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# Vegetable Breeding Strategies

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***Authors' contributions***

*This work was carried out in collaboration between both authors. Author MBG designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author AM managed the literature searches of the study. Both authors read and approved the final manuscript.*

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## ABSTRACT

Variation present in vegetables provides plentiful of opportunity for development of breeding lines and hybrids. Germplasm development depends on incorporation of approaches such as MAGIC population, use of wild relatives, genetic transformation for transferring resistance gene and gene pyramiding. These approaches are most reliable to improve qualitative as well as quantitative characters. Since conventional methods are time consuming, molecular techniques are also utilized at appropriate stages to enhance swiftness of line development. Molecular approaches includes many tools but marker assisted selection is best to use in vegetable breeding programme. It helps to rapid back cross breeding also selection of resistance or qualitative lines from segregating populations. Double haploid is one of finest methods to create diverse pure lines in short interval. There are many ways to incorporate diverse gene in single line through double haploid technology. Hybridization mating such as line x tester or diallel are common methods instead combination breeding method is very result oriented as compared with others. The success rate of specific combination breeding is as much as double as associated with other ones.

*Keywords: Vegetable; germplasm; gene pyramiding; MAS; hybridization.*

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## 1. INTRODUCTION

Vegetable breeding in the turfs is constantly transforming and crafting new strategies and scope for meeting the challenges of new era. In recent years, world is most concern about food quality for health conscious consumers and quantity for rapidly increasing human population. Furthermore, challenges like abiotic and biotic stress cause significant loss for farmers [1]. Irish potato blight in 1840's led to death of millions of peoples, papaya ring spot virus in Hawaii led to collapse of papaya industry, Coffee rust of 2012 caused over one billion dollar damage to coffee plantation and still it tampering agriculture economy and one of latest leaf curl virus of chilli from 2015 to till date in India make huge loss and affects farmers [2-3]. Such kind of epidemics create loops at successive cropping and affects food security as well as economy of particular country or trade over world.

Plant breeding tools with improvisation and latest approaches give direction toward solving such glitches [4]. Artificial selections by human beings have already been effected for 10, 000 years for improving nutritional value and yield [5]. Conventional methods focused to improve the nutritional status of different food plants. Recent advancement in molecular technology pinups wide range of possibility and innovations in plant breeding [6]. Mendelian laws revolutionized plant breeding and crop improvement has been reformed to great extent as a magnitude of contemporary age of genomics [7].

Novel technology such as genomic selection, modern speed breeding, high-throughput phenotyping (HTP) empower the plant breeders to practice breeding with precision. Genetic engineering and molecular approaches play crucial role in developing crops with desirable characters using gene transformation [8-10]. Some of other technologies like large scale sequencing, genomics, rapid gene isolation and high throughput molecular markers also proposed to improve the breeding of commercially important crop species [11-12].

Vegetable breeding is somewhat different from the grain crops which are bind with mostly dominant or recessive traits. Role of the particular gene and its expression at definite environment condition rules in vegetable breeding which make it more complicated and challenging. Here we focus more toward solving quest rather than defining prerequisite discussed breeding methods. In conventional methods, purpose of plant breeding is aimed at yield, quality of crop, agronomic suitability and resistance for pest and disease [13]. In 1990, molecular techniques are introduced in Agriculture which creates a hope of solution toward tough and time consuming breeding approaches [14].

Molecular technology is tool for conventional breeding methods not actual breeding methods, this we forgetting while putting things forward. In 2021, we neglecting the main effect of environment and continuously changing challenges of resistance development which not possible to solve without field evaluations. In case of grain crops breeding is not that much complicated as in vegetables where epistatic gene play crucial role in defining crop for resistance of disease or pest [15].

In view of making complicated things simpler, here some amalgamation of approaches accrual for long term goal and also enhancement of research.

## 2. GERMPLASM BUILDUP

Germplasm is basic requirement for development of any crop. The fundamental objective of collecting plant genetic resources is to capture the maximum amount of genetic variation in the smallest number of samples [16]. Analyses of diversity in the existing collections using GIS tools facilitate identification of gaps to launch targeted collections. Germplasm collecting is expensive. Therefore, we should review the past collections of the crop before embarking on a new collection trip. If others have already explored the area under consideration, we should try to secure the germplasm from the earlier germplasm expedition team [17].

Collector must know about the crop thoroughly, cultivated land races, existing species along with wild species which can be used as bridge species for interspecific hybridization, etc. In case of vegetable germplasm, most of crops species are thermosensitive and photosensitive too [18]. In this case, most of international institute established satellite breeding stations for breeding and research purpose which is lacking in Indian agricultural sectors. As, in case of Bhoot Jolokiya, highest pungent chilli ever known lacks its pungency gradually when taken under cultivation in plains of India. In the same way happens with turmeric from Lakadong, curcumin

percentage highly varies when it cultivated outside of its original place. We must focus toward use of specific traits and its inheritance from area of specific cultivars in commonly cultivated varieties or hybrids. Satellite breeding stations helps in collecting germplasm in their natural state which also helpful to interspecific breeding programmes and other research oriented programmes.

The base collections of germplasm should be maintained at -20°C in vacuum packed standard aluminum foil pouches at 3% - 7% seed moisture content, depending on the crop species and with initial seed viability above 85%. Base collections ensure long-term viability of material (vary with crop and species) as a security to the active collection. This type of setup helps to secure main germplasm source if field germplasm got affected by and biotic or abiotic calamities [19].

The revolution in molecular biology, bioinformatics, and information technology has provided the scientific community with tremendous opportunities for solving some of the world's most serious agricultural and food security issues. The Generation Challenge Program (GCP) on "Unlocking Genetic Diversity in Crops for the Resource-Poor" is helping in molecular characterization of core and mini core collections to discern the diversity at DNA level and identify genetically diverse parents for mapping and use in breeding programs [20].

Germplasm collection is not only confined toward collection and preservation of variation among species. The conception of new germplasm is dedicated toward introduction of qualitative and quantitative traits in parental lines for hybrid development. The approaches for development of germplasm through different techniques are discussed further.

## 2.1 MAGIC Population

In plant breeding, the detection of quantitative trait loci (QTL) is no longer limited by the availability of genetic marker information and genotyping throughput, but rather by the genetic material employed. In an attempt to counteract this fact, Multiparent Advanced Generation Inter-Cross (MAGIC) populations were established. In MAGIC designs, multiple inbred founders are intercrossed several times in a well-defined order to combine the genetic material of all the founders in a single line. This leads to highly diverse genotypes each with a unique mosaic of founder alleles. The higher number of parents and recombination events of a MAGIC population are clear advantages compared to a classical biparental population, while for both designs pedigree and genetic structure are well known. In association mapping (AM) panels, genetic diversity and recombination rates are higher than in MAGIC designs as these panels take advantage of a collection of diverse breeding lines [21-24].

In vegetable breeding, MAGIC population creates marvelous traits combination as breeder needs in line. Vegetable is broad segmented area of research, in Brinjal, there are more than 42 types of segment based on morphological character of fruit is present. Instead of collecting all germplasm, breeder can use MAGIC population to develop required segment along with other important traits such as resistance, yield parameters, etc. to develop new type of entries or lines in germplasm. MAGIC population not only confined to craft traits but also to makeup lines with horizontal resistance which is most desirable for any breeder in making hybrids. With help of this multiple disease resistant hybrids can be made.

## 2.2 Wild Species

In Germplasm, wild species has special place in views of breeder. Wild species harboring genes meant for providing resistance to disease but also quality product. In most of the vegetables like tomato, Brinjal, chilli, okra, cucurbits, etc. several wild species where observed with unique resistance immunity [25]. Wide hybridization as a norm is an attempt of intermating two species of a genus or two genera of a taxon with an intention of introgression of genes of economic value into the cultivated species. Wide hybridization invariably comprises crosses between wild, primitively cultivated species and genera [26]. Wild species not only confer resistance but also helps to transfer male sterility in most of the cultivated crop species [27].

In India, most of agricultural institute having stock of wild relative species but most of them are not useful due to lack of proper supervision, management and research to evaluate them thoroughly. Use of molecular technology not only endeavor its genomics but also the compatibility among wild and cultivated species breeding for resistance and other important traits such as resistance for biotic and abiotic stress [28].

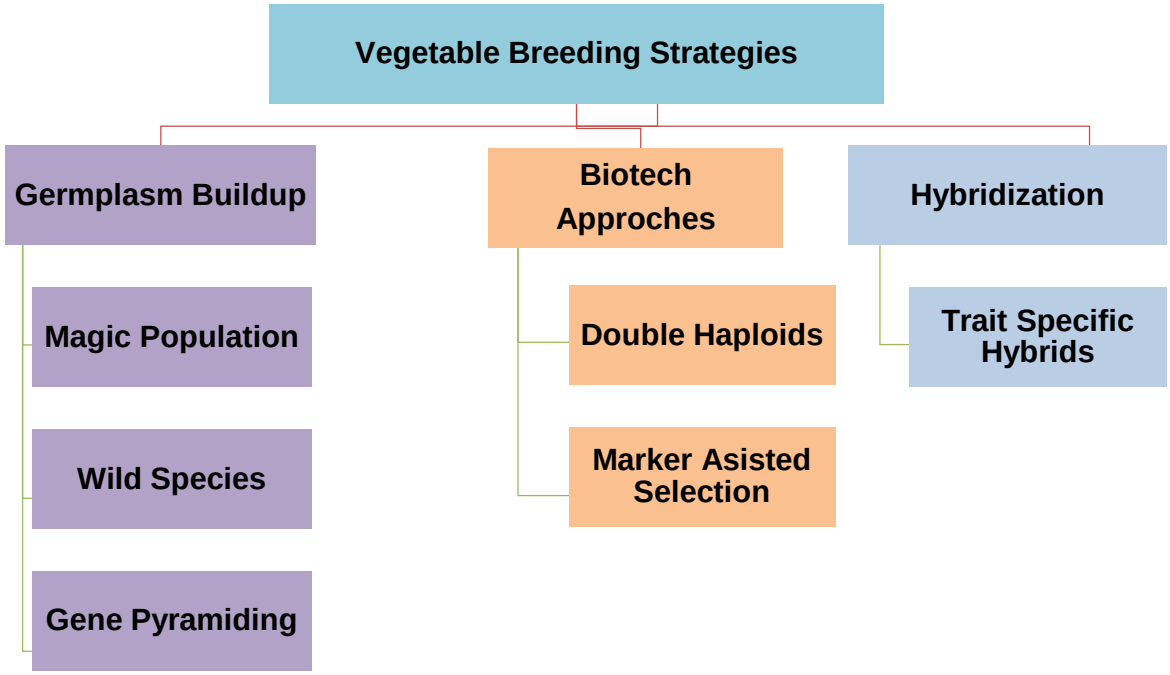


**Fig. 1. Vegetable breeding strategies**

**Table 1. Disease resistance in different vegetable crops**

| Crop | Wild Species | Character transferred |
|---|---|---|
| Okra | *Abelmoschus caillei* | Resistance to (YVMV) |
| Brinjal | *S. stenototum* | Resistance to bacterial wilt |
| Tomato | *Solanum hirsutum* | Resistance to Fusarium wilt |
| Chilli | *Capsicum chinense* | Resistance to fruit rot |
| Onion | *Allium fistulosum* | Resistance to Purple blotch |
| Potato | *Solanum demissum* | Resistance to late blight and leaf roll |
| French bean | *P. flavescens* | Rust resistance |
| Cucumber | *Cucumis Hardwiiki* | Resistance to green- mottle mosaic |

**Table 2. Insect resistance in different vegetable crops**

| Crop | Wild Species | Character transferred |
|---|---|---|
| Potato | *Solanum verni* | Resistance to Nematode |
| Brinjal | *S. incanum* | Resistance to Shoot & fruit borer |
| Cucumbits | *Cucumis trigonus* | Resistance to Fruit fly |
| Okra | *Abelmoschus manihot* | Resistance to Shoot & fruit borer |
| Tomato | *Solanum hirsutum* | Resistance to White fly |

**Table 3. Abiotic stress resistance in different vegetable crops**

| Crop | Wild Species | Character transferred |
|---|---|---|
| Okra | *Abelmoschus angulous* | Tolerance to Low temperature |
| Tomato | *Solanum cheesmani* | Resistance to High temperature |
| Onion | *Allium porrum* | Tolerance to Cold |
| Potato | *Solanum chacoense* | Tolerance to Heat and drought |
| Brassicae | *Brassica chinensis* | Drought and heat tolerance |
| Cucurbits | *Benincasa hispida* | Resistance to salinity |

**Table 4. Quality improvement in different vegetable crops**

| Crop | Wild Species | Character transferred |
|---|---|---|
| Tomato | *Solanum hirsutum* | Carotenoid content |
| Chilli | *Capsicum frutescence* | High capsaicin |
| Onion | *Allium kurrat* | Leaf flavour |
| Potato | *Solanum acule* | Starch content |
| Melons | *Cucumis melo* var. *cantaloupensis* | Thick rind and good keeping quality |

## 2.3 Gene Pyramiding

Gene pyramids are multiple genes controlling different trait, such as pathogen or pest resistance, etc. are accumulated, combined, or stacked into a single genotype [29]. Resistance gene pyramids usually consist of major, virulence-specific genes, but can include minor genes, defeated genes, effective genes, ineffective genes, race-specific genes, non–virulence-specific genes, or any other host genes involved in a resistance response to attack. This approach to increase resistance durability has been accomplished using traditional breeding and selection techniques. Best resistance gene pyramiding strategy is to increase the numbers of qualitative or quantitative resistance genes in a pyramid, or select the best combinations of different resistance genes [30].

The concept of gene pyramiding is similar to MAGIC population but we can use those line in lines improvement along with generation development. Use of dihybrid cross comprises resistance along with quality and yield attributes use for gene pyramiding in vegetables. There is lot of chance of selection of desirable traits in $F_2$ and $F_3$ generation and fixing them in further generations. It would beneficial for development of superior lines used in hybrid development.

## 3. BIOTECH APPROACHES

If we consider convention breeding is on side of coin then surely biotechnology emerges as another side. Conventional breeding methods takes five to six generations to transfer a trait within a species into the high yielding locally adapted cultivars and one has to plant a large number of progenies in order to select the plants with appropriate combination of traits. The improved lines developed then have to go through a set of multi-location tests, before a variety could be identified for cultivation by the farmers. This process takes minimum of 7 to10 years. However, genetic transformation provides access to genes from other species, which can be used for producing transgenic crops, ability to change the level of gene expression, and capability to change the spatial and temporal pattern of gene expression. The genes of interest can be transferred into the target crops/cultivars in a single event, and it takes 5 to 6 years to develop cultivars with stable gene expression [31-34].

In vegetables this timeline goes shorter as life span of most of vegetable crops is much shorter as compared to grain crops. It's easy to take even three generation in year in case of cucurbits and two generation in most of the vegetables. Biotech tools encompasses micro propagation and molecular tools, here we discussed about the effective tools in improvement of vegetables.

## 3.1 Double Haploids (DH)

Development of breeding line or elite germplasm lines takes several generation in vegetables, which is time consuming and also laborious process. Use of double haploids cut time interval [35]. Instead of going in details of procedure we take account of how we can make it fruitful strategy for vegetable breeding.

Double haploid emerges as boom for pure line development with qualitative and quantitative traits. Hybrids can be self and open for segregating generation selections. It takes up to 7 generations, instead if it takes for double haploid we can get plenty of lines with variations [36-37]. The same procedure can be adopted for transfer of biotic or abiotic resistance form wild to cultivated lines. Different aspect are given above such as double hybrids, three way cross and use of wild species along with exposure of male sterility with desire traits. In DH evaluation, one must keep following points into consideration:

1. DH provide large number of lines with variability of characters in 1-2 years.
2. In vegetables, though DH lines are homozygous in nature but most of traits specially resistance or quality traits are controlled by epistatic genes. Linkage factor also important to look after in DH population.

3. In 1st field trial, DH population taken for stringent selection by breeder for desirable characters.
4. The selected plants progeny should be tested for 2-3 seasons for fixation of characters.
5. After conformation of purity and trait fixation, breeder can utilize those lines for various purpose like hybridization, line improvement, CMS or GMS development, etc.

### 3.2 Marker Assisted Selection

MAS is one of best fitted strategy for back cross breeding, trait selection in line development, etc. Marker development is cost intensive process therefore important traits will be selected such as, disease resistance, male sterility and others related to shape, color, and architecture of whole plants and are often of mono- or oligogenic in nature [38]. The marker loci that are tightly linked to major genes can be used for selection and are sometimes more efficient than direct selection for the target gene [39]. In plants QTL mapping is generally achieved using bi-parental cross populations; a cross between two parents which have a contrasting phenotype for the trait of interest. Commonly used populations are near isogenic lines (NILs), recombinant inbred lines (RILs), doubled haploids (DH), back cross and $F_2$. Linkage between the phenotype and markers which have already been mapped is tested in these populations in order to determine the position of the QTL [40].

Such techniques are based on linkage and are therefore referred to as "linkage mapping". QTL based MAS for back crossing is very efficient for identifying concentration of gene governing trait and its saturation in required elite line by using foreground and background selection. Recently, high-throughput genotyping techniques are developed which allows marker aided screening of many genotypes. This will help breeders in shifting traditional breeding to marker aided selection. Gene pyramiding has been proposed and applied to enhance resistance to disease and insects by selecting for two or more than two genes at a time [41-42]. The advantage of use of markers in this case allows to select for QTL-allele-linked markers that have same phenotypic effect [43].

## 4. HYBRIDIZATION

The chief objective of hybridization is to create genetic variation. The degree of genetic variation produced in the segregating generations would, depend on the number of genes that differ in the parent of $F_1$. If the two parent are closely related, they are likely to differ in few genes only. But if they are not related or are distantly related, they may differ for several, even a few hundred, genes [44-47]. Therefore, when it is said that the $F_1$ is 100% heterozygous, it has reference only to those gene for which the two parent differ. The aim of hybridization may be:

1. Transfer of one or few qualitative characters
2. Improvement in one or more quantitative characters.
3. Use of $F_1$ as hybrid variety

Hybridization process includes choice of parents, evaluation of parents, emasculation, hand pollination, tagging, bagging, etc. we focus toward the untouched areas of vegetable hybridization discussed here.

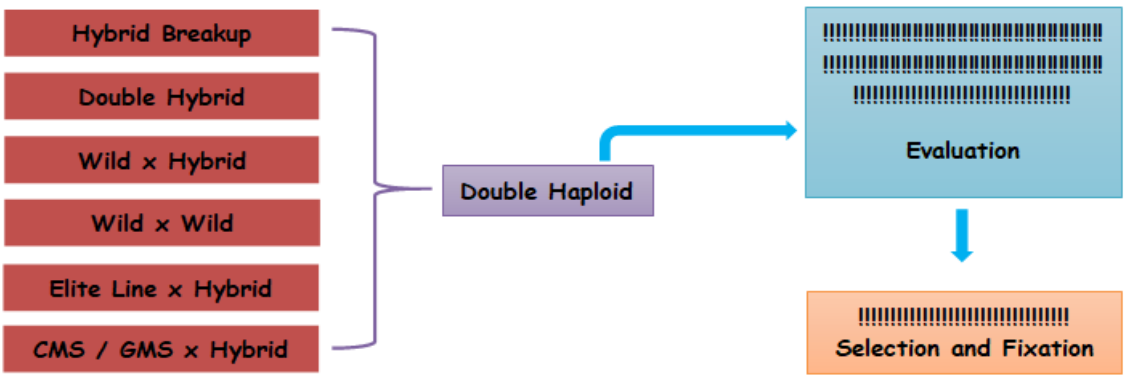


**Fig. 2. Double Haploids development strategy**

### 4.1 Trait Specific Hybrids

Development of hybrids is prime focus of vegetable breeding. Several combination of procedures were followed for making hybrids such as, diallel (partial, half and full), line x tester, etc. This techniques are made for grain crops not for vegetables. Producing hundreds of hybrids using few lines is just waste of time, money and efforts as it results in selection of only 10-15 hybrids can go for next evaluation trials of which hardly one or two fetches goal of hybridization. In India most of institutes are still running on this aspects.

Vegetable breeder must have grater knowledge on the germplasm deeply and also on the pipeline generations under development which he can utilize in further breeding. As we discussed earlier in germplasm chapter, strategic involvement of qualitative and quantitative genes of traits in line will be helpful for development of elite breeding lines. Use of CMS line for hybridization reduce the cost of emasculation process [48-49]. The superior lines should be converted in CMS stock to use in hybridization programme. In vegetables segregating generations, breeder have to study and observed the flow, fixation and expression of epistatic gene governing traits, it helps breeder in selection of parents for hybridization in vegetables [50-52].

Development of trait specific hybrids in vegetables increase the chances of success in $F_1$ production. Most important step is choice of parents, it depend on analysis of expression of traits such as resistance, fruit quality, plant growth, etc. which are epistatically heritable [53]. It's not always be effective as resistance parent taken as female in cross combination of hybrids.

It was seen and noticed that if female carry the resistance the $F_1$ also be resistance. This concept fails in case of vegetables. Vegetable breeding mainly focused on resistance for various biotic and abiotic stress, especially disease. Disease resistance govern by recessive or dominant epistatic is tough to incorporate in elite lines through back crossing or MAS method, it would be incorporate through segregating populations. Parents involve in $F_1$ though possess resistance but it's depend on expression of particular gene in F1 hybrid [54].

The $F_1$ developed in trait specific hybridization must be evaluated where the expression of character such as resistance is at its best. Chilli LCV express at hotter region like Maharashtra, Madhya Pradesh, Rajasthan and Andhra Pradesh but fails to express if trial taken at cooler regions like as Bangalore vice versa for Chilli green mottle virus which express in Bangalore regions only. Stringent testing will helps to section of best $F_1$ for release [55-57]. The success rate in trait specific hybridization is doubled as compared to conventional methods.

## 5. CONCLUSION

Vegetable breeding is one of the important and challenging area of agriculture. The conventional and molecular approaches are helpful for breeding in agricultural crops. Improved and well developed germplasm is key factor in developing $F_1$ hybrids. Quality of segregating population can be levelled up by introduction of approaches like MAGIC population, introgression of wild species and gene pyramiding. All these strategy creates needful variation in germplasm population, which also helps breeder to study character flow within segregating population and gene behavior with respect to environment which is very important.

Application of only those molecular tools that gives result like utilization of double haploid technology for prompt development of stable and pure line with demanding variations. Molecular techniques are costlier markers are to be developed only for those traits which possess prominence then others as well as not detected by morphologically such as, resistance for disease, qualitative traits (color, pungency, acids, pigmentation, oleoresin content, etc.).

Marker assisted selection based on QTL's, which make this tool useful in fast-track back cross breeding for resistance. The primary stage selection with use of MAS in $F_2$ population for specific traits development in segregating generations. When we deal with vegetable breeding, breeder must concentrate for trait specific hybridization. This helps breeder to develop limited crosses with more probability of getting success.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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