SATSA Mukhapatra - Annual Technical Issue **28** : 2024 ISSN 0971-975X **Invited Paper**

Distant Hybridization : Achievements and Problems in Modern Plant Breeding

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(Received : December 15, 2023; Revised : January 04, 2023; Accepted : January 15, 2024)

ABSTRACT

Green revolution had immense impact on the agricultural development which add on impacts on plant breeding and production of crops. Though the side effects of green revolution resulted reduction in the varietal diversity in major cultivated crop species and increased uniformity in appearance and harvestable products. To cope up with the abiotic stresses in crops through climate change plant breeders have to use the wide hybridization techniques involving wild species those inhabiting noble characters. Wide hybridization encompasses conventional breeding and modern molecular techniques as its effective tool in crop improvement.

Keywords : Wide hybridization, Adaptation, Commercial hybrids, Barriers in fertilization and way out.

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 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 upto 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 cultivated crop species and increased uniformity in appearance and harvestable products, to feed the ever-increasing population and to ever-increasing population and to fight with malnutrition, wild species offers the scope for quality improvement in cultivars. In order to restore the characteristic of ecological sustainability and to combat the biotic and abiotic stress in cultivated crops, wide hybridization has been advocated a strong tool in the hand of plant breeders (Singh, 2010) 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 (Harlan, 1976) have been used from decades for breeding, in particular to transfer genes of resistance

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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.

Wide hybridization

Wide hybridization as a norm is an attempt to 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)

Particulars	**Inter specific Hybridization**	**Inter generic 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 inter specific crosses
Release of hybrid Varieties	Possible in some crops	Not possible
Evolution of new Crops	Not possible, but evolution of new species is sometimes possible	Sometimes possible

1. Significant role distant hybridization

a) Improving the crop plants for

i. Disease and insect resistance-Jassid resistance, Bacterial Blight resistance, Black arm resistance, white mould resistance (Abawi *et al.,* 1978) etc.

ii. Quality-Fibre length, Oil content, Cartenoid content etc.

iii. Adaptation-Cold tolerance, Drought tolerance, Frost tolerance etc.

iv. Yield

v. Mode of reproduction-Apomixis, Cleistogamy

vi. Several other characters-Dark green colour, excellent leaf texture, bright red thin flesh

b) Developing commercial hybrids in some crops.

c) Creation of new crops.

d) Characters Improvement

i) Disease and Insect Resistance - :

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*

Crop	**Character transferred**	**Species transferred from**	**Species transferred to**
Cotton	Jassid resistance, Black arm resistance	*G. tomentosum,* *G. arboreum*	*G. hirsutum* *G. barbadense*
Okra	Resistance to YMV	*Abelmoschus manihot*	*A. esculenta*
Groundnut	Resistance to Leaf chewing insect	*Arachis monticola*	*A. hypoge*
wheat	Rust resistance	*Agropyron*	*T. aestivum*
Sugarcane	Sereh disease resistance	*Saccharum spontaneum*	*Saccharum officinarum*

Crop	**Character transferred**	**Species transferred from**	**Species transferred to**
Tomato	White fly Rootknot nematode	*Solanum hirsutum* *S. peruvianum*	*S. esculentum* *S. esculentum*
Potato	Nematode	*S. vernei*	*S. tuberosum*
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*

Crop	**Wild species along with gene**	**Resistance source Disease**
Rice	*O. longistaminata(Xa21)* *O. rufipogon(Xa23)* *O. minuta(Xa27)* *O. officinalis(Xa29)*	Bacterial blight
Wheat	*Ae. umbellulata(Lr9)* *T. tauschii(Lr32)* *T. boeoticum(Sr22)* *Ae. speltoides(Sr22)* *T. dicoccoides(Yr15)*	Leaf Rust Stem rust Yellow rust
Zea mays	*Zeadiplo perennis*	Immunity to major diseases of maize

Crop	**Wild species**	**Resistance against**	**Reference**
Rice	*O. officinalis* *O. minuta* *O. latifolia* *O. nivara* *O. brachyantha* *O. ridleyi*	Brown plant hopper Yellow stem borer	Brar and Khush (1995)
Sorghum	*S. purpeosericeum* *S. versicolor*	Sorghum Shoot fly and Stem borer	Mote (1984)

2. Improvement in Adaptation

i) Tolerance to cold in rye, wheat, onion, potato, tomato, grapes, strawberry and peppermints etc. has been transferred from wild species of these crops in Russia.

ii) In wheat, increased winter hardiness has been transferred from Agropyron.

iii) In grape, hardier vines have been developed through the use of wild species Viti samurensis in the breeding programme.

iv) In sugarcane, cold tolerance has been transferred from wild species in USA.

v) Drought tolerance in peas and wheat

vi) Salt tolerance in tomato

Crop	Character transferred	Species transferred from	Species transferred to
Cotton	Fibre length Male sterility	*G. Thurberi* & *G. Raimondii* *G. harkenssii*	*G. hirsutum* *G. hirsutum*
Potato	Starch content Frost resistance	Wild species *Solanum acaule*	Cultivated Spp. *S. tuberosum*
Tomato Palm	Carotenoid content Oil quality	Lycopersicon Wild Spp.	*L. esculentum* Cultivated Spp.

4. Improvement in Yield

Improvement in yield has also been achieved through the use of wild species in some crops. For example, in oat yield increase of 25-30% over the recurrent parent was obtained from across between *Avena sativa*x *A. sterilis*. High yielding transgressive segregants were obtained after 4 back crosses.

In tobacco, yields were increased by the use of wild species *Nicotian cidebneyi*. Yields of sugarcane and octaploid strawberries have been increased by the use of their wild species.

Increase in yield has been reported in several crops such as Vigna, Zea, Ribes, vanilla, arachis, potato and tobacco through inter specific hybridization.

5. Mode of Reproduction

Use of wild species in the hybridization programmes sometimes leads to alteration in the mode of reproduction.

Apomictic genes have been transferred from maize—Tripsacum cross to maize and from wild species of Beta to cultivated species.

The cleistogamy and self-fertility traits of wild Secale have been transferred to cultivated rye (secale cereale).

6. Other Characters

There are several other desirable characters which have been transferred from wild species to cultivated plants.

For example, wild species have been used to transfer dark green colour and excellent leaf texture in lettuce and bright red thin flesh in red peppers.

Semi-dwarf wheat has obtained from *Triticum* x *Agropyron* hybrid derivatives.

Short statured oil palms resulted from interspecific hybrids. Earliness has been achieved from use of wild species in soybean.

7. Developing commercial hybrids in some crop]

Hybrid Varieties

Improved hybrid cultivars have been developed through the use of wild species mainly in sugarcane, potato and some forage crops.

Most of the modern cultivars of sugarcane and potato are the derivatives of interspecific hybridization.

In cotton, commercial interspecific hybrids have been developed both at tetraploid and diploid levels but between cultivated species only.

Some of the varieties of upland cotton (MCU2, MCU5, Deviraj, Devitej, G67, Khandwa1, Khandwa2, Badnawar1 PKV081, Rajat and Arogya) are derivatives of interspecific hybridization.

A hybrid between Pearl-millet and napier grass has been developed which has become very popular by virtue of its high fodder yield potential and superior fodder quality.

8. Achievements of wide hybridization

i) Rice

In rice four genes namely Xa21 (O. longistaminata), Xa23 (O. rufipogon), Xa27 (O .minuta) and Xa29 (t) (O. officinalis) have been introgressed from wild species of Oryza which show broad spectrum of resistance to several virulent races of bacterial blight caused by Xanthomonas oryzae pv. oryzae.

Traits for conferring cytoplasmic sterility was also transferred from the wild rice O. rufipogon into cultivated species (Katsuo and Mizushima1958).

QTLs for yield and yield component and other agronomic characters were identified from O. rufipogon and transferred to elite varieties of O. sativa through wide crosses. (Moncada et al., 2001)

NERICA (New Rice for Africa), NERICA has been developed by assistance from Japan, UNDP and other organization.

WARDA succeeded for the first time in crossing Oryza Glaberrima and Oryza Sativa in 1994. (MonteyJones)

Because the different species do not naturally inter breed, a plant tissue culture technique called embryo-rescue was used to assure that cross between the two varieties survive and growth to maturity.

ii) Triticale

Triticale Level	**Cross**	**Genome**	**Ploidy**
Triticosecale semisecale	Triticum monococcum X Secale cereale	AARR	Tetraploid
Triticosecale neoblaringhemii	Secale cereal X Triticum turgidum	AABBRR	Hexaploid
Triticosecale rimpaui	Secale cereal X Triticum aestivum	AABBDDRR	Octaploid

iii) Maize

Zea perennis tetraploid teosinte (2n=40) x *Tripsacum dactyloides* (2n=72) F1 hybrid plants were obtained by embryo rescue and induction of somatic embryogenesis / organogenesis. These hybrids showed *Tripsacum*-like phenotypes, tolerance to stresses such as NaCl salinity and low temperatures.

iv) Sorghum and Bajra

The interspecific sorghum × sudan grass annual multi-cut hybrids are grown for greenfodder.

The interspecific pearl millet × napier grass hybrids are perennial and yield green fodder throughout the year.

v) Cotton

Varalaxmi : (*G. hirsutum* x *G. barbadense*) : This is a first interspecific hybrid released by UAS, Dharwad which made a preamble in changing fibre quality scenario of Indian Cotton.

DCH-32 : (*G. hirsutum* x *G. barbadense*) : This another interspecific hybrid released from UAS Dharwad has characteristic good quality long fibre.

vi) Pigeon Pea

Hybrid ICPH-8 Was bred by hybridizing a gemetic male sterile line ms Prabhat (DT) and a male fertile inbred line ICPL-161, both developed at ICRISAT centre. To develop the male sterile line, the ms1 gene was transferred from the original medium duration source genotype (MS3A) to a short duration pigeonpea cultivator Prabhat by back crossing.

vii) Sugarcane

Interspecific hybrids of *Saccharum officinarum, Saccharum barberi/ sinense* and *Saccharum spontaneum* are better interms *of* sugar content, disease resistance, hardiness and ability to withstand adverse conditions. *S. barberi / sinense* to be derivatives of natural hybridization between *S. officinarum a*nd *S.spontaneum* might have contributed gene complexes for adaptiveness and sucrose content. First Indian commercial cane Co205 was a interspecific hybrid between S. *officinarum* x S. *spontaneum*

viii) Radish

Auxinic herbicide resistance was transferred from wild mustard (*Sinapis arvensis*) into radish (*Raphanus sativus*) through Wide hybridization.

ix) Brinjal

Interspecific hybrids between cultivars of eggplant (*Solanumm elongena* L.) and its wild relative *S. torvum*, which has disease resistance and desirable traits for crop improvement, were obtained by cross hybridization followed by embryo rescue.

x) Okra

Prabhani kranti- *A. esculentus cv.* Pusa sawani x *A. manihot* resistance against YMV.

xi) Papaya

Brien and Drew have reported use of bridge species *Vasconcellea parviflora* in between two incompatible species *viz*., *Carica papaya* and *V. cundinamrcensis* (resistant to PRSV and Cold). When they crossed *C. papaya* with *V. cundinamarcensis* resulted in inviable hybrid embryo. However, F1 of *C. papaya* with *V. parviflora* was crossed with *V. cundinamarcensis* resulted inviable hybrid embryo. So in this, *V. parviflora* act like a bridge species between two incompatible species *(C.papaya* and *V. cundinamarcensis*).

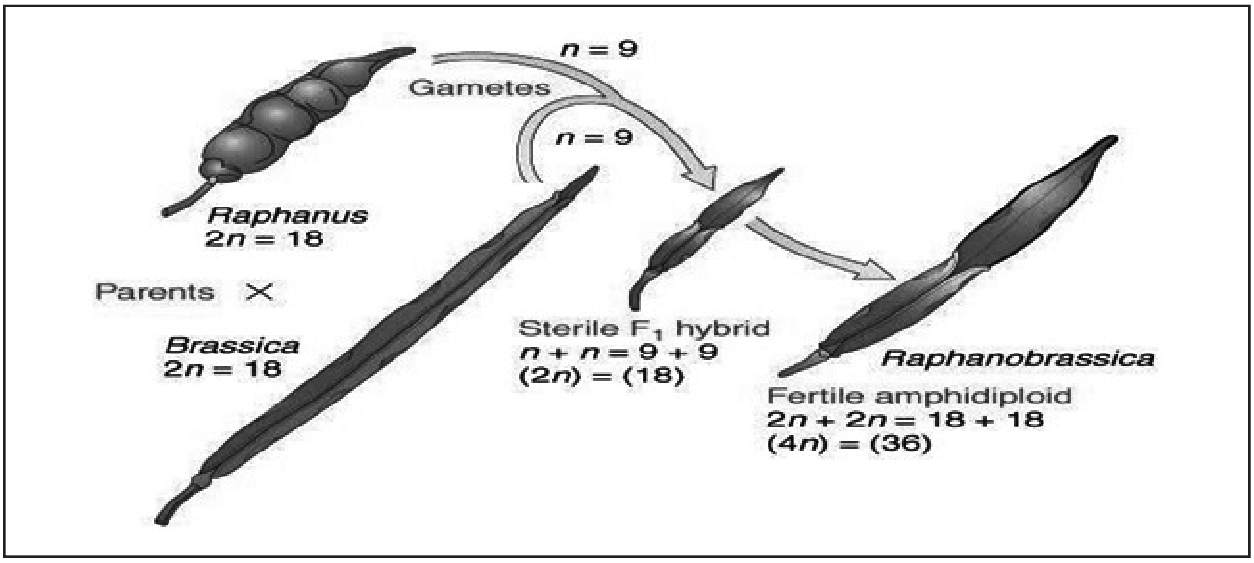
xii) Strawberry

Yunnan resulted in transfer of peach flavor from wild species (*F. nilgerrensis* var. Yunnan) to cultivated species (*F.* x *ananassa* cv. Toyonoka). But resulted progeny is triploid without anyseeds that can be overcome by restoring fertility by doubling chromosome with treatment of colichicne (mutagen). This resulted in development of hybrid TN-13-125 which is having fruit quality and taste similar to cultivated strawberry species with added advantage of peach flavor from wild species.

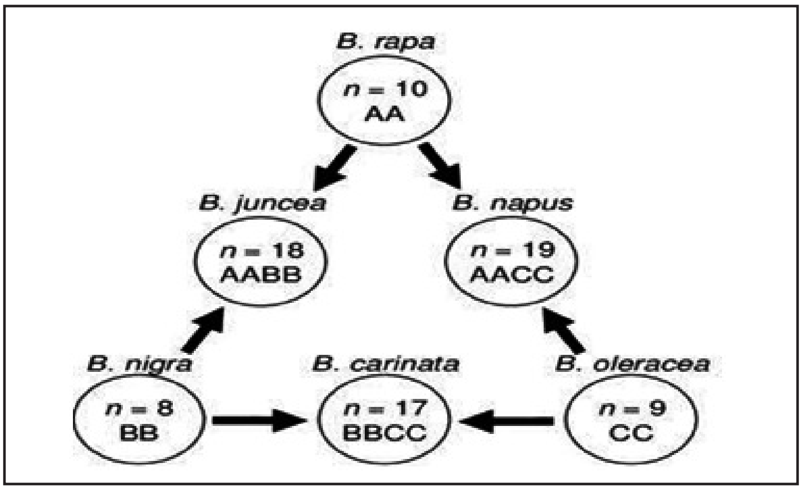
xiii) Citrus

Smith *et al.* 2013 has produced first inter generic hybrid between *Citrus* and *Citropsis*. *Citropsis*is producing less number of seeds per fruit (4/fruit) and showing resistance to many diseases. They successfully produced viable intergeneric hybrid between *citrus* and *citropsis* and confirmed through molecular marker.

Creation of new Crop species



The triangle of U By Nagaharu in1935



9. 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 (Gowda *et al.*, 2020).

A. 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 incompatability 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.

i) 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.*

ii) 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.

iii) 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.

B. 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.

i) 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).

ii) Hybrid inviability

It results from various factors and manifests from zygote development to seed formation. It operates in F1 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 F1 hybrids. e.g. Cross between *Cucumis sativus* × *C. melo and Abelmoschus esculentus* × *A. tetraphyllus.*

iii) Hybrid sterility

F1 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.

iv) 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.

v) Hybrid breakdown

It is manifested in the form of inviable and weak F2 generation or later generation. In some wide crosses seed setting is normal, even F1 plant progenies show normal development and good fertility but in the F2 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.*

10. The difficulties encountered in the production of distant hybrids may also be grouped into three classes-

A) Failure of zygote formation

Failure of fertilization pollen tube is unable to reach embryo sac

The pollen tube may burst in the style of different species.

The style of the species used as female is longer than the pollen tube.

Sometimes difference in ploidy of the parent cause failure to zygote formation

When a diploid species is used as female and a polyploid one as a male, the polyploid tube grows at a slower rate and hence suppress fertilization.

B) Failure of zygote development

Fertilization does take place and zygote is produced, but development of zygote is blocked at various stages.

1) Lethal gene - Some species carry a lethal gene, which causes death of zygote during early embryonic development. Such lethal genes generally do not have any effect in the species carrying them, but they affect only interspecific hybrid embryos. For example Aegilops umbelluata has a lethal gene that has three allele showing lethality, late lethality and non lethality.

2) Genotypic disharmony Between the two parental gene - In some cases, the death of embryo may occur due to genetic imbalance occur. For example, in interspecific cross involving Gossypium gossypiodes hybrids die at various stages of development ranging from early embryonic development to reproductive phase of the F1 plants.

3) Chromosome elimination : Chromosomes are gradually eliminated from the zygote. Mitotic irregularity

4) Incompatible Cytoplasm : Incompatibility because of cytoplasm of the species used as female and the genome of the species used as male.

5) Endosperm Abortion : The endosperm may abort during early embryo development preventing further development of the embryo. Poorly developed seed Non viable seed Dead seed.

C) Failure of F1 seedling development

Failure of hybrid seedling development : Some distant hybrids die during seedling development or even after initiation of flowering. Some interspecific hybrids are chlorophyll deficient and fail to survive.

Some cases of abnormal seedling development involve complementary lethal genes. For example, interspecific and intergeneric hybrid of wheat show both chlorosis and necrosis, which is often lethal.

Chlorosis refers to improper chlorophyll development resulting in variable degrees of chlorophyll deficiency; in an extreme case, very little chlorophyll may be produced.

11. Techniques to Overcome Fertilization Barriers

A. Techniques to overcome pre-fertilization barriers

i. 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.

ii. 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.

iii. 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 incrossing *S. acuale* and *S. tuberosum.*

iv. 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.

v. 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 cellwall 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.

vi. Use of growth regulators and immune suppressants

Growth Regultors (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.

vii. 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).

viii. Protoplast fusion

It involves the fusion of protoplast of two incompatible species or genera and the fused hetero karyon 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.

B. Techniques to overcome post-fertilization barriers

i. 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.

ii. Ovary culture

In some wide crosses, embryo abortion occursat 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.

iii. 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 beback 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*). Embryorescue 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. 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.

Reciprocal crossing

Reciprocal differences in wide crosses are also very common, and can be due to chromosomal imbalance in the endosperm, the role of the sperm nucleus in differential endosperm development or the alteration of endosperm development by pollen through the effects of antipodal cells, which are assumed to supply nutrients during early endosperm development (Beaudry, 1951). If disharmony between the genome of one species and cytoplasm of the other is a cause of a fertilization barrier, reciprocal crosses can be successful in recovery of hybrids. In case of distant hybridization with different ploidy level parent is used than reciprocal cross should be taken mostly using a female parent with higher ploidy than male.

Important points summarizing this research

- Three blackgram varieties namely Mash 338, UG 562 and UG 844 were crossed with one ricebean variety RBL 1.

- Blackgram was susceptible to MYMV (Mungbean yellow mosaic virus), CLS (Cercospora leaf spot) and BLS (Bacterial leaf spot), whereas ricebean was resistance against all these three diseases.
- Blackgram was selected as female whereas ricebean was selected as male. Only Mash 338 and RbL 1 cross were proceeded forward because of highest crossability percentage (3%). All segregating generation were sown at Gurdaspur (hot spot for MYMV, CLS and BLS).
- After every 10 progenies rows, 1 infector row was planted.
- For screening, the standard 1-9 scale is used (Kaur and Singh 1998), 1 score means highly resistance and 9 means highly susceptible.
- In F7, 24 selected lines were send in PYT (Preliminary Yield Trial) against check Mash 338. 15 progeny rows that have shows 10% superiority over checks was send to MYT (Multilocation Yield Trial).
- KUG 114 was found to be most suitable line as it has resistance (2) highly resistance (1) against MYMV, CLS and BLS.
- KUG 114 was released for general cultivation in Punjab.

Disease	KUG 114	Mash 338	RBL1	Susceptible Check
Mungbean Yellow Mosaic Virus (1-9)scale	2.0	5.0	1.0	9.0
Cercospora Leaf Spot (1-9)scale	1.0	3.0	1.0	7.0
Bacterial Leaf Spot (1-9) scale	1.0	3.0	1.0	7.0

Name	Year	Contribution
Thomas Fairchild	1717	First authentic record of distant hybridization between sweet William and carnation which results in Fairchild mule.
Rimpau	1890	First intergeneric hybrid was triticale produced by crossing between wheat and rye.
Karpenchiko	1928	Raphanobrassica was produced by crossing raddish and cabbage.
B.H. Kataraki	1972	First interspecific cotton hybrid Varalaxmi.

Conclusion

Wild species are the store house of novel genes, which can be utilized for wide hybridization. So, wild species forms the heart of wide hybridization programme. 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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