, CO₂, gun powder and by gases and DNA can be introduced into a tissue. This method has some limitations such as it reveals a complex pattern of transgene integration, the delivery of long fragment DNA is challenging and it is more expensive in terms of equipment (Hansen and Wright, 1999).

Microinjection

The microinjection technique is a direct physical approach, for introducing substances under microscopic control into defined cells without damaging them (Neuhaus and Spangenberg, 1990). By means of micropipettes, DNA solution is introduced into plant protoplasts. Microinjection can be used with crop species from which whole plant can be obtained from single transformed cells.

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

(Maluszynski, 2001). Mutations are the source of changes in the genome and can be either permanent or temporary. Spontaneous mutations are occurring naturally with very low frequencies of 10⁻⁶ due to transposable elements which move into the genome and cause alteration in DNA sequence (Wessler, 2006). Induced mutations are caused by either chemical mutagens or other agents like UV radiation, X-rays α -particles and β -particles. 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 conservation and preservation of crop biodiversity. 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 (Roychowdhury and Tah, 2013). 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. Those were not selected as cultivators, but can be used in cross breeding program for the desired allele. (Roychowdhury and Tah, 2013)

Mutational breeding shows great potential over genetic engineering and due to economic disparities this technology may not readily be operational in developing countries. Other major problem is the regulation and positional insertion of introduced gene (Jain, 2010).

According to the FAO/IAEA Mutant Varieties Database (<http://www-mvd.iaea.org>), there are 1,357 crop species which are officially released as mutant cultivars, 490 mutant varieties of ornamental and aesthetic 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' (Roychowdhury and Tah, 2013).

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 fruits with nutritional value. RNAi includes the sequence specific gene silencing at post transcription level (Kamthan *et al.*, 2015). Two major players 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) (Bernstein *et al.*, 2001; Hutvagner *et al.*, 2001). Hence, RNA induced silencing complex (RISC) is

activated by the incorporation of these single stranded RNAs. RISC contains protein which has ribonuclease activity to degrade the mRNA and RNA binding domains (Hammond *et al.*, 2000). RISC contains another important protein, argonaute that has been reported. In *Arabidopsis thaliana*, it is made in the catalytic core of RISC and is involved in silencing (Vaucheret, 2008). Activated RISC-RNA (antisense strand) that binds to target sequence specifically by complementary base pairing and degrades the mRNA (Sledz and Williams, 2004).

siRNAs can also regulate gene expression at transcription level by regulating the chromatin siRNA to maintain the transcription rate at minimal level by controlling histone modification including the cytosine methyl transferase CHROMOMETHYLASE3 (CMT3) which keeps the DNA into transcriptional inactive state (Ossowski *et al.*, 2008).

The phenomena of RNA interference can be used for producing desirable traits. The process of RNAi can be triggered by the entry of siRNA into a cell by several different ways, such as *Agrobacterium*-mediated transfer, viral-mediated dsRNA transfer bombardment or by infiltration (Sijen and Kooter, 2000). An RNAi vector is used to transform cell and produce stable dsRNA *in vivo*.

Biotic resistance

The strategy of RNA interference was firstly used to develop resistance against virus in plants. Mechanism of pathogens with derived resistance was developed whereby expression of three different viruses derived protein viral coat protein (CP) and replication-associated proteins (REPs) antisense and hairpin RNA (hpRNA) have been used for gene silencing to produce resistance against viruses (Shepherd *et al.*, 2009). Transgenic potato with resistance against Spindle Tuber Viroid (PSTVd) infection has been developed which produce dsRNA against PSTVd sequences (Schwind *et al.*, 2009). RNAi is also effective against DNA viruses. Zhou *et al.* (2013) developed resistance in rice by using sequences from disease specific protein gene and CP gene from *Rice Stripe Virus*.

RNAi is also effective against bacterial diseases. Crown gall disease was managed by using RNAi against tumor formation gene in *Arabidopsis thaliana* (Escobar *et al.*, 2001). In resistance management against fungal diseases fatty acid genes were targeted. Suppression of gene *OsSSI2* in rice resulted in an increased resistance to blast fungus *Magnaporthe grisea* and leaf blight bacterium *Xanthomonas oryzae* (Jiang *et al.*, 2009).

Plant varieties with resistance against pest and nematode also have been developed. These insects are feed with dsRNA as dietary component, which results in decreased expression of the targeted gene. This strategy has been applied to corn plant to produce transgenic corn plant by targeting tubulin or vacuolar ATPase genes to

develop western corn root worm resistance.

Abiotic stress

One of the major problems that affect crop yield and quality is drought. RNAi can provide a solution to this problem. Activated C-kinase 1 receptor gene was targeted in transgenic rice plants to enhance drought tolerance (Li *et al.*, 2009). A family of miRNAs miR393 shows expression in stress condition. A transgene *Osa-miR319a* when over expressed in rice plant shows enhance tolerance against drought and salt stress (Zhao *et al.*, 2007).

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. Male sterile tobacco lines have been developed by targeting the expression of TA29, a gene necessary for pollen development (Mao, 2007). Male sterility was also generated by RNAi by controlling the Msh1 gene expression in tobacco and tomato that led to rearrangements in the mitochondrial DNA that was associated with natural cytoplasm male sterility (Baum *et al.*, 2007).

Modified metabolic pathways

Basic metabolic pathways of plants can be manipulated through RNAi to get nutritionally improved fruits and crops. Some improved plant varieties with target gene are summarized in Table 1.

Next generation sequencing

The term next-generation sequencing (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 (Service R.F, 2006).

NGS technology is the cutting-edge technology for genome sequencing of several species. It has been proved as an essential tool for development of novel or atypical molecular markers and determining genes of agricultural importance (Edwards and Batley, 2010). 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-consuming experiments (Ariyadasa and Stein, 2012). GS-FLX and Illumina HiSeq, are leading NGS methods for utilizing the whole genome shotgun (WGS) approach for sequencing of several crops

Table 1. Trait improvement in different plants with specific targeted genes (Kamthan *et al.*, 2015).

Traits improvement	Targeted gene and plant	References
Biomass	<i>OsDWARF4</i> , Rice	Feldmann (2006)
Grain yield	<i>OsGA20ox2</i> , Rice	Qiao <i>et al.</i> (2007)
Carotenoid and flavonoid	<i>DET1</i> Tomato	Davuluri <i>et al.</i> (2005)
Low glutenin content	<i>GluB</i> , Rice	Kusaba <i>et al.</i> (2003)
Tearless onion	Lachrymatory factor synthase (<i>LFS</i>)	Eady <i>et al.</i> (2008)
Increased shelf life	α -Man/ β -Hex, Tomato	Meli <i>et al.</i> (2010)
Seedless fruit	tChalcone synthase, Tomato	Schijlen <i>et al.</i> (2007)
Reduction of toxic terpenoid gossypol	Delta-cadinene synthase, Cotton	Sunilkumar <i>et al.</i> (2006)
Amylose	<i>SBE IIa and SBE IIb</i> Wheat	Regina <i>et al.</i> (2006)

as massive amounts of data is being generated within a short time using these platforms. There are a number of companies that have made third generation sequencing technologies available to the market (Egan *et al.*, 2012).

A dramatic uplift in the number of completely sequenced plants has been observed after the replacement of traditional Sanger Sequencing by NGS. First completely sequenced plant was *Arabidopsis thaliana*, this project was carried out by the Arabidopsis Genome Initiative (AGI, 2000). Rice genome sequencing followed that of *Arabidopsis thaliana* (Yu *et al.*, 2002; International Rice Genome Sequencing Project, 2005). The sequences of many valuable crop species such as grape, sorghum, maize and soybean have been studied since then using the traditional Sanger method and NGS (Jaillon *et al.*, 2007; Paterson *et al.*, 2009; Schnable *et al.*, 2009; Schmutz *et al.*, 2010). Genome sequencing projects associated with sequencing of many other food and cash crops (e.g. banana, cotton, barley, wheat and oil palm) are in pipeline. The tomato genome sequence has been also published recently by exploiting NGS as well as Sanger technology (Tomato Genome Consortium, 2012).

NGS has its applications in Genome Assisted Breeding in which it is an integrated approach for identification and selection of genetic variations (Varshney *et al.*, 2005). Molecular markers are used for the physical mapping and tracking of genes or quantitative trait loci for marker-assisted breeding (MAB) (Varshney *et al.*, 2006).

Commercially available leading NGS techniques like Roche/454 (<http://www.454.com/>), Solexa/Illumina (<http://www.illumina.com/>) and AB SOLiD (http://www3.appliedbiosystems.com/AB_Home/applications/technologies/SOLiDSystemSequencing/index.htm) are considered superior to Sanger method. For example, sequencing can be multiplexed to a much greater extent by many parallel reactions at a greatly reduced cost (Hudson, 2008). Roche/454, Solexa and AB SOLiD find their applications predominantly in the area of crop genetics and breeding. Roche/454 is better than Solexa and AB SOLiD because it can obtain longer sequence reads, maximum data output is higher for both Solexa and AB SOLiD (Gupta, 2008). Roche/454 is more

expensive than both Solexa and AB SOLiD technologies in terms of cost per run.

NGS has valuable applications in development of SNP-based markers, for characterization of de-novo sequence drafts of orphan-crops where no previous data is available such as complex genomes of barley and wheat and for resequencing of those crops which already have been sequenced previously. An assembly of 11700 and 8700 contigs was generated by using Wheat Roche/454 ESTs for two hexaploid wheat lines and compared with sequences for progenitor species of polyploidy wheat; 2500 contig assemblies were assigned to one of the homologous wheat genomes and 1000 SNPs were discovered (http://www.intl-pag.org/17/abstracts/P03e_PAGXVII_144.html). The study indicates that NGS could be the lead tool for SNP identification in polyploidy crops (Cronn *et al.*, 2008).

Association mapping was done either by candidate gene sequence (CGS) or by whole genome sequencing. Both CGS and WGS-based association mapping approaches can be elucidated well by NGS techniques (Nordborg *et al.*, 2008).

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 (Varshney, 2009). Assembly and analysis of data generated through next generation sequencing is challenging because of limited availability of powerful bioinformatics resources for this technology.

However, speed of crop improvement has shifted to a new pace because of HeliScope, Ion Torrent, single molecular real-time sequencing and Oxford Nanopore like techniques of third generation sequencing (Van *et al.*, 2013).

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 research purposes. With the passage of time, the technology has been enhanced to surprising levels. Bioinformatics is providing crucial information about the genomic data of crops and the sequence data generated for many genes are being explored by this technology. This could possibly help us to sequence crops, which are economically important and traits that are more beneficial. Whole genome comparisons have accelerated the rate of competent research.

Genome sequence projects of economically important crops have been completed and are regarded as a gateway to further research. The database housing focused dataset together in a compiled form with rich annotations can help to study gene families more precisely. Genome wide comparisons of different crops can lead to the identification of conserved regions among crops for elucidation of adaptation strategies used by plants (Mochida and Shinozaki, 2010). After completing the process of sequencing of these crops, data generated can be used to create modeled proteomic data to determine the content of certain gene families. Major events like gene duplication along with other abnormalities are being manipulated by the help of bioinformatics tools. Furthermore, advances in the technology and data acquisition sites can make it easier to access crucial data necessary for the improvement in traits of crops. Hence effective use of genetic data supports sustainable improvement of crops. Different techniques like high through put sequencing can generate a large data about crops. Omics research is working on the prediction of candidate genes and consequently enables to determine their predicted functions (Moco, 2006).

The recent accumulation of nucleotide sequences of model organisms and other applied species like domesticated animals and crops has improved our understanding. Data obtained from transcriptomic and metabolomics have also contributed to the elucidation of regulatory networks that are crucial against plant stressors. Hence various crops have been protected from biotic and abiotic stressors and yield has been restored. It is evident that bioinformatics tools are accelerating the speed of innovations and have led to improved different crop varieties of economic importance.

Nanotechnology in crop improvement

Nanotechnology is going to be the next amazing thing in agriculture. In future our food will have the ability to detect presence of contaminants and spoiling agents. Nanotechnology is a novel, exploratory, vast scientific technology that involves designing, development and application of materials at molecular level at nanometer scale. It is a broad spectrum and an emerging field of science, which has applications in fields of science and

agriculture (Rahong, 2014). There are many reports which have showed the involvement of nanoparticles or nanotechnology in crop improvement. Mostly used or studied nanoparticles are carbon and metal-oxide based particles. The positive effects observed by using these nanoparticles 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 which physiological parameters have been observed such as photosynthetic activity and nitrogen metabolism. In 2009, it was reported that the germination of seeds from 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. TiO₂ nanoparticles have been known to enhance the growth of spinach. These nanoparticles contributed to enhanced Rubisco activase activity and have improved light absorbance. In 2010, it was reported that ZnO nanoparticles retarded germination of seed in corn and rye grass. It was also reported that silicon nanoparticles when used in some plants have increased disease and stress resistance. Recently, it was discovered that photosynthetic activity can be increased three times by SWCANT'S containing cerium nanoparticle. The use of magnetic fluid after exposure to magnetic field during germination of seed showed a visible increase in nucleic acid. This was due to the process of regeneration of plant metabolism. Iron oxide when used in pumpkin increased root elongation (Ali *et al.*, 2014). This was due to dissolution of iron. The genetic implications of nanoparticles-induced positive changes have been confirmed through decreased oxidative stress in spinach chloroplast under ultraviolet-B radiation by nano-titanium dioxide. This was also demonstrated in rice through transmission of fullerol in seeds for generations. Nanoparticle also aided to change the genetic expression in potato and tomato through carbon nanotube.

FUTURE PERSPECTIVES

As described previously the population of this planet is rapidly growing and in the next two decades it is expected to cross the figure of 9 billion. So in the coming days it is going to be the greatest challenge to feed 9 billion people and to deal with hunger of such a huge population. The biggest hurdle is the rapidly changing climate with time. There is need to introduce better seeds which can survive under this changing climate and give maximum yield. 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 other established stakeholders should also invest more resources to make it a successful venture 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 natural science.

Author’s comment

In personal opinion, it is impossible to attain the future of agriculture crops without taking biotechnological gadgets into account. Though the methods of molecular plant breeding continue to evolve with time but to shift the conventional agriculture practices to a new landscape, a multidisciplinary collaboration is required between multiple research areas like plant biology, plant genetics, plant physiology and plant system biology. Furthermore, we emphasize how application of molecular farming is now contributing to discoveries of genes and their functions that open new avenues for basic plant biology research. These and many other products of molecular farming have contributed to the numerous benefits global society has received from greater sustainable supplies basic needs. What need to be done is the implementation of these latest technical approaches in those areas which have not been able to afford these yet and still practicing the conventional ways.

The above review emphasizes that despite recent advances and successful examples of molecular plant breeding, one of the current grand challenges in plant biotechnology remain identifying those gene combinations that lead to significant crop improvement. This comment closes by suggesting that the most effective approach is to bring all the agriculture related research disciplines at a single platform in an integrated way where it can not only boost the modern farming practices but also uplift the agriculture economics.

REFERENCES

- Ali MA, Rehman I, Iqbal A, Din S, Rao AQ, Latif A, Samiullah TR, Azam S, Husnain T (2014). Nanotechnology, a new frontier in Agriculture. *Adv. life Sci.* 1(3):129-138.
- Arabidopsis Genome Initiative (2000). Analysis of the genome sequence of the flowering plant *Arabidopsis thaliana*. *Nature*, 408(6814):796.
- Ariyadasa R, Stein N (2012). Advances in BAC-based physical mapping and map integration strategies in plants. *J. BioMed Res.* 2012.
- Baum JA, Bogaert T, Clinton W, Heck GR, Feldmann P, Ilagan O, Johnson S, Plaetinck G, Munyikwa T, Pleau M (2007). Control of coleopteran insect pests through RNA interference. *Nature biotechnol.* 25(11):1322-1326.
- Bernstein E, Caudy AA, Hammond SM, Hannon GJ (2001). Role for a bidentate ribonuclease in the initiation step of RNA interference. *Nature* 409(6818):363-366.
- Brown AHD (1989). The use of plant genetic resources. Illustrated edn. Cambridge University Press.
- Brown D, Thorpe T (1995). Crop improvement through tissue culture. *World J. Microbiol. Biotechnol.* 11(4):409-415.
- Consortium TG (2012). The tomato genome sequence provides insights into fleshy fruit evolution. *Nature* 485(7400):635-641.
- Cronn R, Liston A, Parks M, Gernandt DS, Shen R, Mockler T (2008). Multiplex sequencing of plant chloroplast genomes using Solexa sequencing-by-synthesis technology. *Nuc. Acids Res.* 36(19):e122-e122.
- Davuluri GR, Van Tuinen A, Fraser PD, Manfredonia A, Newman R, Burgess D, Brummell DA, King SR, Palys J, Uhlig J (2005). Fruit-specific RNAi-mediated suppression of DET1 enhances carotenoid and flavonoid content in tomatoes. *Nature Biotechnol.* 23(7):890-895.
- De Filippis L (2014). Crop improvement through tissue culture. *Improvement of Crops in the Era of Climatic Changes*, edn: Springer. pp. 289-346.
- Dunwell JM (2000). Transgenic approaches to crop improvement. *J. Exp. Bot.* 51(suppl 1):487-496.
- Eady CC, Kamoi T, Kato M, Porter NG, Davis S, Shaw M, Kamoi A, Imai S (2008). Silencing onion lachrymatory factor synthase causes a significant change in the sulfur secondary metabolite profile. *Plant Physiol.* 147(4):2096-2106.
- Edwards D, Batley J (2010). Plant genome sequencing: applications for crop improvement. *Plant Biotechnol. J.* 8(1):2-9.
- Egan AN, Schlueter J, Spooner DM (2012). Applications of next-generation sequencing in plant biology. *Am. J. Biotech.* 99(2):175-185.
- Escobar MA, Civerolo EL, Summerfelt KR, Dandekar AM (2001). RNAi-mediated oncogene silencing confers resistance to crown gall tumorigenesis. *Proceedings of the National Acad. Sci.* 98(23):13437-13442.
- Feldmann KA (2006). Steroid regulation improves crop yield. *Nature Biol.* 24(1):46-47.
- Gamborg OL, Phillips G (2013). Plant cell, tissue and organ culture: fundamental methods. edn. Springer Science.
- Godfray HCJ, Beddington JR, Crute IR, Haddad L, Lawrence D, Muir JF, Pretty J, Robinson S, Thomas SM, Toulmin C (2010). Food security: the challenge of feeding 9 billion people. *Science* 327(5967):812-818.
- Goodman RM, Hauptli H, Crossway A, Knauf VC (1987). Gene transfer in crop improvement. *Science* 236(4797):48-54.
- Gupta P (2008). Ultrafast and low-cost DNA sequencing methods for applied genomics research. *Proceedings of the Nat. Acad. Sci. Ind. Sec. B-Biol. Sci.* 78:91-102.
- Hamilton CM, Frary A, Lewis C, Tanksley SD (1996). Stable transfer of intact high molecular weight DNA into plant chromosomes. *Proceedings of the National Acad. Sci.* 93(18):9975-9979.
- Hammond SM, Bernstein E, Beach D, Hannon GJ (2000). An RNA-directed nuclease mediates post-transcriptional gene silencing in *Drosophila* cells. *Nature* 404(6775): 293-296.
- Hansen G, Wright MS (1999). Recent advances in the transformation of plants. *Trends in plant Sci.* 4(6):226-231.
- Hazell P, Wood S (2008). Drivers of change in global agriculture. *Philosophical Transactions of the Royal Society B: Biol. Sci.* 363(1491):495-515.
- Hudson ME (2008). Sequencing breakthroughs for genomic ecology and evolutionary biology. *Mol. Ecol. Res.* 8(1):3-17.
- Hutvagner G, McLachlan J, Pasquinelli AE, Bálint É, Tuschl T, Zamore PD (2001). A cellular function for the RNA-interference enzyme Dicer in the maturation of the let-7 small temporal RNA. *Science.* 293(5531):834-838.
- Jaillon O, Aury J-M, Noel B, Policriti A, Clepet C, Casagrande A, Choisne N, Aubourg S, Vitulo N, Jubin C (2007). The grapevine genome sequence suggests ancestral hexaploidization in major

- angiosperm phyla. *Nature* 449(7161):463-467.
- Jain SM (2001)**. Tissue culture-derived variation in crop improvement. *Euphytica* 118(2):153-166.
- Jain SM (2010)**. Mutagenesis in crop improvement under the climate change. *Roma Biotechnol. L.* 15(2):88-106.
- Jiang C-J, Shimono M, Maeda S, Inoue H, Mori M, Hasegawa M, Sugano S, Takatsuji H (2009)**. Suppression of the rice fatty-acid desaturase gene *OsSSI2* enhances resistance to blast and leaf blight diseases in rice. *Molecular plant-microbe interactions* 22(7):820-829.
- Kamthan A, Chaudhuri A, Kamthan M, Datta A (2015)**. Small RNAs in plants: recent development and application for crop improvement. *Frontiers in Plant Sci.* 6:208.
- Kusaba M, Miyahara K, Iida S, Fukuoka H, Takano T, Sassa H, Nishimura M, Nishio T (2003)**. Low glutelin content1: a dominant mutation that suppresses the glutelin multigene family via RNA silencing in rice. *The Plant Cell* 15(6):1455-1467.
- Li D-H, Hui L, Yang Y-L, Zhen P-P, Liang J-S (2009)**. Down-regulated expression of *RACK1* gene by RNA interference enhances drought tolerance in rice. *Rice Sci.* 16(1):14-20.
- Maluszynski M (2001)**. Officially released mutant varieties - the FAO/IAEA Database. *Plant Cell, Tissue and Organ Culture* 65(3):175-177.
- Mao Y-B, Cai W-J, Wang J-W, Hong G-J, Tao X-Y, Wang L-J, Huang Y-P, Chen X-Y (2007)**. Silencing a cotton bollworm P450 monooxygenase gene by plant-mediated RNAi impairs larval tolerance of gossypol. *Nature biotechnol.* 25(11):1307-1313.
- Meli VS, Ghosh S, Prabha T, Chakraborty N, Chakraborty S, Datta A (2010)**. Enhancement of fruit shelf life by suppressing N-glycan processing enzymes. *Proceedings of the Nat. Acad. Sci.* 107(6):2413-2418.
- Mochida K, Shinozaki K (2010)**. Genomics and bioinformatics resources for crop improvement. *Plant and Cell Physiol.* 51(4):497-523.
- Moco S, Bino RJ, Vorst O, Verhoeven HA, de Groot J, van Beek TA, Vervoort J, De Vos CR (2006)**. A liquid chromatography-mass spectrometry-based metabolome database for tomato. *Plant physiol.* 141(4):1205-1218.
- Mroginski L, Kartha K (1984)**. Tissue culture of legumes for crop improvement. *Plant Breed. Rev.* 2:215-264.
- Neuhaus G, Spangenberg G (1990)**. Plant transformation by microinjection techniques. *Physiologia Plantarum* 79(1):213-217.
- Nordborg M, Weigel D (2008)**. Next-generation genetics in plants. *Nature* 456(7223):720-723.
- Ossowski S, Schwab R, Weigel D (2008)**. Gene silencing in plants using artificial microRNAs and other small RNAs. *The Plant J.* 53(4): 674-690.
- Paterson AH, Bowers JE, Bruggmann R, Dubchak I, Grimwood J, Gundlach H, Haberler G, Hellsten U, Mitros T, Poliakov A (2009)**. The *Sorghum bicolor* genome and the diversification of grasses. *Nature* 457(7229):551-556.
- International Rice Genome Sequencing Project, IRGSP (2005)**. The map-based sequence of the rice genome. *Nature* 436(7052):793-800.
- Qiao F, Yang Q, Wang C-L, Fan Y-L, Wu X-F, Zhao K-J (2007)**. Modification of plant height via RNAi suppression of *OsGA20ox2* gene in rice. *Euphytica* 158(1-2):35-45.
- Rahong S, Yasui T, Yanagida T, Nagashima K, Kanai M, Klamchuen A, Meng G, He Y, Zhuge F, Kaji N (2014)**. Ultrafast and wide range analysis of DNA molecules using rigid network structure of solid nanowires. *Sci. Rep.* 4:5252.
- Regina A, Bird A, Topping D, Bowden S, Freeman J, Barsby T, Kosar-Hashemi B, Li Z, Rahman S, Morell M (2006)**. High-amylose wheat generated by RNA interference improves indices of large-bowel health in rats. *Proceedings of the National Academy of Sciences of the United States of America* 103(10):3546-3551.
- Roychowdhury R, Tah J (2013)**. Mutagenesis - A potential approach for crop improvement. In: (ed)(eds). *Crop Improvement*, edn: Springer. pp. 149-187.
- Schmutz J, Cannon SB, Schlueter J, Ma J, Mitros T, Nelson W, Hyten DL, Song Q, Thelen JJ, Cheng J (2010)**. Genome sequence of the palaeopolyploid soybean. *Nature* 463(7278):178-183.
- Schnable PS, Ware D, Fulton RS, Stein JC, Wei F, Pasternak S, Liang C, Zhang J, Fulton L, Graves TA (2009)**. The B73 maize genome: complexity, diversity, and dynamics. *Science* 326(5956):1112-1115.
- Schijlen EG, de Vos CR, Martens S, Jonker HH, Rosin FM, Molthoff JW, Tikunov YM, Angenot GC, van Tunen AJ, Bovy AG (2007)**. RNA interference silencing of chalcone synthase, the first step in the flavonoid biosynthesis pathway, leads to parthenocarpic tomato fruits. *Plant Physiol.* 144(3):1520-1530.
- Schwind N, Zwiebel M, Itaya A, Ding B, Wang MB, Krczal G, Wassenecker M (2009)**. RNAi-mediated resistance to Potato spindle tuber viroid in transgenic tomato expressing a viroid hairpin RNA construct. *Mol. plant Pathol.* 10(4):459-469.
- Service RF (2006)**. Gene sequencing: The race for the \$1000 genome. *Science (New York, NY)* 311(5767):1544.
- Sharma HC, Gill BS (1983)**. Current status of wide hybridization in wheat. *Euphytica* 32(1):17-31.
- Shepherd DN, Martin DP, Thomson JA (2009)**. Transgenic strategies for developing crops resistant to geminiviruses. *P. Sci.* 176(1):1-11.
- Sijen T, Kooter JM (2000)**. Post-transcriptional gene-silencing: RNAs on the attack or on the defense? *Bioessays* 22(6):520-531.
- Sledz C, Williams B (2004)**. RNA interference and double-stranded-RNA-activated pathways. *Biochem. Soc. Transact.* 32(6):952-956.
- Sunilkumar G, Campbell LM, Puckhaber L, Stipanovic RD, Rathore KS (2006)**. Engineering cotton seed for use in human nutrition by tissue-specific reduction of toxic gossypol. *Proceedings of the Nat. Acad. Sci.* 103(48): 18054-18059.
- Van K, Rastogi K, Kim K, Lee S (2013)**. Next-generation sequencing technology for crop improvement. *SABRAO J Breed. Genet.* 45(1):84-99.
- Varshney RK, Graner A, Sorrells ME (2005)**. Genomics-assisted breeding for crop improvement. *Trends in Plant Sci.* 10(12):621-630.
- Varshney RK, Hoisington DA, Tyagi AK (2006)**. Advances in cereal genomics and applications in crop breeding. *Trends in Biotechnol.* 24(11):490-499.
- Varshney RK, Nayak SN, May GD, Jackson SA (2009)**. Next-generation sequencing technologies and their implications for crop genetics and breeding. *Trends in Biotechnol.* 27(9):522-530.
- Vaucheret H (2008)**. Plant argonauts. *Trends in Plant Sci.* 13(7):350-358.
- Wessler SR (2006)**. Transposable elements and the evolution of eukaryotic genomes. *Proceedings of the Nat. Acad. Sci.* 103(47):17600-17601.
- Yu J, Hu S, Wang J, Wong GK-S, Li S, Liu B, Deng Y, Dai L, Zhou Y, Zhang X (2002)**. A draft sequence of the rice genome (*Oryza sativa* L. ssp. *indica*). *Science* 296(5565):79-92.
- Zhao B, Liang R, Ge L, Li W, Xiao H, Lin H, Ruan K, Jin Y (2007)**. Identification of drought-induced microRNAs in rice. *Biochem. Biophys. Res. Com.* 354(2):585-590.
- Zhou M, Li D, Li Z, Hu Q, Yang C, Zhu L, Luo H (2013)**. Constitutive expression of a miR319 gene alters plant development and enhances salt and drought tolerance in transgenic creeping bentgrass. *Plant Physiol.* 161(3):1375-1391.