

Plant Biology Techniques for Smart Agricultural Crop Production: Tradition to Advanced Technology – A Review

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ABSTRACT

Plant breeding improves the physiological health of the plants by targeting to the genotypes of the species and immunize them for particular pathogen; this naturally increases nutritional quality and productivity of agricultural lands. This superior quality of crops can tolerate abiotic or biotic stresses of the environment, synthesizes energy-rich biomolecules and supplements through organized metabolism, and equally productive for better human health. Current article is mainly focused on practices and techniques used in plant breeding and tissue culture methods, advance genome editing tools and their significance and efforts made by scientific communities during path of 'Green Revolution to Gene Revolution' for sustainable agriculture in India.

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Introduction

Plant breeding (PB) allows the meaningful editing of plant species at morphological and genotypical level. Conventional PB practiced back to ~10,000 years ago, after establishment of human civilization. Many domesticated varieties of crops also used to produce new variety as modern crops, that have both domesticated and advanced genetic traits. It is part of crop domestication. Another approach, classical plant breeding deals with crossing-over and linkage or artificial recombination or hybridization of pure lines, followed by artificial selection to produce advanced plants varieties. Such plants many transgenic or hybrid varieties produced through back-crossing or self-crossing and executed the traits of desirability like more nutritious in pigments, carotenoids or vitamins, trait for defending specific pathogen or draught resistant. With advancements in molecular techniques and bioinformatics tools, tissue culture experiments are now productive and useful for transforming the 'test-tube plants' on the real ground.

What are the major steps of breeding in production of new genetic varieties?

In India, Agriculture accounts for 33% of India's GDP and employs nearly 62% of the population. After

1947 of independence, one of the main challenges of country was to fulfill the food demand and food supply for the population of 350 million at that time, later massive population explosion also increased the agriculture pressure. As only limited land is fit for cultivation, India has to strive to increase yields/area from existing cultivating land (current agriculture area in our country (60.43%). In mid-1960, the experiments of test-tube culture of plants, intra ovarian pollination and artificial culture of embryo's lead the milestone of PTC in India and several high yielding varieties of wheat and rice were successfully introduced, these plant breeding techniques led to dramatic increase in food production in India. In the same window of time, autotetraploid origin of *Solanum tuberosum*, hexaploidy wheat, and development of C4 fixing rice varieties was studied²⁸, now these efforts are called Green Revolution. Some Indian hybrid crops of high yielding varieties are:

Wheat and Rice: In last six decades, the production of wheat has increased by nearly seven folds (11 million tons to 75 million tons). Similarly, the production of rice also raised by from 35MT to 89.5 MT. These crops were semi-dwarf phenotypically. Nobel laureate Norman E. Borlaug, at CIMMYT-Mexico,

developed semi-dwarf wheat. The high biomass yield or disease-resistant crops are *Sonalika* and *Kalyan Sona* also produced in India. IR-8 and Taichung Native-1 fusion produced semi-dwarf rice varieties. Jaya and Ratna are semi-dwarf rice now cultivated in rice-belt of India. Mutational breeding developed 200 varieties. *In vitro* cultured plants subjected to radiation therapy and their traits are mutated and expressed in expected time-line, e.g.; semi dwarf rice by X-ray induced mutation²¹, and γ -irradiation of rice variety Pelite-1 has produced high yielding and brown hopper resistant variety Atomita-2. Reimei, high yield rice is produced through γ -irradiation. Amber wheats Sharbati-Sonara, Pusa-Lerma were developed from 'red-grained' varieties Sonara-64, LermaRojo-64A same radiation treatment of plants. The multiple cross in (C-591 X P-19XC-281) fusion developed into wheat C-306. ADT-37 rice is another example (*Oryza japonica* x *Oryza indica*).

Sugarcane: Interspecific hybridization in *Saccharum barberi* X *Saccharum officinarum* leads to the development of hybrid sugarcane with high yields. Currently In India, 8.17m/ha area has 19.73 mt production of maize. Hybrid maize such as fusion of HKI-1128 X HKI-163 pedigree produce orange grain HM11, BML 6 x BML 15 pedigree produce yellow grain DHM-111, BML 6 x BML 7 pedigree orange-yellow grain DHM-117 etc. few varieties of hybrid maize are resistant to 'downy mildew and moderate tolerance to stem borer', for e.g. COH₃.¹³

Plant Breeding for Disease Resistance: Most of crops are microbial and nematode sensitive in tropical regions of world, their infection to the crops influences the crop productivity and yield. Thus 25% crop productivity may be influenced or sometime total. In this situation, breeding trials, trans gene cloning and tissue culture can experiment, can produce disease resistant plants thus, enhances food production. Nearly all plants have Resistance genes or 'R-genes' for disease resistance and immunity. Control of disease transmission among crops may bring by crop rotation, tillage, disease-free seeds etc., and exposure of pesticides to the farming land. Pure lines of crops should be selected to avoid inherent disease pressures. It is noticed that, cross pollinated crops have high degree of inbreeding depression, thus sterile crops may produce.²⁸ To overcome this, self-pollination may reduce the inbreeding depression in plants, probably.

Can we make disease-resistant or pathogen-free crops?

Inbreeding, backcrossing, mutation breeding, interspecific hybridization, hybrid breeding, and Genetic engineering are laboratory experiments on plants, that integrate genome of cells and enhance immunity to resist

the pathogen.²⁷ Conventional breeding includes hybridization and selection among selected plants this increases yield of crops as well disease-resistance. Germplasm screening, hybridization or cross between selected parents, hybrids selection and evaluation or testing and release of new varieties are major steps in this procedure. Disease-resistant plants traits may inherit from disease-resistance parent, thereby offspring chiefly produced by selection of a good source of resistance and a dependable disease test. Disease testing is performed by exposure of plant varieties to a particular pathogen under artificial conditions, thus diseased traits may express, morphologically. Among such a group of plants, those which are pathogen defending are identified and selected by determining 'disease triangle'. Special receptors called pattern recognition receptors 'PRR' distributed intracellularly and extracellularly on plant cells, which counteract specific intruder and produce immune response by secretion of anti-pathogenic or anti-microbial peptides and metabolites. Another set of R-genes of plants actively involved in defense mechanism by interfering to avirulence gene of pathogen. R-gene products destroy the toxic compounds of pathogen and remove the pathogen. Disease-resistant varieties may have resistance for wheat rust, fungal or viral diseases, early or late blight of potato, Tikka disease (*Cercospora arachidicola*, *C. personata*) etc. Few important disease/pathogen-resistant variety of crops are 'Himgiri' (wheat variety) resistant to 'wheat yellow rust' caused wind-blown spores of *Puccinia striiformis*, Pusa Swarnim (*Brassica* variety) can defend white rust *Albugo cruciferarum*, Pusa shubra and Pusa snowball-K1 (cauliflower) resistant for *Xanthomonas campestris* black rot and curl, Pusa komal *Vigna unguiculata* for bacterial blight *Xanthomonas axonopodis* pv *phaseoli*, and Pusa sadabahar (chilly) have specific resistance for CMV and TMV.

Other breeding methods are inducing mutation in plants through diverse mutagens, somaclonal variations, and genetic engineering. Mutation induces nucleotide or peptide orders thus metabolic order and signaling alters this resulting in the expression of a new traits in variety that is not observable in parental generation. Mutation breeding allows the filial generations to accept new traits, and improves their quality.¹⁷ Spontaneous mutations both germinal and somatic types. Somatic mutations are significant in vegetative propagation of seedless *Vitis* or Citrus production. Vegetative propagation is also useful in maintaining spontaneous germinal mutations,⁵ e.g., *Malus*, *Mangifera indica*, *Saccharum officinarum*, and *Solanum tuberosum*. <0.1% mutant alleles are beneficial, because mostly are recessive and produce harmful effects. The only limitation of spontaneous mutation (SM) is their slow mutation rate and frequency is poor $f\% < 1$

to 10^5 genes and benefit of SM is, they provide genetic variations. Rate of mutations is increased by mutagens. Induced mutations were first produced by X-rays on *Drosophila* and maize. Temperature and radiations are physical mutagens. During, mutational breeding selected seeds are treated with a selective and specific mutagen that seed can tolerate. After mutagenic treatment, seeds are screened and cultivated on field followed by self-pollination. There F1 generations cultivated on field in next crop season. Mutant crops are considered to produce mutant lines. The most popular example of mutational breeding is mung bean, resistant to MYMV (Circular-SS-DNA yellow mosaic virus) and powdery mildew were induced by mutations. *Abelmoschus esculentus* is introduced with resistant genes against **YMV** and developed as a new variety Parbhani kranti. **Atomic gardening** techniques are advanced plants mutation breeding where radioactive substances such as ^{60}Co are used. Few varieties of atomic gardening are tulips, snapdragons *etc.* Plant pathogens normally control over the host cellular machineries by damaging the metabolic networks. With the UV-radiation certain disease specific genes of pathogens are made inoperative *i.e.* knocked-out or KO for determining the virulence of pathogen. Chemi-mutagens are those alkylating substances that induce point mutation or transition mutation in purines especially guanine in DNA sequences for *e.g.* EMS (ethyl-methanesulphonate). X-rays induced mutation breeding successful for cotton plants in India, *e.g.* for world's 1st high yielding cotton mutant MA-9 and O -rays induced Calrose-76 rice in U.S.

Development of insect resistance in crops:

Insect resistance in host crops may be due to morphological, biochemical or physiological characteristics. Sometimes certain morphological traits of plants repulse the pest to attack on them. Such as plants those with a normal leaf and venation, attracts the insects while hairy or rough leaves repel insects. *e.g.* resistance to Hemipterans in *Gossypium*, Coleopterans in *Triticum aestivum*. In *Triticum*, external rigidity of stem and extra-toughness lead to the natural retention of Hymenopterans. Smooth leaved and nectar-free *Gossypium* non-fascinate the Lepidopterans. Biochemicals present in crops *viz.* high concentration aspartic acid, reduced nitrogen, increased phytotoxins, polysaccharides, or antifeedants (saponins) content in *Zea mays* leads to resistance to Lepidopterans (maize stem borers *Busseola fusca*). At recent, certain Arthropod-resistant plant varieties are produced by breeding. Such as *Abelmoschus esculentus* varieties Pusa Sawani, Pusa A-4 are resistant to *Earias vittella* probably. Pusa sem 2 and Pusa sem 3 are 'Helda varieties' of *Phaseolus vulgaris* are resistant to Jassids, Aphids, and fruit borers. Pusa

Gaurav (*Brassica*) shows aphids resistance.

Plant Breeding for Improved Food Quality:

Over 3 billion people of the world are suffering from 'hidden hunger' due to poverty or unaffordability to buy proper healthy food.²⁴ Nutritional quality of a crop may be defined as to the optimum nutrition essential amino acids or fatty acids for health and must not contain an antinutritional factors (ANF) that could toxic to consumer health. Glucosinolates (rapeseeds mustard), a neurotoxin, cyanolanine (*Lathyrus sativus*), **lectins** (Concanavalin-A), are ANF. Glucosinolates may act as goitrogens prevent the uptake of iodine in thyrocytes thus disable the synthesis of tri-iodothyronine or thyroxines, and thyrocalcitonin from thyroid gland, thus cretinism, and exophthalmic goiter in humans, and spontaneous abortion in pregnant woman. Ca^{2+} is important in neuron functions, cytokinesis, osteoblast, and fertilization mechanism in humans. Bohn; ANF such as oxalic acid and phytic acid strongly bind to Ca^{2+} and reduce its absorption in duodenum, thus above significant activities of Ca^{2+} ions are inhibited. However, phytic acids may digest in GI-tract of ruminant animals and excreted in phosphate form with manure, because these animals produce enzyme phytase that splits the phytic acids.² Trypsin of pancreatic juices and pepsin of gastric juices are proteases and their proteolytic acids digest the proteins by breaking peptide bonds present in the structure. Few legumes and soyabeans possess Bowman-Birk trypsin protease inhibitors and reduce the protein digestion, thus undigested proteins might be excreted with faecal matters. Cereal and millet contain less lysine and tryptophan, while pulses have less sulfur containing cysteine or methionine. Less dependence on cereals and pulses in diet result into less protein in cells, which may be scrutinized from lysine rich cereals (maize) *Shakti*, *Rattan* and *Protina*. Oil quality determines through concentration of saturated or unsaturated fatty acids. Compared to LCFA erucic acid (22C) PUFA rich seeds are in development. The interspecific hybridization between *Brassica oleracea* and *Brassica rapa* produced *Brassica napus* (an digenomic amphidiploid), now used for oil, biodiesel, and anticoagulant hirudin production. This oil has high ANF content, and poor storage property thus harmful to human health. Canola and Hoya are other varieties of rape seed with low quantity of both erucic acid or ANF.

◆ **Biofortification:** It is conventional selective breeding process for improving the nutritional value of plant by genetic modification or seed prime 'iron oxide'. Protein, Oil or triglycerides, vitamins, and micronutrient or mineral content is increased in host crop. Such biofortified crops can be promising solution against many diseases including Kwashiorkor, and Marasmus

[PEM]. Hybrid maize with high lysine and tryptophan value and useful for the people who are myoglobin, melatonin or serotonin deficient. Atlas 66 (*Triticum*) has higher protein, iron-fortified rice (IR68144 = IR8 X Taichung Native-1) variety has 5fold more iron than normal rice, and has significant value in pernicious anemia²⁴, and retinol rich rice (A_1 -golden rice) important in nyctalopia. IARI (New Delhi) has released several nutrient-rich crops *viz.* retinol enriched carrots, ascorbic acid rich *Momordica charantia*, *Lycopersicum*, Fe²⁺ and Ca²⁺ enriched *Spinacia oleracea*; and protein enriched *Vicia faba*, *Dolichos lablab* and many others. Biofortified plants may be source for production of edible vaccines also.

Plant tissue culture (PTC): PTC has vast application in horticulture, floriculture, agriculture and conservation biology. Normal or modified plant cells are cultured on nutrient media/slant, and grown into a new plant variety, that serve as phyto-bioreactor and produces more amount of secondary metabolites or phytochemicals such as drugs, toxins, pigments, lectins, gum and polymers. PTC increases the chances of production of artificial seeds, clones, or transgenic and hybrid plants with advance chromosomal and morphological characters. Tissue culture technique was of old concept & successfully attempted talk to mate roop. Plant growth regulators (PGR's) *viz.* auxin and cytokinin influence the plant growth. Embryoids are non-zygotic or somatic embryo-like structures and have potential to develop as full-fledged plants.¹³ The ability of cells to get divide and differentiate in variety of cells are called pluripotency and such cells are called pluripotent, this is basis of PTC practices. The descendent are called Clones or Ramet. The part of plant excised from parent and use for tissue culture is called explant, that must be surface sterilized and transfer it to culture media in aseptic environment 'Laminar Air flow'. Culture medium contain inorganic salts, vitamins, sucrose, and the PGR's *viz.* 2,4-D and cytokinins like benzylaminopurine.¹ The cultures shall be subjected to optimum temperature 24°C and illumination. PTC practices are of various types on the basis of *in vitro* growth as;

a) Callus culture or Suspension Cultures: In callus culture, explant meristematic cells will divide mitotically and produce an undifferentiated mass or cluster of cells 'callus' on agar medium. However, this is time-dependent growth period of 10-15 days for callus formation. Another strategy may adopt, when scanty cells or single colony is available during experiment. Colony is transferred or suspended in a liquid media enriched with auxin or 2,4-D. All these suspension cultures containing flasks must be placed on mechanical agitator or shaker at 100-250 rpm thus

agitation will create aeration, proper and constant inter-mixing of suspension and segregation of cell groups. If cellular biomass increases and the proportion of nutrient medium or volume declines, it indicates as tissue growth sign. These tissues are timely transferred to the fresh medium and vessels for irreversible growth *i.e.* subculture. Both, callus and suspension cultures increase the chances of increase in organic matter and biomass, regeneration of plantlets, isolation of protoplasts and production of transgenic plants.⁹

◆ **Regeneration of Plantlets:** Regeneration in cultured tissues promotes the development of root, shoot or somatic embryo. Plantlets are obtained from cultured cells by shoot regeneration followed by rooting of the shoots⁵ and regeneration of somatic embryos and their germination. Shoot regeneration is promoted by a cytokinin (BAP)³ and root regeneration by auxin (NAA). There must be Cytokinin-Auxin balance. Transfer the cultured callus on BAP, it will form shoots. As shoot elongates 2-3cm, excised and transferred them on auxin-containing media, its lower part regenerate into Roots. Plantlets can also be developed through somatic embryo regeneration, in which the pattern of somatic embryo development is similar to the zygote embryo. The somatic embryo develops from a sexual or vegetative part of the plant. The somatic embryo grows under high concentration of auxin [2,4-D], BAP, or Gibberellic acid. Somatic embryos or embryoids in plenty of growth medium germinate to yield plantlets. Developed plantlets selected from culture vessels and transferred in the field followed by hardening (< light + > humidity for a period of time *t*). Hardening increases plantlets tolerance and make them pre-adaptive for sudden environmental changes or abiotic stresses. Somatic embryogenesis is very useful for clonal propagation, elimination of viruses from tissues, protoplast and synthetic seed development.

Applications of Plant Tissue Culture

Rapid Clonal Propagation: Plantlets produced from callus or suspension culture share the genetic similarities, thus used for rapid clonal propagation.²³ The somaclonal variation in plants caused by mutation in chromosomal information, thus altered genotypic or phenotypic information expresses in plants.⁶ Change in ploidy, arm-position of chromosomes, structural change in chromosome, altered nucleotide orders are major factors resulting in somaclonal variation (SCV). SCV provides genetic variation in plants those regenerated from a single culture thus diverse varieties are produced. Plants inherit these genetic traits to offspring thus they are **somaclones**. This technique equally benefits to the plants regenerated

from callus.⁴ Genetic genotypic variations may change polyploidy and aneuploidy, chromosome aberrations (insertions, inversion, deletions, duplications, or translocations), and DNA sequence base mutations or frame-shift point mutation. A typical epigenetics-related event would be gene methylation.¹⁸ One advantage of SCV is that, somaclonal mutants can be enriched during *in vitro* culture includes resistance to biological toxins viz. mycotoxins, herbicides (2,4-D), high [salt Na⁺, K⁺, Cl⁻], mineral toxicity and tolerance to environmental or chemical stress, as well as for increased production of 2⁰ metabolites alkaloids, drugs, phytochemicals, gum, and polymers *etc.* SCV may genetically unstable and may pleiotropic. *Saccharum* somaclones are resistant to Fiji disease pathogen, *Solanum tuberosum* resistant to *Alternaria solani* toxins are selected examples of somaclonal variants.

Transgenic Plants: Transgenic plants are produced by cloning experiments under high level of aseptic conditions. Transgenic plants are produced by interkingdom or inter-phylum gene transfer in host *i.e.* natural plant with genes from another species like bacteria, yeast, single cell protozoan, or multicellular animal with desired trait. The genes for desired products are selected and cut out from one organism using molecular scissors, and ligated through molecular glue in host DNA *i.e.* recombinant-DNA. Host r-DNA has dual gene expression property one as self-gene and another acquired-gene. Such genetically modified plants with 'breeder's gene of interest' are transgenic plants, that may serve as 'bioreactor or factory of gene-expression' for production of hormones, drugs, vaccines, antibodies or plantibodies, carotenoids, energy-rich biomolecules, and biofuels. The transgenes are cloned into individual plant cells by gene-gun methods or suitable physical transformation methods. Such transformed cells expressing transgenes are selected *in vitro* among the non-transformed cells. Ultimately, plantlets can be regenerated from transformed cells. These plantlets grow as transgenic plants. Transgenic plants are remedy in health and therapeutics in many lethal diseases. One classical example that satisfies their importance is, an homozygous recessive inheritable 'Gaucher disease' where individual has reduced level of β -glucocerebrosidase thus poor sphingomyelin are synthesized. Such patients have many neural complications which are prevented by transgenic carrots cloned with taliglucerase- α . Zinc, arsenic, plumbum heavy metals and common environmental pollutants, their bioremediation is now possible through genetic strain of duckweed plants. HPV vaccine for cancer, mosquirix for malaria is produced from transgenic *Chlamydomonas reinhardtii*. Cowpea trypsin inhibitor (CpTi) is used for production of transgenic cotton

varieties. 'SunUp' is PRSV resistant transgenic papaya are produced from transgene experiments in plant breeding and tissue culture. Transgenic tobacco, Bt cotton are most selected examples used in biotechnology processes²². Production of such transgenic varieties is now referred as Gene Revolution.

Meristem Culture or MSC: This practice includes the shoot tips or internodes or nodal segments in explant¹¹ and cultured on BAP media to promote axillary branching. Small shoots (2-3cm) are harvested and rooted on a nutrient-rich media which develop as plantlets, followed by hardening and field trials. MSC practices enhance the plant immunity, biomass productivity, and disease resistance for *e.g. Elettaria, Fragaria*,²² *Musa*, Orchids, *Saccharum*, *Solanum tuberosum*, *etc.*

Embryo Culture (EC): Young embryo from developing seeds excised and cultivated on nutrient media propagate into fresh seedlings. EC has following applications: In some interspecific crosses, it is observed that the endosperm of developing hybrid seeds degenerate at an early stage of their life. Developing embryos procure nutrition from the endosperm. As endosperm reduces/declines, the hybrid embryo starts degenerating and died. Therefore, such interspecific crosses cannot be normally made. The embryo rescue may be alternative in such premises.¹⁶ Several interspecific hybrids have been produced by EC such as *Corchorus*, *Lycopersicum*, *Oryza sativa* *etc.* In orchids, EC used for rapid clonal propagation. Seed dormancy is another factor that resist the germination of embryo within seed such as hard cotyledon, lack of nutrition, adverse abiotic stresses lack of moisture, water, or temperature or enzyme-inhibition in cells. EC in such cases suppresses the barrier by providing optimum environment or chemical constituents, thus dormancy-breakage. Macapuno nut is example of embryo culture where egg nucleus has MM genes are dominant and germinating embryo, and mm recessive and non-germinating embryo. mmm polar nuclei produces Macapuno endosperm.

Anther culture or Haploid production: In nature, haploid (n) plants originate from unfertilized ovum, this phenomenon is parthenogenesis, but in laboratory, plants produced from both male and female gametes. During anther culture, the anthers from selected plants are cultured on an appropriate slant to cultivate haploid plants *in vitro* as studied in case rye.¹⁰ In India, Haploid production technique (HPT) was used to produce haploids of *Datura stramonium*.⁸ Haploid plants are always pure line due to single gene each trait, *i.e.* no dominant and no recessive. The mutation in such genes are easy to screen under expression studies. Jinghua-I (winter wheat) and Guan-18 (Rice variety) are also produced by HPT in China. Following anther culture, the pollen nucleus may continue

to divide and develop as pollen embryo. Alternatively, the continued division in pollen grows into callus that further regenerates shoots.¹⁹ In several species pollens are sources to obtain haploids or either haploid is produced by culturing unfertilized ovaries/ovules. Haploids are completely sterile and are used to produce homozygous lines in 2-3 years. This strategy can be easily integrated into breeding programs. Anthers from F1 plants (obtained by crossing >2 lines) are cultured to obtain haploid plants.²⁵ The ploidy of these haploid plants may double on colchicine treatment that result in homozygous plants. The progeny from these plants used to determine superior homozygous lines. HPT is productive for immediate expression of mutations.¹⁷

Protoplast Culture and Somatic Hybridization:

Fusion of two different somatic cells from two separate species form somatic hybrid (SH) is done by somatic hybridization.⁹ Hybrids are developed by the genetic fusion between two different varieties of same species (e.g. Non-flowering *Solanum tuberosum* X Flowering *Solanum tuberosum*) OR by genetic fusion or recombination of DNA between two different species. or between two different species (e.g. *Triticum aestivum* X rye *Secale* to produce Triticale).⁸ Workers performed protoplasm fusion in animals. Somatic hybrids of tobacco plants were first obtained by two different species (*Nicotiana glauca* × *N. langsdorffii*).⁴ The first step in somatic hybridization (SH) is the enzymatic digestion of cell wall components using pectinase or cellulase. Thus protoplasts are obtained.⁷ Protoplasts are widely used for uptake of foreign DNA in host. Generally, plants cells protoplasts undergo caulogenesis and regeneration thus callus or shoots are produced from PTC. Growth of protoplasts into callus and regeneration of shoots requires the proper balance of PGR in the growth media that must be customized for each species [Auxins, IAA, IBA, NAA, NOA, 2, 4-D] to support cytokinesis and callus growth (2,4-D), somatic embryo induction and rooting, cytokinins like kinetin, FAP, BAP, zeatin, 2-IP (Isopentenyl adenine), TDZ (Thidiazuron) are employed to promote cytokinesis, regeneration of shoot, somatic embryo induction, and proliferation and growth of axillary buds.²⁶ ABA promotes somatic embryogenesis and shoots bud regeneration in plants. GA₃ promote shoot elongation. The concentrations used of various PGR's are auxins 0.1-3mg/l; cytokinins 0.1-3mg/l; ABA ~0.2mg/l; and GA₃ 0.1-1mg/l. Protoplast fusion is mediated in presence of polyethylene glycol (PEG), and chile saltpeter or by electroporation. It produces hybrid protoplasts *i.e.*, synkaryon or heterokaryon. Sometimes one of the two nuclei of a heterokaryon degenerates (cybrid). When the protoplasts are cultured, they regenerate cell walls, perform mitosis and produce plantlets.⁹ SH allows the production of

hybrids between lines and species that cannot be produced normally by means of sexual hybridization. For example, fertile flowers in potato [non-flowering clone of potato X Flowering potato clone], or **Pomato**¹² [Potato X Tomato] or Topatoes.^{10, 20} Somatic hybrids may be used for cytoplasm or gene-transfer, or production of allopolyploids. Hybrid cells can be detected from non-hybrid cells using isoenzyme analysis amylase, esterase, aspartate aminotransferase, phosphodiesterase, isoperoxidase, and ADH/MDH/LDH. If the enzyme is dimeric, somatic hybrids contain an isoenzyme with an intermediate mobility property. The isoenzymes are variable in the same plant. Hence, it is important to use the same enzyme from each plant (parents and somatic hybrids), from a particular tissue with the same age. Chromosomal OR Molecular markers¹¹ RFLP, AFLP, RAPD, microsatellites, and PCR are also employed. Cybridization allows the desired cytoplasm to be transferred in a single step. Cybrids can transfer cytoplasmic male sterility (CMS), antibiotic and herbicide resistance in agriculturally crops including rice. Cybrids of *Brassica rapanus* contain nucleus, atrazine resistant chloroplasts, and CMS respectively from *B. napus*, *B. campestris*, *Raphanus sativus*.

Conclusion and Future Perspective

As we know, agriculture is broadly classified in organic and inorganic or conventional agriculture. We use manure or compost to nourish soil as they act as natural soil fertilizers and promote plant growth, crop rotation, use mulch, eradication of pests, and reduce diseases to grown plants while in inorganic agriculture we promote plant growth in presence of synthetic fertilizers, or herbicides, insecticides, antibiotics, or PGR's. In traditional indian farming, manure is first choice of farmers because it is easily available, cheap, and most important - manure can restore the soil textures and and improves retention capacities of water from soil particle spaces, thus soil quality remain sustained while, fertilisers compostition rich in N₂, K⁺, and P so enhances auxentic growth of crops. Organic agriculture is quite low crop yielding or productivity. However, part of that poor performance may be the result of growing poorly adapted varieties.¹² It is estimated that >95% of organic agriculture is based on conventionally adapted crops, even though the production environments found in organic vs. conventional farming systems are vastly different due to their distinctive farming practices.¹⁵ Most notably, organic farmers have fewer inputs available than conventional growers to control their production environments. Industrial agriculture is now dealing with single cell proteins production from algae and fungi. Agriculture can reduce water pressures at global level.¹⁷ Breeding varieties specifically adapted to the unique conditions of organic

agriculture is critical for this sector to realize its full potential. This requires selection for traits such as abiotic stress tolerance, pest or disease resistance, water or nutrient use efficiency *etc.* There are many classical and modern breeding techniques that can be utilized for crop improvement in organic agriculture despite the ban on GMO's. For instance, controlled crosses between individuals allow desirable genetic variation to be recombined and transferred to seed progeny by natural processes. Marker assisted selection (MAS), a diagnostics tool to facilitate selection of progeny along with the desired trait(s), significantly speed up the breeding process.¹⁴ Now a days, advancement in genetic engineering techniques is useful in development of disease resistant or pest-resistant plants. But, now there is emergence of computational sciences in agriculture that has provided a new direction to agriculture. At latest, the methods of artificial intelligence are applied in agriculture. The deep machine learning practices are used, where the species-specific data's are processed through algorithms, and valid interpretations are brought out for further use. With the use of deep neural networks and convoluted neural networks and image processing tools the crops and its environmental adaptation is understood. Let us assume, if crops are infected by pathogens, then infected crops will express particular symptoms such as change in leaf pigmentation, leaf-venation, chlorosis, or necrosis, curling pattern, dried leaf, spots-formation *etc.* These qualitative and quantitative informations are feeded in computers and associated operating tools. With this, utility of tools may provide prior knowledge of disease growth to the breeders, researchers, or farmers. Another, advancement in agriculture is the use of artificial sensors. Sensors are sensitive for abiotic changes *viz.* increase or decrease in temperature, moisture, water availability, light index, or photosensitive active radiation, soil nutrients level, soil pH, and other soil fertility parameters.²³ These sensors may also control the water sprinklers systems in green houses also. Complete growth and development studies of 'test-tube plants' in experimental field (green houses) also be performed by flying robotic drones. The capturing of crop-field images is carried out by drones. Thus disease prone area or disease-prone crops are mapped. Harvesting of diseased crops from field will

prevent the transmission of pathogen in healthier crops. Nematodes may be removed from the soil by artificial soil steam sterilization techniques. Quantity of pesticides, manure, or compost measurement may be possible by advance soil sensors. Culturing of plants in test-tubes or flasks by callus has few limitation, but now PTC lab's are spreading their research roots in molecular science. The bacterial cells produce special restriction enzyme called CAS9 to defend the bacteriophages and prevent their lytic or lysogenic action within their cytoplasm. CAS9 enzymes are expressed from 'clustered regularly interspaced short palindromic repeats' CRISPR genes in bacterial genome.³¹ In modern approaches in breeding, CRISPR or CAS9 are used for editing of plant genome. Thus edited DNA sequence information will not allow the progress of disease, creates barrier in plant-pathogen interaction, increases the immunity-assisting proteins in plant cells. Transgenic cum genome-edited plants may have special resistance against the particular diseases. The example of this advance genomic strategies for enhance crop production are Indica rice line IR58025B, in which the dense and erect panicle-1 gene (DEP-1) edited. This gene enables the rice plants to fix more nitrogen comparatively than normal rice, thus more nitrogen metabolism improves the rice grain yield.³⁰ The window of photoperiod in soyabean is now increased by inducing mutations on *Glycine max* flowering locus T-2a genes (GmFT2a)². Like soyabean, similar photoperiod and flowering experiment was successful on *Lycoopersicum* by the flowering suppressor SELF-PRUNING5G (SP5G) gene editing, thus enhanced flowering and fruting resulted.²³ Phytoene desaturase enzyme is important in yellow, orange or red pigments 'carotenoid' synthesis in *Citrus*, editing of CIPDS gene change the normal pink phenotype and replaced by white phenotype thus also called 'Albino Citrus'.²⁹ *Xanthomonas citri* subsp. *Citri* (Xcc) causes citrus canker thus genome editing and targeting of CsLOB1 gene provides the pathogen-specific resistance.¹⁸ All these advancement in genome editing and gene targeting, methods of deep machine learning, neural networking, and artificial intelligence are transforming the traditional farming into '**SMART AGRICULTURE**' and are now useful and equally significant for the crop and human health, positively.

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