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Review Article

Wide Hybridization in Vegetable Crops

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A B S T R A C T

Green revolution has transformed India into a food grain surplus country from a deficit one. No other activity has such an immense impact on the agricultural development as the green revolution has done. It also, has reflected its impact on breeding and production of vegetable crops. Side effects of green revolution were witnessed largely in the form of reduced varietal diversity in major cultivated crop species and increased uniformity in appearance and harvestable products. This predisposed improved agriculture to natural calamities. Emergence of new pathogen races lead to outbreak of diseases and pest attack caused yield losses up to 50 percent. Changing climatic condition has caused abiotic stresses like drought, flood, salinity and high temperature, which in turn results in reduction of yield and quality. To feed the ever-increasing population and to fight with malnutrition, wild species offers the scope for quality improvement in vegetable cultivars.

Keywords
Wide hybridization, Vegetable crops, Green revolution

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Introduction

Green revolution has transformed India into a food grain surplus country from a deficit one. No other activity has such an immense impact on the agricultural development as the green revolution has done. It also, has reflected its impact on breeding and production of vegetable crops. Side effects of green revolution were witnessed largely in the form of reduced varietal diversity in major cultivated crop species and increased uniformity in appearance and harvestable products. This predisposed improved agriculture to natural calamities. Emergence of new pathogen races lead to outbreak of diseases and pest attack caused yield losses up to 50 percent. Changing climatic condition has caused abiotic stresses like drought, flood, salinity and high temperature, which in turn results in reduction of yield and quality. To feed the ever-increasing population and to
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fight with malnutrition, wild species offers the scope for quality improvement in vegetable cultivars.

In order to restore the characteristic of ecological sustainability and to combat the biotic and abiotic stress in cultivated vegetable crops, wide hybridization has been advocated a strong tool in the hand of plant breeders as wild species are the rich pool of noble characters, better quality and processing traits as well as imparting resistance against biotic and abiotic stress. Crop wild relatives have been used from decades for breeding, in particular to transfer genes of resistance or tolerance to pests, diseases or abiotic stress to the cultivated species. Wide hybridization comprises the efficient conventional breeding and modern molecular techniques as its effective tool in crop improvement (Table 1–8).

**Wide hybridization**

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. Interspecific hybridization means hybridization between individuals from different species belonging to same genus. Intergeneric hybridization means hybridization between individuals from different genus belonging to same family (Liu et al., 2014).

**Interspecific and Intergeneric hybridization**

**Role of wide hybridization**

Disease resistance against various pathogens

Insect resistance
Abiotic stress resistance
Quality improvement
Yield enhancement
Development of new crop species
Transfer of sterile cytoplasm for hybrids production
Rootstock breeding

**Barriers associated with wide hybridization**

**Spatial isolation**

It is associated with geographical distance, physical separations of time and environment. Sensitivity to photoperiod, introduction to different latitudes and separation by geographical and political barriers can isolate two species reproductively. Development of different maturity groups in knol khol, radish and cauliflower are consequences of spatial isolation.

**Pre-fertilization barriers**

These barriers are operative between the parental species. They prevent crossability by hindering the fertilization process. Such disturbance of fertilization in interspecific crosses is also termed as interspecific incompatibility or incongruity. Lack of pollen-stigma recognition is mainly responsible for incongruity. The reaction is sporophytic in nature and reported to be associated with pollen wall substances.

**Failure of pollen germination**

Interspecific hybrid in *Cucumis* species through conventional breeding procedures was unsuccessful because of the existence of a pre-fertilization barrier. The barrier was characterized by non-germination of pollen even up to 72 hours after pollination (Chatterjee and More, 1991). Kaneko and
Bang (2014) reported the same problem in case of *Brassica campestris* and *Raphanus sativus*.

**Swollen pollen tube growth**

This has been widely reported in case of failure of wide crosses. Incompatibility in *Cucumis spp.* is characterized by delayed growth of pollen, or arrested pollen tube growth in the stigma, or inability of pollen tubes to reach the ovules (Chen and Adelberg, 2000). Based on pollen tube growth behaviour, crosses between African groups of *Cucumis* were classified into three groups, namely bilateral congruity, bilateral incongruity and unilateral incongruity.

**Lack of fertilization**

In such cases, pollen tube effectively delivered the two sperm nuclei to embryo sac. In one case, zygote was formed but no endosperm development while in second case 10 percent crosses developed endosperm but no embryo.

**Post fertilization barriers**

These barriers are operative in the distant hybrids and their progenies. These are also known as post syngamic barriers and include embryonic breakdown, failure of zygote development, abnormal fertilization, inhibition of endosperm and embryo development.

**Hybrid embryo abortion**

Arrest of embryo development or its abortion has been noticed in several interspecific hybrids. The major barriers to interspecific hybridization in *Phaseolus* sp. *i.e. Phaseolus vulgaris* × *P. acutifolius* is embryo abortion. In case of *Phaseolus coccineus* × *P. vulgaris*, abnormal embryo development is observed (Andradf- Aguilar and Jackson, 1988).

**Hybrid inviability**

It results from various factors and manifests from zygote development to seed formation. It operates in *F*₁ seedlings causing high mortality, hybrid plants are characterized by poor growth, chlorotic leaves and die before maturity. The reason may be disharmony between nucleus of one species and cytoplasm of other or between nucleus of both the species or due to action of specific genes known to cause lethality, chlorosis and weakness in *F*₁ hybrids. e.g. Cross between *Cucumis sativus* × *C. melo* and *Abelmoschus esculentus* × *A. tetraphyllus*.

**Hybrid sterility**

*F*₁ hybrids are characterized by lack of seed set. Hybrid sterility is due to manifestation of lethal genes, genetic imbalance due to chromosomal non-homology, chromosomal elimination and endosperm abortion. Several interspecific hybrids of *Abelmoschus esculentus* × *A. ficulneus* and *Abelmoschus esculentus* × *A. tuberculatus* show very poor seed set.

**Failure of flowering in the progenies**

Either hybrid is devoid of reproductive structure or highly deformed non-functional structures are formed. It is due to failure of physiological differentiation while non-functional reproductive systems are attributed to failure of meiosis in either one or both the sexes.

**Hybrid breakdown**

It is manifested in the form of inviable and weak *F*₂ generation or later generation. In some wide crosses seed setting is normal, even *F*₁ plant progenies show normal development and good fertility but in the *F*₂ generation performance of hybrids fall below satisfaction and sometimes may be complete
sterility. Afful et al., (2018) observed failure in fruit set, when the wild accessions were used as female parents in the crossability study of cultivated brinjal with *Solanum torvum*, *S. anguvi* and *S. aethiopicum*.

**Techniques to overcome pre-fertilization barriers**

**Taxonomic position of parental species**

An ease in hybridization is expected when the species more resemble phenotypically. Taxonomic classification is based on morphological features but by and large is the outcome of genetic factors in association with environment.

**Doubling of chromosome number**

When the failure of hybridization is due to different ploidy level then this technique is followed. In many polyploid cultivated species, their wild progenitors are diploid and crossing attempts are difficult. One may increase ploidy level of wild type by colchicine treatment. This enhances the success rate in crops like potato, *Cucumis* and *Brassica* spp.

**Bridging species technique**

In many crops, two species which are otherwise incompatible, may be hybridized with the help of third species. The third species acts as bridge in recombining the two incompatible species so known as bridging species. This technique has been used in making wide crosses in potato, lettuce and sugar beet.

Hayes et al., (2005) were successful in using *S. verrucosum* as a female bridging parent to access 2x (1EBN) *S. pinnatisectum*. *Solanum simplicifolium* was used as bridging species in crossing *S. acuale* and *S. tuberosum*

**Shortening of the style**

In some species, the incompatible reaction can be overcome by reducing the length of style. Incompatible reaction in radish can also be overcome by reduction of stylar tissues.

**Mentor pollination**

Pollen grains of distant species do not germinate on the stigma of cultivated species. However, when these pollen grains are mixed with killed maternal pollen grains, germination of the incompatible pollen grains take place as in case of Cucumis (Beharav and Cohen, 1994). It happens because cell wall proteins of pollen play pivotal role in pollen-pistil interaction. Pollen killed in ethanol and mixed with incompatible pollens, release the proteinaceous recognition factors, thereby masking rejection reaction of the recipient stigma. The killed maternal pollen is known as mentor pollen.

**Use of growth regulators and immunosuppressants**

Growth Regulators (GRs) enhance the zygotic formation in distant hybrids of many cucurbits, okra and tomato. Commonly used GRs are IAA, IBA, GA3. GA3 75ppm application to maternal plant 1 or 2 days before and after pollination improved zygote formation, faster pollen tube growth, more embryo survival and more seed set in wide crosses. Application of auxin in *Solanum* prevented flower abscission and enhanced fruit set.

**In vitro fertilization**

It is effective when stigma and style inhibit pollen tube growth and embryo abortion occurs at early stages of development. The whole gynoecium is excised and placed on MS medium followed by dusting of pollens on the stigma and fertilized ovule is reared to maturity (Ondrej et al., 2002).
**Protoplast fusion**

It involves the fusion of protoplast of two incompatible species or genera and the fused heterokaryon is placed on artificial nutrient medium and regenerated into hybrid plant. This approach has particularly more potential to generate new genotypes in vegetatively propagating species. Some examples are tomato+potato, *Solanum nigrum*+ *S. tuberosum*. Chandel et al., 2015 studied interspecific potato somatic hybrids between cultivated *S. tuberosum* dihaploid C-13 and wild species *S. cardiophyllum* via protoplast fusion. The use of somatic fusion for improving valuable agronomic traits in cultivated potatoes is done for traits like atrazine resistance, frost tolerance, quality improvement and pest and diseases resistance.

**Techniques to overcome post-fertilization barriers**

**Embryo rescue**

Embryo abortion is the major barrier in distant hybridization. It can occur at any stage of development depending on the genomic relationship of two parental species. *Solanum sitiens* is a rare endemic plant of the Atacama Desert of Chile having tolerance to drought, salinity and low temperatures, resistances to certain pathogens, and modified fruit ripening. To overcome sterility and unilateral incompatibility of *Solanum lycopersicum* × *S. sitiens* hybrids, embryo culture was used (Chetelat, 2016).

*Capsicum chinense*, *C. annuum* and *C. frutescens* were crossed with each other and embryo rescue was done between 27-33 days after pollination by Debbarama et al., 2013. Hybrid plants were obtained and their hybridity was confirmed using both morphological and RAPD markers.

**Ovary culture**

In some wide crosses, embryo abortion occurs at early stages of development when it is difficult to excise and culture embryos. To overcome this problem, ovaries are cultured. Depending on cross combination, ovaries 2-15 days after pollination are excised and cultured on nutrient medium. Flower is pruned by removing calyx, corolla and stamen. Distal part of the pedicel is cut and ovary planted on simple semi-solid MS nutrient medium. When the embryos become visible, they are aseptically taken out and cultured in a manner of embryo rescue technique.

**Ovule culture**

Ovule culture is an elegant experimental system by which ovules are aseptically isolated from the ovary and are grown aseptically on chemically defined nutrient medium under controlled conditions. It is used when barrier impedes growth of zygote at earlier stages of development.

**Backcrossing**

Wide crosses showing poor fertility can be back crossed to cultivated species to improve the fertility of hybrids. Backcrossing can be applied to balance cytoplasmic interaction by producing cybrids or alloplasmic lines.

Chamola et al., 2015 used cytoplasmic male sterile (CMS) lines of *Brassica juncea* and *B. napus* with the mitochondrial genome of *Moricandia arvensis* and *Erucastrum canariense*, respectively, were used to transfer CMS to cauliflower (*B. oleracea*). Embryo rescue was also done in BC1 and BC2 to obtain progenies. Recovery of the recurrent parent phenotype was faster in *B. napus* x *B. oleracea* than *B. juncea* x *B. oleracea*. BC3 generation plants of *B. napus* x *B. oleracea*
showed good curd formation and complete male sterility and nine bivalents at meiosis whereas those of *B. juncea* x *B. oleracea* were male sterile but still had genetic elements of *B. juncea*.

**Table.1 Interspecific v/s Intergeneric hybridization**

| Particulars             | Interspecific Hybridization                                                                 | Intergeneric hybridization                                                                 |
|-------------------------|-------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------|
| Parents involved        | Involve two different species of the same genus                                            | Involve two different genera of the same family                                           |
| Fertility               | Such hybrids vary from completely fertile to completely sterile                           | Such hybrids always sterile                                                               |
| Use in crop improvement | Frequently used                                                                           | less than interspecific crosses                                                           |
| Release of hybrid       | Possible in some crops                                                                     | Not possible                                                                              |
| Varieties               |                                             |                                                                                            |
| Evolution of new crops  | Not possible, but evolution of new species is sometimes possible                           | Sometimes possible                                                                        |

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

| Crop      | Character transferred | Wild Species          | Species          |
|-----------|-----------------------|-----------------------|------------------|
| Okra      | Resistance to YVMV    | *Abelmoschus caillei* | *A. esculenta*   |
| Brinjal   | Bacterial wilt        | *S. stenotomum*       | *S. melongena*   |
| Tomato    | Fusarium wilt         | *Solanum hirsutum*    | *S. lycopersicum*|
| Chilli    | Fruit rot             | *Capsicum chinense*   | *C. annum*       |
| Onion     | Purple blotch         | *Allium fistulosum*   | *A. cepa*        |
| Potato    | Late blight, leaf roll, virus-x | *Solanum demissum* | *S. tuberosum*   |
| French bean | Rust resistant      | *P. flavescens*      | *P. vulgaris*    |
| Cucumber  | Green-mottle mosaic   | *Cucumis hardwickii* | *C. sativus*     |

**Table.3 Insect resistance in different vegetable crops**

| Crop       | Character transferred | Species transferred from | Species transferred to |
|------------|-----------------------|--------------------------|------------------------|
| Tomato     | White fly             | *Solanum hirsutum*       | *S. esculentum*        |
| Potato     | Root knot nematode    | *S. peruvianum*          | *S. esculentum*        |
| Okra       | Fruit and shoot borer | *A. manihot*             | *A. esculentus*        |
| Brinjal    | Shoot and fruit borer | *S. incanum*             | *S. melongena*         |
| Epilachna beetle |                   | *S. nigrum*             | *S. melongena*         |
| Cucurbits  | Fruit fly             | *Cucumis trigonus*       | *C. sativus*           |
**Table.4** Abiotic stress resistance in different vegetable crops

| Crop    | Character transferred | Species transferred from | Species transferred to |
|---------|-----------------------|--------------------------|------------------------|
| Tomato  | High temperature      | Solanum cheesmani        | S. lycopersicum        |
| Potato  | Frost tolerance       | Solanum acaule           | S. tuberosum           |
|         | Heat and drought      | Solanum chacoense        | S. tuberosum           |
| Onion   | Tolerance to cold     | Allium porrum            | A. cepa                |
| Cucumber| Salinity              | Benincasa hispida        | Cucumis sativus        |
| Brassicae|Drought and heat    | Brassica chinensis        | B. oleracea            |
| Okra    | Low temperature       | Abelmoschus angulosus    | A. esculentus          |

**Table.5** Quality improvement in different vegetable crops

| Crop    | Character transferred | Species transferred from | Species transferred to |
|---------|-----------------------|--------------------------|------------------------|
| Tomato  | Carotenoid content    | Solanum hirsutum         | S. esculentum          |
|         | Soluble solid         | Solanum chmielewskii     | S. esculentum          |
| Melon   | Thick rind and good keeping quality | Cucumismelovar. cantaloupensis | C. melo |
| Potato  | Starch content        | Solanum acaule           | S. tuberosum           |
| Chilli  | High capsaicin        | C. frutescence           | C. annum               |
| Onion   | Leaf flavour          | Allium kurrat            | A. cepa                |

**Table.6** The use of somatic fusion for improving valuable agronomic traits in cultivated potatoes

| Trait                                    | Fusion partner                        |
|------------------------------------------|---------------------------------------|
| Atrazine resistance                      | S. tuberosum (+) S. nigrum            |
| Frost tolerance                          | S. tuberosum (+) S. commersonii       |
| Reduced glycoalkaloid aglycones          | S. tuberosum (+) S. brevidens         |
| Resistance to bacterial wilt             | S. tuberosum (+) S. phureja           |
| Resistance to late blight                | S. tuberosum (+) S. brevidens; S. tuberosum (+) S. bulbocastanum |
| Resistance to potato leaf roll virus (PLRV) | S. tuberosum (+) S. brevidens; S. tuberosum (+) S. verrucosum |
| Resistance to potato virus Y (PVY)        | S. tuberosum (+) S. brevidens         |
Table.7 Inter-specific hybridization in vegetable crops

| Tomato | Pusa Red Plum | S. lycopersicum x S. pimpinellifolium | Rich in Vit-C |
|--------|---------------|--------------------------------------|--------------|
|        | Hisar Anmol   | Hissar Arun x S. hirsutum f. glabratum| Resistant to TLCV |
| Potato | Kufri Kuber   | (Solanum curtilobum x S. tuberosum) x S. andigenum | High tuber yield |
| Cucumber | C. hystivus | Cucumis sativus x C. hystrix | Resistant to downy mildew |
| Amaranthus | Pusa Kiran | A. tricolour x A. tristis | Rainy season |
| Okra | Pusa A4 | A. esculentus x A. manihot ssp. manihot | Tolerant to jassids, Fruit and shoot borer |
| Punjab-7 | A. esculentus (Pusa Sawani) x A. manihot ssp. manihot (Ghana)(2n=194) | Resistance to YVMV |
| Punjab Padmini | A. esculentus (Rashmi) x A. manihot ssp. manihot (Ghana) |  |
| Parbhani Kranti | A. esculentus (Pusa Sawani) x A. manihot |  |
| Arka Anamika | A. esculentus(2n=130) x A. tetraphyllus (2n=138) | Resistance to YVMV and tolerant to fruit borer |
| Arka Abhay | A. esculentus(2n=72) x A. tetraphyllus (2n=130) |  |

Table.8 Inter-generic hybridization in vegetable crops

| New Crop | Parents | Special feature |
|----------|---------|-----------------|
| Hakurana | Cabbage x Chinese cabbage (Developed by Embryo culture) | New leafy vegetable in Japan Resistant to soft rot, drought and heat |
| Nabicol | Kale x Turnip |  |
| Caulicob | Cabbagex Cauliflower |  |
| Swede | Turnip x Cabbage | Root vegetable |
| Raphanobrassica | Radish x Cabbage | Fodder crop |
| Baemooccha (Brassicoraphanunus) (2n=38) | B. rapa ssp. pekinensis (Big head Chinese cabbage) x R. sativus (big root radish) | New leafy vegetable |

**Chromosome doubling**

Distant hybrids exhibit high degree of sterility, which is mainly due to genic or chromosomal difference between parental species. This problem in many cases has been overcome by doubling the chromosome number of F1 hybrid. Bharathi et al., (2014) crossed natural tetraploid *Momordica subangulata* subsp. *renigera* (2n = 56) with induced tetraploid *Momordica dioica* (2n = 4x = 56) to produce new species *Momordica x suboica* Bharathi. This hybrid maintains its morphological characteristics, superior agronomic traits making it a good choice as a new vegetable crop.
In conclusion, wild species are the storehouse of novel genes, which can be utilized for wide hybridization. So, wild species forms the heart of wide hybridization programme. Wide hybridization is an effective tool for genetic improvement in vegetables crops. It is a highly technical and knowledge intensive process. Even though, wide hybridization has so many barriers, it can be overcome through the conventional and biotechnological approach. To broaden the genetic base, wide hybridization following in vitro and biotechnological approach is being used. It can be used for disease and insect resistance breeding and quality improvement in the cultivars, which is need of the hour.

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