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Short Communication

Widening the Horizons of Agriculture with Sustainable and Modern Scientific Techniques and Practices

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Most of the farmers are practiced in their conventional methods and they are not interested to upgrade or modify themselves towards the improved and long term cropping due to their illiteracy. This conventional methods have lots of drawbacks as following:

- Unnecessary use of high dose of chemical fertilizers herm the soil nutrients as well as the crop. Use of high amount of Nitrogen fertilizers makes the plants succulent and more prone to insect and pathogens. In other hand large dose of nitrogen hampers the uptake of other essential nutrients, shows deficiency of others.
- More use of concentrated chemical fertilizers alter the soil pH, soil structure and after all the soil health. That kind of soil will be fail to long term crop production, also reduction of nutrient use efficiency, further application will not give any satisfactory increase in yield. Over fertilizing also increase the weed growth and their populations. Also produce ground and other water pollutions by residual chemicals.
- In many areas farmers apply large amount of strong insecticides to control pests, initially they are managed but in next or successive seasons. Resurgence may occur, and in that case attack and pest populations will be tremendous. It will also kill many parasitoids, useful insects, pollinators.
- Over flooding the lands under cropping will increase leaching loss of nutrients, soil erosion, hamper root respiration, cause death of aerobic organisms.
- Frequent cropping, monoculture harms the ecosystem.
- High yielding varieties and Disease resistant varieties require judicial application of nutrients and water management, which is not followed by local farmers.
- Over tillage disrupts the soil structure. Over tilled soil will be more dense (high bulk density) after rain.
- Burning of crop residue will decrease soil fertility.

Practices for proper soil management

In recent days our main objective is to improve crop yield without compromising current and future generation of ecosystem.

Conservation tillage

Conservation tillage is a tillage in which soil surface will be covered with stubbles or previous crop residues. It is also known as vegetative mulching. By this method soil erosion due to rain water, desiccative wind and other forces are checked. The soil moisture is conserved by the reduction of evaporation due to vegetative mulch, and soil temperature fluctuation is also checked. Many nutrients are added to the soil, especially nitrogen; if green manuring crops (Dhaincha, sunhemp) are used for conservation tillage. Also, different microbial populations are increased in soil. Due to conservation of soil moisture and temperature; soil pH is buffered. Sometime different cover crops (forage crops like Oat) are used for vegetative mulch to cover the soil surface rather for harvesting. This method influences the long term maintenance of soil structure and soil health towards sustainable agriculture.

Application of organic matter (FYM, Composts, etc.)

- Organic matter have bulky nature, and also contain huge population of bacteria (produce slime); through their adhesive nature they produce soil aggregates, and porosity will increase. As a result good soil structure and increased water holding capacity is maintained.
- Organic matter provide a huge percentage of macronutrients and essential micronutrients, thus they can reduce use of chemical fertilizers.

- By the microbes present in organic matter the nitrification (Nitrosomonus, Nitrobacter) process is carried out and NO_3^- is produced which is available form for the plants.

- Through microbial decomposition, Mineralization (mainly of nitrogen), solubilisation (for phosphorus), mobilization (for potassium) of nutrients are influenced by different microbes, and the nutrients will be in soluble pool and available for plants.

- Microbes produce different kind of organic acids (citric acid, malic acid) which chelates the heavy metals and remove heavy metal toxicity. It also chelates Fe, Al which prevent the formation of metal-nutrient complex and prevent the precipitation of essential nutrients and bring them in soil solution. It also chelates the essential nutrients and prevent their loss, and microbial breakdown of those chelate compounds will release the nutrients for plants.

- Organic matter helps to increase the effective surface area of clay, through which it increase the cation exchange capacity (CEC) [ability to hold cations].

- Organic matter increase the use efficiency of chemical fertilizers, and reduce their application, also supply much amount of micronutrients.

It is recommended to mix fully decomposed organic matter during land preparation. In partially decomposed organic matter microbes are in dynamic state and they take up the soil nutrient for their own metabolism and produce deficiency for plants.

Paira cropping/utera cropping

Sowing of second crop before harvesting of the main crop is called paira cropping or utera cropping. A concept of Residual Soil Moisture and nutrient is coming. All the fertilizers and irrigations applied for the first crop is not completely used. Left over water and nutrients can be used for the second crop. By this method the excess nutrient loss (Leaching loss) is prevented. In normal cropping system excess nutrients pollute the ground water and the nearest water bodies. Paira cropping can minimize the excessive use of chemical fertilizers. Simply we can say amount of water and fertilizers for single crop is used for two crops here. As a result soil structure, nutrient use efficiency, moisture content are retained for much longer time. As for example lentil, pea and such crops are sown in paddy field.

Induced deficiency problems: (Antagonistic effect)

In our area there is a tendency of farmers to apply huge amount of fertilizers without any proper investigation. As for example we can say, a farmer applied a greater amount of nitrogen fertilizer than recommendation for better result, he also applied recommended dose of phosphorous. But the plant will show purple discoloration due to anthocyanin accumulation, which is a deficiency symptom of phosphorous. Here due to large nitrogen dose, the growth of plant cells were vigorous, so there phosphorous requirement increased much, as a result plants were showing phosphorous deficiency. It is a Nitrogen induced phosphorous deficiency. Here recommendation is to reduce the nitrogen fertilizer rather than increase in phosphorous. If they apply large amount of phosphorous there will be chance of increase in loss of nutrients, water pollution, and destruction of soil structure and cost of farming. There are so many pairs of such induced deficiency. Such as in reduced soil condition Fe^{2+} induced Zn deficiency occurs (as in reduced condition Fe^{3+} is reduced to Fe^{2+}, and there is only one oxidation state of Zn, naturally Fe^{2+} is dominated). So, farmers and technical field analysts should have to follow the universal DRIS chart for certain crops, proper soil testing, plant analysis to get rid of this kind of faults (Is the nutrient is originally deficit or it is suppressed by high dose of any other nutrient).

Prevention of excessive chemical fertilizers' application

- Excessive use of chemical fertilizers change the ratio of nutrients in soil, and soil structure. Also increase cost of cultivation.

- Higher the readily available soluble nutrient lower will be the microbial activity).

- Due to more solubility; excess and leached out fertilizers pollute the water bodies.

- Excessive use of Urea or any ammoniacal fertilizers produce NH_4^+ in soil system, which reduce the soil pH. In the other hand by cation exchange process NH_4^+ replace other base forming cation which adsorbed in soil colloid, causes the leaching and deficiency of those base formation cations. Excessive urea also induce the nitrification (NH_4^+ □NO_2^-□ NO_3^-) process, but due to acidic condition nitrobacters are inactive and the final reaction (NO_2^-□ NO_3^-) is prevented, thus Nitrite toxicity produce and harm seeds and seedlings. Excessive nitrogen fertilizers near sown seed also affect germination. To prevent such problems and leaching loss we have to use slow release N-fertilizers such as Sulphur/neem coated urea.

- Phosphorus have a tendency to bind with Fe, Al in acidic soil, and with Ca in calcareous soil and precipitate out. To check this, before application of fertilizers; chelating organic matter may be used to chelate those metal, one can apply lime to check soil acidity (OH- of lime replace the fixed phosphate and bring them on soil solution), gypsum application is recommended for calcareous and basic soil.

- So we shall reduce the amount of chemical fertilizers. Chemical fertilizers are now successfully replaced by biofertilizers like N_2 fixing - *Azotobacter, Azospirillum*, Cyanobacteria, *Rhizobium Bacillus megatherium* (phosphate solubilizer), *Pseudomonas* (k-mobiliser), etc.

Crop rotation, inter culture operation and contour strip cropping

Crop rotation is the practice of growing a series of different types of crops in the same area across a sequence of growing seasons. Due to different crop residues several nutrients are added in soil, changed crop environment develop dynamic microbes. It increase crop yield, improve soil structure and water holding capacity, reduce soil erosion, soil fertility and nutrients are sustained for a longer time, reduce the application of chemical fertilizers, it also reduce the outbreak of pathogen and pest due to different crop in successive season, suppress weeds, more cost effective. example- legumes with maize.

Intercropping is the practice for growing two or more crops in same season at close proximity (same row/beds, or rows that are close enough). By this process biodiversity is maintained, complementary sharing of plant resources (nitrogen fixing legume plants provide nitrogen to others), proper land/field utilization, weed-pest suppression, yield maximization, effective utilization of soil nutrient-reduction of additional chemical fertilizers applications, retention of soil moisture, maintenance of soil structure, reduction of soil erosion, proper cash utilization can be done. In this process to reduce the inter-competition between two crop we have to plant to different kind of crop. Example- Alfalfa (deep rooted) with cauliflower (shallow rooted). They take nutrition from different depth of soil, thus the competition among them is reduced. Also, association of cereals and legumes may be practiced.

Contour strip cropping is a farming with row patterns (equal-width strips) that run nearly level around the hill, not up down the hill, combined with crop rotation. It slow down the runoff water which increase water holding capacity, also reduce soil erosion.

- **Grassed waterway:** Native grassland strip of green belt, to control erosion.

- **Buffer strip:** A area of land maintained in permanent vegetation to control soil, air, water content, and ecosystem.

- **Agroforestry aspects:** Forest land is full of organic matter/microbes, soil moisture, and due to presence of large trees it is suitable for shade loving crops such as tea. We have to use the forest land for agriculture purpose, it will reduce the cost of cultivation, help is proper nutrient management, reduce soil erosion (strong tap root system of large trees), trees also act as windbreaks.

ZFNs and its applications

ZFN (Zinc Finger Nucleases) s are fusions of zinc-finger-based DNA-recognition modules and the DNA-cleavage domain of the Fok I restriction enzyme Each individual zinc finger typically recognizes and binds to a nucleotide triplet, and fingers are often assembled into groups to bind to specific DNA sequences to date, ZFNs have been used to modify *Arabidopsis*, *Nicotiana*, maize, petunia, soybean, rapeseed, rice, apple, and figure. In one example of the application of ZFNs to crop breeding, the endogenous maize gene *ZmIPK1* was disrupted by insertion of *PAT* ***gene*** cassettes, and this resulted in herbicide tolerance and alteration of the inositol phosphate profile of developing maize seeds. As a proven technology, ZFN-mediated targeted transgene integration was also used for trait stacking in maize, that is for assembling a number of useful traits together to create an even greater potential for crop improvement. Later, Cantos., *et al*. used ZFNs to identify safe regions for gene integration in rice, and these identified sites should serve as reliable loci for further gene insertion and trait stacking. Nevertheless, the design of ZFNs remains a complicated and technically challenging process, and one that often has low efficacy.

TALENs and its application

- The second type of GENOME EDITING tool for the purpose of crop improvement is TALENs. They are fusions of Transcriptional Activator-Like Effector (TALE) repeats and the Fok I restriction enzyme. However, each individual TALE repeat targets a single nucleotide, allowing for more flexible target design and increasing the number of potential target sites relative to those that can be targeted by ZFNs. Genome editing by TALENs has been demonstrated in varieties of Arabidopsis, Nicotiana, Brachy podium, barley, potato, tomato, sugarcane, flax, rapeseed, soybean, rice, maize, and wheat.

- The first application of TALEN-mediated genome editing in crop improvement was in rice, where the bacterial blight susceptibility gene *OsSWEET14* was disrupted and the resulting mutant rice were found to be resistant to bacterial blight.
- By knocking out the maize *GL2* gene, Char., *et al*. obtained mutants with the glossy phenotype, with reduced epicuticular wax in the leaves and the potential to be surface manured. In sugarcane, cell wall composition and saccharification efficiency have been improved by TALEN-mediated mutagenesis.

CRISPR Cas9 and its application

Introduction

CRISPR-Cas9 is a gene editing technology that offers the potential for substantial improvement over other gene editing technologies in ease of use, speed, efficacy, and cost. These characteristics led Science magazine to name *CRISPR-Cas9* gene editing technology "Breakthrough of the Year". Many in the scientific, engineering, and business communities believe that *CRISPR-Cas9* may offer revolutionary advances in the investigation, prevention, and treatment of diseases; understanding of gene function; improving crop yields and developing new varieties; production of chemicals used in biofuels, adhesives, and fragrances. CRISPR is an acronym for "Clustered Regularly Interspaced Short Palindromic Repeats", which are unique DNA sequences found in some bacteria and other microorganisms which help them to protect themselves from various phage (Bacteriophages) viruses. These sequences, along with the genes (Cas gene) that are located next to them, known as CRISPR-associated or Cas genes, form an immune system that protects against viruses and other infectious DNA. The CRISPR system identifies, cuts, and destroys foreign DNA. Researchers have identified five different types of CRISPR systems. The most studied CRISPR system is associated with the Cas9 protein and is known as *CRISPR-Cas9*. During 2012 and 2013, researchers modified *CRISPR-Cas9* to serve as an effective and efficient technology for editing the genomes of plants, animals, and microorganisms. Since then, *CRISPR-Cas9* has been used to modify the genomes of a variety of species-ranging from mice and fruit flies to corn and yeast. Many in the scientific community believe *CRISPR-Cas9* has shifted the paradigm with its simplicity and low cost relative to other methods of gene editing-removing barriers to widespread adoption and creating new research opportunities.

Mechanism (Principle)

CRISPR-Cas9 is a gene editing technology that uses a combination of an enzyme that cuts DNA (Cas9, a nuclease) and a guiding piece of genetic material (guide RNA) to specify the location in the genome. Generally, the guide RNA targets and binds to a specific DNA sequence, and the attached Cas9 enzyme cleaves both strands of DNA at that site. This cut may be used to insert, remove, or edit the DNA sequence as per the requirements. The cut is then repaired and the changes incorporated. This specificity of modification is one of that features, which differentiates *CRISPR-Cas9* from predecessor genome editing systems. Scientists can create a guide RNA (g RNA) corresponding to almost any sequence within an organism's genome. This special flexibility allows for the potential application of the technique to a very wide range of genomes, including microorganisms, animals, or plants. If the sequence of the desired target or gene (and its function) is known, in theory, *CRISPR-Cas9* could be used to alter the function of a cell or organism.

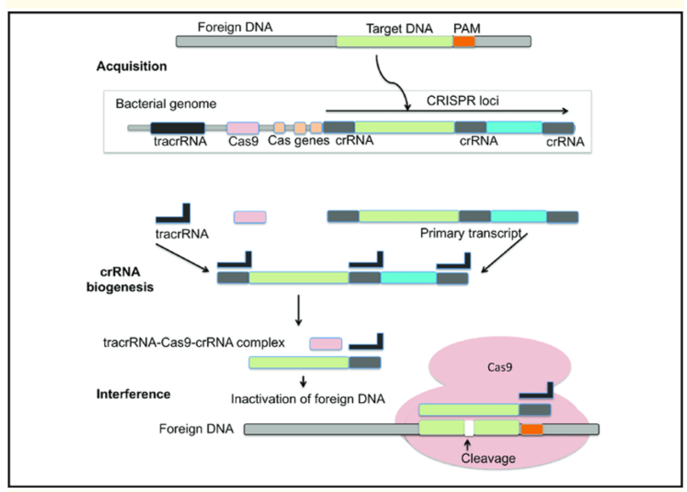

Figure 1

Application of CRISPR Cas9 (Specifically for agriculture purpose - crop improvement)

- *CRISPR-Cas9* technology and other genome-editing tools have generated substantial international interest in their potential for biomedical research and clinical innovations, the versatile technology may also make significant contributions to global agriculture. As *CRISPR-Cas9* permits the introduction or deletion of genetic sequences with much greater precision than traditional plant and livestock breeding techniques or earlier methods of genetic engineering (GE).

- Researchers see the *CRISPR-Cas9* technology as offering the capacity to engineer changes in major food crops by substituting existing plant DNA sequences with desired ones, or by enhancing or suppressing particular gene expression.

- Conventional plant breeding for desired traits often involves cross-breeding with related wild species of the target plant. However, this approach also introduces genes that are not wanted. *CRISPR-Cas9* allows the breeder to take only the gene of interest from the wild species and insert it at a precise location in the target organism to produce a new plant variety. In addition, this precision also reduces the plant breeding cycle by years through eliminating the time-consuming backcrossing procedure in conventional plant breeding and older GE techniques.

- Through more precisely altering DNA, *CRISPR-Cas9* and other genome engineering technologies have the potential to provide a level of control over plant genetic material that is unprecedented. Future crops created through these technological systems could include those with higher degrees of plant-pest control, plants with new and enhanced nutritional characteristics, and varieties that could be grown on marginal lands or in poor quality soils.

- Transgenesis-the introduction of foreign DNA into a plant genome-has characterized most commercial plant biotechnology innovation over the past 25 years. Most of the global acreage planted to GE (GENETIC ENGINEERING) crops today is in corn, cotton, soyabean, and canola production. Pest resistance and/or herbicide tolerance traits are the dominant features engineered into these GE crops.

- While *CRISPR-Cas9* permits similar transgenic manipulation, it does so with greater precision in the genome, and can involve more than a single gene insertion. New genetic variation can be created by identifying the precise DNA sequence modifications that are wanted in the cultivated variety, and then introducing them via the *CRISPR-Cas9* system. By controlling the specific genetic variation introduced into the cultivated plant, *CRISPR-Cas9* opens up a fundamentally new method of creating novel plant cultivars. For example, in 2014, Chinese researchers published a paper claiming the development of a strain of wheat that is resistant to powdery mildew, a fungal disease that affects a wide range of plants. CRISPR has also been used to modify the genes of a variety of other agricultural products, including rice, soyabeans, potatoes, sorghum, oranges, and tomatoes.

Major breeding methods and their utilization

Plant breeding can be broadly defined as alterations caused in plants as a result of their use by humans, ranging from unintentional changes resulting from the advent of agriculture to the application of molecular tools for precision breeding. There are so many breeding methods for both self-pollinated and cross-pollinated crops. Here we have discussed major plant breeding methods and their relevance in current condition of our country where the population is relentlessly increasing and there is a consistent decline in the environment to which crops are exposed.

- **Plant introduction:** Plant introduction consists of taking a genotype or a group of genotypes of plants into a new area or region where they were not being grown before.

- **Merits:** Introduction provides entirely new crop plants. It may provide superior varieties either directly, after selection or through hybridization. Introduction and exploration are the only feasible means of germplasm collection. Crops may be introduced to new disease-free areas to protect them, e.g. coffee and rubber.

- **Demerits:** The disadvantages of plant introduction are associated with the entry into the country of weeds, diseases and insect pests along with introduced material that may be a threat to ecological balance.

- **Mass selection:** In mass selection, a large number of plants of similar phenotype are selected and their seeds are mixed to constitute the new variety.
- **Merits:** This a good method for improvement of old varieties and land races. This is also used for purification of improved varieties. Mass selected varieties are more stable in their performance than purelines. In other words, they have more buffering capacity than purelines due to heterogeneity.
- **Demerits:** The selection is based on phenotypic performance. The superior phenotype is not always an indication of superior genotype. The real breeding value of single plants can be judged from the performance of their progeny. Progeny test is not carried out in mass selection.
- **Pureline selection:** In pureline selection, a large number of plants are selected from a self-pollinated crop and harvested individually; individual plant progenies from them are evaluated, and the best progeny is released as pureline variety.
- **Merits:** This is a good method of isolating the best genotype for yield, disease resistance, insect resistance, earliness, quality etc. from a heterogeneous population of an old variety.
- **Demerits:** this method can isolate only superior genotypes from a mixed population but can not develop new genotypes. This method is applicable to self-pollinated species only but can not be used for development of varieties of cross-pollinated species.
- **Hybridization:** Natural variability present in self pollinated population is exhausted quickly when they are subjected to selection. For further improvement, therefore, new genetic variability has to be created, which is easily and most commonly achieved by crossing two different purelines. This is called hybridization. It can be used to develop hybrid varieties for commercial cultivation.

There are some methods of handling segregating populations (viz. F_2, F_3, F_4 etc.) after hybridization.

Pedigree method

In pedigree method, a detailed record of the relationships between the selected plants and their progenies is maintained; such a record is known as pedigree record or simply pedigree. As a result, each progeny in every generation can be traced back to the F_2 plant from which it originated:

- **Merits:** Pedigree method provides information about the mode of inheritance of various qualitative characters, which is not possible by other breeding methods. There are chances of recovering transgressive segregants by pedigree method.
- **Demerits:** The selected material becomes so large that handling of the same becomes very difficult. Since large number of progeny are rejected in this method, there are chances of elimination of some valuable material.

Bulk method

In the bulk method F_2 and the subsequent generations are harvested in mass or in bulk to raise the next generation. At the end of the bulking period individual plants are selected and evaluated in a similar manner as in the pedigree method of breeding.

Merits: In this method natural selection operates which results in elimination of undesirable genotypes from the bulk population and increases the frequency of desirable plants. The chances of obtaining transgressive segregants are more in this method than pedigree method, because the materials are grown in large plots in this method.

Demerits: This method doesn't provide the information about the mode of inheritance of various oligogenic characters which is obtained in pedigree method. It is difficult to access the variability in the population and genotypic frequencies in this method, because they change in each generation of bulking.

Single seed descent method

A breeding procedure used with segregating populations of self-pollinated species in which plants are advanced by single seeds from one generation to the next is referred to as single seed descent method.

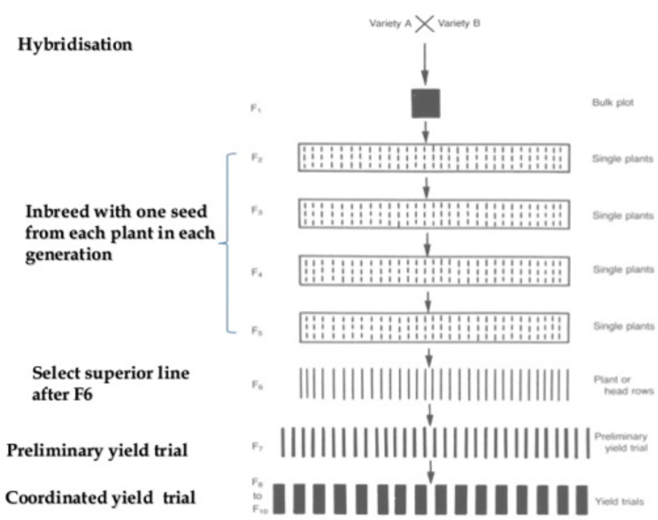

Figure 2

This is a modified form of bulk method. In this method, only one seed is selected randomly from each plant in F2 and subsequent generations. The selected seed is bulked and is used to grow the next generation. This process is generally continued up to F5 generation. By this time desired level of homozygosity is achieved. In F6, large number of single plants are selected and their progeny is grown separately. In F7 and F8 selection is practiced between progeny and superior progeny are isolated based on preliminary replicated trial. The superior progeny then tested in multilocation trials and the best progeny is identified for release.

Merits

- Rapid generation advance; 2-4 generations/year.
- Requires less space, time and resources in early stages, therefore accommodates higher crosses.
- Superior to bulk/mass selection if the desired genotype is at a competitive disadvantage, natural selection usually has little impact on population.
- Delayed selection eliminated confusing effects of heterozygosity.
- Highly amenable to modifications and can be combined with any method of selection.

Single seed descent method has aroused considerable interest among plant breeders and offers a unique opportunity for rapidly advancing segregating generations.

Backcross method

In this method, the hybrid and the progenies in the subsequent generations are repeatedly backcrossed to one of the parents of the F1. As a result, the genotype of backcross progeny becomes increasingly similar to that of the parent to which the backcrosses are made. The objective of backcross method is to improve one or two specific defects of a high yielding variety, which is well adapted to the area and has other desirable characters.

Merits: This is a useful method for transfer of oligogenic character like disease resistance. It is also useful in the incorporation of genes for quality such as protein content. Multiline varieties carrying resistant genes for different races of pathogen are also developed by backcross method.

Demerits: It involves lot of crossing work. The backcross has to be made for 6-8 generations but in pedigree and bulk method, hybridization is done only once. Sometimes, undesirable character is tightly linked with desirable one, which is also transferred to the new variety.

In these days as a responsible agriculturist our foremost objective is to- Increase crop yield, maintain the overall ecosystem and natural resources, control pollutions, overcome the various problems of soils and improve the soil health for a long span, make available the genetic improvement methods (CRISPER CAS9) to the farmers, who are not able to access those, proper utilization of modern breeding techniques for development of biotic and abiotic stress resistant varieties.

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