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Wide hybridization for fruit crop improvement: A review

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Abstract

Wild plant species related to crop plants form an important source of useful traits for fruit quality improvement and biotic and abiotic stress tolerance. Wide hybridization involving crop wild relatives and related taxa has gained momentum in the recent fruit crop improvement programs. It breaks the species barrier for gene transfer and makes it possible to transfer the genome of one species to another, which results in changes in genotypes and phenotypes of the progenies. The incompatibilities or barriers in wide hybridization occur as prezygotic and postzygotic barriers. Techniques of chromosome doubling, bridging species, protoplast fusion and embryo rescue are highly beneficial in recovering fertile progenies by overcoming various barriers in wide crossings. The present article reviews the techniques involved in distant crossing and their applications in fruit crop improvement.

Keywords: Wide, hybridization, improvement, hybridization

Introduction

Improvement of perennial fruit crops is largely dependent on conventional methods of introduction, selection or hybridization by utilizing the cultivated genotypes of a species. However, in majority of crops, most cultivars are developed with relatively narrow genetic diversity. An estimated 75 per cent crop genetic diversity has been lost in the twentieth century (Singh, 2017) ^[20]. When the desired traits are not available in the cultivated species, breeders have resorted to methods such as mutation, polyploidization or recombinant DNA technology. Wild plant species related to crop plants are key resources for adapting agriculture to the challenges posed by climate change. They form an important source of useful traits such as agronomic, quality, biotic, and abiotic stresses, which are identified as critical component for food security and environmental sustainability in the 21st century (Maxted *et al.*, 2006) ^[12]. Thus, wide hybridization involving crop wild relatives and related taxa has gained momentum in the recent fruit crop improvement programs.

Wide hybridization: The concept

Mating between individuals from different species belonging to the same genus (interspecific hybridization) or two different genera of same family (intergeneric hybridization) is termed as distant hybridization and such crosses are known as distant crosses or wide crosses (Pujar *et al.*, 2017) ^[17].

Wide hybridization breaks the species barrier for gene transfer and thus makes it possible to transfer the genome of one species to another, which results in changes in genotypes and phenotypes of the progenies. Repeated backcrossing of wide hybrids to their parental species has also contributed to the evolution and speciation of some species by gene introgression, i.e., the infiltration of chromosomes or chromosome fragments from one species into another through repeated backcrossing of wide hybrids to their parental species (Liu *et al.*, 2014) ^[10].

In an attempt to produce intergeneric hybrids in *Citrus*, hybridization of *Citrus wakonai* P. I. Forst. & M. W. Sm. with a related genus *Citropsis gabunensis* (Engl.) Swing. & M. Kell. resulted in high rates of fruit set and seed formation (Smith *et al.*, 2013) ^[22]. Over 90 per cent germinated without the need for embryo rescue techniques and 35 plants flowered within two years of sowing. Of these, two hybrids (12Q031 and 12Q032) began setting fruits and the plant and fruit characteristics were intermediate to the parents.

Smisha and Sabu (2018) ^[21] have developed a new interspecific *Musa* hybrid *Musa* × *parahaekkinenii* (Musaceae), by manual crossing of two wild parent plants *Musa coccinea* Andrews (female) and *Musa haekkinenii* N.S. Lý & Haev. (male).

Not all the interspecific and most of the intergeneric hybridizations confer success in achieving the target. Crossability relations determine the potential gene exchange between crop plants and their wild relatives under either natural conditions or by experimental techniques. Crossability relations of crop plants with alien species are of the following types:

1. Intercross is complete or nearly complete and is in both cross-directions
2. Successful crosses are unilateral
3. Crosses are only partially successful or must be aided by embryo culture;
4. Crosses are incompatible.

Crop plants differ markedly from each other by their crossability relations. With their wild progenitors, crop plants are completely or nearly completely interfertile. More distantly related wild species can be hybridized with crop plants with greater difficulty, and many others are cross incompatible (Ladizinsky, 1992) ^[8].

Interspecific crossability of *Zizyphus mauritiana*, *Z. jujuba* and *Z. spina-christi* was studied by Asatryan and Tel-Zur (2014) ^[1]. Following interspecific hand pollination, in vivo pollen grain germination, formation of pollen tubes and their growth to ovule were observed in compatible crosses of *Z. mauritiana* × *Z. jujuba*, *Z. mauritiana* × *Z. spina-christi* and *Z. spina-christi* × *Z. mauritiana* crosses, wherein fruits, seeds and viable embryos were obtained. The crosses *Z. jujuba* × *Z. mauritiana* and *Z. spinachristi* × *Z. jujuba* were incompatible and no fruit set was noted.

Pujar *et al.* (2019) ^[18] made intergeneric hybridization to know the crossability between papaya varieties (Arka Prabhath, Arka Surya and Red Lady) with *Vasconcellea* species (*V. cauliflora*, *V. cundinamarcensis* and *V. parviflora*). Arka Prabhath was found to be a good combiner with all the three wild species (*V. cauliflora*, *V. cundinamarcensis* and *V. parviflora*) which has recorded good fruit set and more mean number of fertile seeds per fruit. Among male parents, *V. cauliflora* was found to be good combiner with all three female parents (Arka Prabhath, Arka Surya and Red Lady) which has resulted better fruit set and more mean number of fertile seeds per fruit.

Barriers in wide crossings

The difficulties encountered during the production of wide hybrids are majorly due to incompatibilities. The incompatibilities or barriers in wide hybridization are broadly classified into prezygotic and postzygotic barriers. The incompatibility which emerges before fertilization is defined as prezygotic incompatibility, i.e., the inability to cross, whereas the incompatibility which emerges after fertilization is defined as postzygotic. In the latter case, crossing takes place although hybrid seeds do not develop or weak development, nonviability, or sterility of F1 hybrids or subsequent generation hybrids is observed. Lack of pollen-stigma recognition, and hence failure of pollen germination, arrest of pollen tube growth inside the stigma or stylar tissue or failure of pollen tubes to penetrate the ovule and lack of pollination are the prezygotic incompatibilities whereas poor development or abortion of embryos (hybrid unviability),

abnormal growth or death in germinated seed or juvenile plants, hybrid sterility and the hybrid breakdown constitute the postzygotic barriers. Hybrid unviability may be due to abnormal endosperm development resulting in embryo abortion. The hybrid seedlings are sometimes lethal or sublethal. Hybrid sterility may be due to chromosomal or genic differences. Hybrid breakdown occurs in F₂ or later generations (Ladizinsky, 1992; Dickinson *et al.*, 2012; Pershina and Trubacheeva, 2017) ^[8, 2, 16].

In intergeneric crosses between *Duchesnea indica* and *Fragaria* × *ananassa* many putative hybrids were produced when *D. indica* was used as female but a few achenes and plants when used as male; therefore, to study the breeding barriers, pollen-pistil compatibility relations were analysed by fluorescence microscopy in this direction of the cross. Of the genotypic combinations, 78.6 per cent were incompatible at the stigma level and 17.2 per cent at the first third of the style. Only 3.6 per cent were pollen-pistil compatible and produced fruits with achenes of which seven did not germinate or originated short-lived plants and nine produced normal plants (Marta *et al.*, 2004) ^[11].

Techniques for overcoming the barriers

Techniques for Overcoming Pre-Fertilization Barriers

Manipulation of Chromosome Number

Crosses between cultivated and wild species with different ploidy levels are difficult to achieve. Chromosome doubling in either of the putative parents or the F₁ progeny are found helpful in making successful wide hybrids (Khush and Brar, 1992 and Pujar *et al.*, 2017) ^[7, 3].

Breeding to introduce a new aroma from a diploid wild strawberry *Fragaria nilgerrensis* was tried in cultivated strawberry, *F. x ananassa* (octaploid). All lines derived from the interspecific hybridization of *F. nilgerrensis* var. Yunnan with *F. x ananassa* var. Toyonoka were sterile. After doubling the chromosome of the interspecific hybrid 'TN13' by colchicine treatment, 152 plants were regenerated. Among the progenies, 15 plants did not flower or set fruits (Group I), 28 bloomed but did not set fruits (Group II) and 109 lines (Group III) bloomed and fruit setting was also observed. Further, a superior progeny 'TN13-125' with peculiar peach like aroma was identified for future use in breeding programs (Noguchi *et al.*, 2002) ^[14].

Bridging Species Technique

When direct crosses between two species with the same or different ploidy levels are difficult or impossible to accomplish, a third species (bridging species) is used to produce such crosses (Khush and Brar, 1992 and Pujar *et al.*, 2017) ^[7, 17].

For PRSV-P resistance breeding in papaya, *Carica papaya* when crossed with the PRSV-P immune species *Vasconcellea pubescens*, all progeny plants were infertile. *V. parviflora* is susceptible to PRSV-P but, when crossed to papaya, produced hybrids with some pollen fertility. Crosses between *V. pubescens* and *V. parviflora* have resulted in fertile F₁, F₂ and F₃ populations. Backcrosses to *V. parviflora* are producing a PRSV-P resistant *V. parviflora* which will be crossed with papaya. Thus *V. parviflora* can be effectively utilized as bridge species introgressing PRSV-P resistance from *V. pubescens* to cultivated *C. papaya* (O'Brien and Drew, 2009) ^[15].

Use of nutrient solution or growth regulators

Various growth hormones and nutrients have found to stimulate pollen tube growth and embryo development. They

also prolong the receptivity of stigma and prevent early abscission of pollinated flowers (Khush and Brar, 1992 and Pujar *et al.*, 2017)^[7, 17].

To break the crossing barrier in intergeneric crosses of *C. papaya* and *V. cauliflora*, the pollination was carried out by smearing sucrose solution in the concentrations of 1, 2, 3, 4 and 5 per cent on to the stigmatic surface of the flower. At 5 per cent sucrose concentration, maximum viable seed set (13.73) was obtained by enhancing the pollen germination. There was drastic reduction in the effect of sucrose with the decrease in the concentration levels. The *in vitro* pollen germination studies carried out with and without sucrose clearly demonstrated the efficacy of sucrose in enhancing pollen germination and pollen tube growth (Dinesh *et al.*, 2007)^[3].

To break the intergeneric hybridization barrier in the intergeneric hybridization involving nine *Carica papaya* cultivars as female and *Vasconcellea cauliflora* as male, various nutrient combinations were used. Among the combinations used, sucrose (5%), sucrose (5%) + boron (0.5%) and sucrose (5%) + CaCl₂ (0.5%) improved the fruit set and seed set percentage (Jayavalli *et al.*, 2011)^[6].

Use of recognition mentor pollen

Sometimes the pollen grains of one species do not germinate on the stigmas of another. If these incompatible pollen grains are mixed with the killed maternal pollen grains, germination of the incompatible pollen grains is obtained. When the compatible pollen is killed with ethanol and mixed with incompatible pollen for use in pollination, the proteinaceous recognition factors released from the walls of the killed compatible pollen grains mask the rejection reaction of the recipient stigma, thus allowing the alien pollen grains to germinate. The killed maternal pollen is called recognition or mentor pollen (Khush and Brar, 1992)^[7].

Wenslaff and Lyrene (2000)^[25] reported use of mentor pollination in wide hybridization in blueberry wherein two diploid yellow leaf *Vaccinium elliotii* Chapmn. clones were pollinated with pollen from the tetraploid southern highbush cultivar 'Misty' (*V. corymbosum* L.). These interspecific crosses, which normally yield few hybrids because of a triploid block, were made with and without the use of *V. elliotii* mentor pollen mixed with *V. corymbosum* pollen. Mentoring had no effect on the number of hybrids produced when *V. elliotii* 'Silverhill' was the seed parent, but when *V. elliotii* 'Oleno' was the seed parent, no hybrids were produced unless mentor pollen was utilized.

Protoplast Fusion

The barriers to hybridization among some species are so strong that sexual hybridization is impossible. To overcome such barriers, protoplast fusion can be attempted followed by regeneration of somatic hybrids (Khush and Brar, 1992)^[7].

Ruiz *et al.* (2018) reported the successful production of two somatic hybrids SMC-58 and SMC- 73 by the fusion of protoplasts derived from leaf mesophylls of *Citrus macrophylla* and Carrizo citrange [*Citrus sinensis* (L.) Osb. x *Poncirus trifoliata* (L.) Raf.]. Genetic characterization of the somatic hybrids was carried with SSR and SNP markers and both the somatic hybrids SMC-58 and SMC- 73 displayed allelic configurations that correspond with the addition of both genome parents as seen with the JK-TAA15 SSR marker.

Techniques for Overcoming Post-Fertilization Barriers

Several techniques such as embryo rescue, *in vitro* embryo culture, ovary/ovule culture, reciprocal crossing, grafting, back crossing and chromosome manipulation are widely adopted in annual crop plants. However, embryo rescue, back crossing and chromosome are the most common methods in fruit crop improvement (Khush and Brar, 1992)^[7].

Embryo rescue

Embryo abortion can occur at different stages of development, depending upon the genomic relationships of two parental species. Such abortive embryos can be dissected from the developing seed, cultured on nutrient medium in test tubes, and grown into mature hybrid plants.

Tian *et al.* (2008)^[24] conducted hybridization using *V. vinifera* as female parents and the wild Chinese *Vitis* spp. (*V. amurensis*) as male parents. In-ovulo embryo rescue was used to develop hybrid plants from the seedless females. The effect of medium type on *in vitro* embryo formation, germination and plant development was studied using liquid, solid and double phase ER media (embryo rescue media). The double-phase medium yielded 31.8 per cent of embryo formation rate, which was significantly higher than the liquid (21.1%) and solid medium (21.1%). The double-phase and liquid media resulted in similar percentages (94.3% and 95.0%, respectively) of embryo germination, which were significantly higher than that from solid medium (73.7%). The double-phase medium produced 23 per cent total plants compared to 16 per cent and 12 per cent for liquid and solid media, respectively.

In the same study, the effect of culture duration of ovules on *in vitro* embryo formation, germination and plant development was also evaluated. Culture duration of ovules largely affected plant development, though it did not seem to affect embryo formation (27.0–34.0%) and embryo germination (70.4– 91.2%). Although frequencies of plant development were not significantly different in ovules cultured for a period ranging from 8 (81.8%) to 12 (77.4%) weeks, prolonged culture of ovules for 16 weeks resulted in only 52.6 per cent of plant development rate. The total plants produced were 18 per cent and 24 per cent for the 8- and 12-weeks culture period respectively and determined to be the optimal ovule culture period.

Applications

i. Improvement of fruit quality

Annona reticulata was hybridized with atemoya (*A. cherimola* Mill. x *A. squamosa* L.), and 250 trispecies hybrids were studied for 28 traits (12 tree traits and 16 fruit traits) with the objective of salvaging useful genes from the three edible annonas and determining the extent of variation in the progeny. Variation in fruit shape, skin colour and skin surface in addition to wide range of values for total soluble solids (17–32°B), acidity (0.16–2.2%) and number of seeds per/100 g fruit (3–49) showed that exciting opportunities existed in terms of selecting for desirable traits. Among the segregating population were slow fruit ripening genotypes that required up to 12 days from harvesting to ripening (Jalikoop, 2010)^[5]. Blueberries are handpicked because of irregular maturation of fruits within clusters. Therefore, harvesting is labor intensive and results in a short shelf life of the product. Cluster harvesting could solve these problems. Miyashita *et al.* (2019)^[13] investigated the degree of parthenocarpy and suitability of

two interspecific hybrids of *Vaccinium corymbosum* and *Vaccinium virgatum* for cluster harvesting and considered the use of parthenocarpic hybrids to breed cultivars for cluster harvesting. It was found that the two hybrid individuals had higher frequencies of parthenocarpy and their fruit set and weight were close to those of pollinated fruits; moreover, the pollinated fruits of these hybrids were seedless.

The uniformity of flowering and fruit maturation of the interspecific hybrids of *Vaccinium corymbosum* and *Vaccinium virgatum* were evaluated, and the two hybrids showed a relatively uniform maturity of fruits in a cluster with a delayed fruit dropping. Comparison of cluster and individual harvesting showed that the percentages of mature fruits within clusters in the two hybrids were markedly higher than those in the HB cultivars.

ii. Abiotic stress tolerance

Citrus is among the most salt-sensitive perennial crops. One of the main rootstocks used worldwide is Carrizo citrange (CC) [*C. sinensis* (L.) Osb. × *Poncirus trifoliata* (L.) Raf.] which is tolerant to CTV but sensitive to iron chlorosis in alkaline soils. *C. macrophylla* is suited for saline soils because they restrict ion transport to the aerial part, whereas CC is sensitive to this condition as it quickly accumulates the ions and reaches toxic concentrations. Attempts to combine the beneficial characters of both rootstock species resulted in two somatic hybrids SMC-58 and SMC-73. The hybrids were tested for tolerance to iron chlorosis and salinity (Ruiz *et al.*, 2018) [19]. Both hybrids were tolerant to iron chlorosis under chlorosis inducing treatment showing intermediate iron concentration in the leaves. The parameters *viz.*, leaf greenness, increase in shoot biomass and iron concentration in the leaves were intermediate in the somatic hybrids as compared to their susceptible and resistant parents.

The differences in behaviour between somatic hybrids and their parents under salinity (+S) were evaluated according to the growth rates, leaf symptoms, ion accumulation, and gas exchange parameters (Ruiz *et al.*, 2018) [19]. Carrizo citrange plants subjected to salinity had 25 per cent lower DW than control plants at the end of the experimental period, indicating their sensitive behaviour. In contrast, *C. macrophylla*, that is salt-tolerant, showed similar growth in both, salinity or Control treatments. Growth of SMC-73 hybrid was also not affected by salinity while SMC-58 had 16 per cent lower dry weight under salinity treatment than in control conditions. Leaf symptoms induced by salt toxicity were intense in Carrizo Citrange plants that had 20 per cent of their leaf area burned. Meanwhile, leaves of the tolerant CM were free of burns and somatic hybrids showed very mild leaf toxicity symptoms with only 2 per cent. Leaf abscission was lower in SMC-73 or SMC-58 than in Carrizo citrange with 6, 3, and 7 per cent of leaves affected, respectively. The sodium, chloride and potassium ion contents of leaves in the two citrus parents and somatic hybrids were also evaluated. Overall, Cl^- and Na^+ molar concentrations in leaf tissue water were higher in the saline treatment (+S) than in the control treatment for all the genotypes. The parent *C. macrophylla*, which is tolerant to salinity, had the lower Cl^- and Na^+ concentrations under the +S treatment, and were 2.2 and 1.4-fold, respectively, higher than in control plants. Carrizo citrange, which is considered salt-sensitive, raised Cl^- and Na^+ leaf concentrations that were 4.1 and 2.6-fold higher in +S treatment than in control treatment, respectively. Somatic hybrid SMC-58 subjected to +S treatment had lower Cl^- concentration and similar Na^+ concentration than CC. Specifically, Cl^- and Na^+ leaf

concentration in SMC-58 were 4.2 and 1.8-fold, respectively, higher in +S than in control plants. The SMC-73 plants subjected to +S treatment had leaf Cl^- concentrations similar to CC, whereas leaf Na^+ concentration was higher than in CC plants. More precisely, leaf Cl^- and Na^+ concentrations in SMC-73 increased by 3.9 and 2.3-fold, respectively, in salt-treated plants when compared to control plants. Therefore, the data show that both somatic hybrids had lower Cl^- exclusion capacity than the salt-tolerant parent CM. However, SMC-58 had greater exclusion capacity than the salt-sensitive parent CC, whereas SMC-73 had similar exclusion capacity to CC. Regarding Na^+ exclusion, the behaviour observed in SMC-58 was similar to CC, whereas SMC-73 plants accumulated less Na^+ in their leaves, showing more tolerance than the sensitive parent CC.

iii. Biotic stress tolerance

Sudha *et al.* (2013) [23] made intergeneric hybridization using *C. papaya* as female and *V. cauliflora* as male parent to transfer the desirable genes for PRSV and the progenies of F2 population were evaluated under polyhouse and field conditions. Screening of F2 progenies through artificial inoculation against PRSV under polyhouse conditions was done 27 days after inoculation. Among the crosses, Pusa Nanha × *V. cauliflora* recorded 52.59 per cent of disease-free seedlings followed by CP 50 × *V. cauliflora* (44.15%) and in CO 7 × *V. cauliflora* recorded 42.79 percent of disease-free seedlings. However, all the parents except *V. cauliflora* showed typical PRSV symptoms after artificial inoculation.

Under field conditions, among the crosses, CO 7 × *V. cauliflora* exhibited the symptoms from 120th day after transplanting which increased gradually and reached the maximum score of 1.42 by 270th day after transplanting. However, the cross, CP 50 × *V. cauliflora* did not show any symptom till 120 days and the progenies expressed minor symptoms from 150th day and recorded disease intensity score of 1.20 at harvest. In the case of cross involving Pusa Nanha × *V. cauliflora*, no symptoms were noticed till 120 days. F2 progenies of Pusa Nanha × *V. cauliflora* showed minor symptoms (0.3) at 150th day after transplantation. At harvest, the cross recorded a disease score of 1.06.

The advanced generation intergeneric hybrid progenies of the cross Arka Surya × *V. cauliflora* numbering 38 and two parents *viz.*, Arka Surya and *V. cauliflora* were evaluated field performance and PRSV-P resistance. Plant height (185 and 195 cm), canopy spread (180 and 185 cm), stem circumference (40 and 43 cm) were superior in the selected progenies S6-2 and S6-4 respectively as compared to parents *viz.*, Arka Surya and *V. cauliflora*. The selected progenies also registered higher values for the fruit characters *viz.*, fruit weight (794.15g and 537.75g), fruit volume (704.00 and 528.35 ml) and pulp thickness (3.18 and 2.83 cm) than the parents. The TSS (11.80, 11.98°B) of the selected progenies was close to the female parent (12.45°B). The total sugars (10.03, 9.54 g 100g⁻¹ FW), total carotenoids (6.08, 7.62 mg 100g⁻¹ FW) and lycopene (2.33, 2.34 mg 100g⁻¹ FW) were found to be higher in the selected progenies as compared to the male parent (*V. cauliflora*). The selected progenies were found to be moderately resistant (2) compared to the susceptible female parent Arka Surya (Gowda *et al.*, 2019) [4].

Li *et al.* (2020) [9] made crosses between the stenospermocarpic *Vitis vinifera* varieties 'Flame Seedless' and 'Ruby Seedless' (susceptible to downy mildew) and the disease resistant 'Beichun' (*V. vinifera* × *V. amurensis*) for developing seedless disease resistant cultivars and the

plantlets were recovered by embryo rescue technique. The S382-615 molecular marker of disease resistance was found in the male parent 'Beichun'. Two F1 progenies in the cross of 'Ruby Seedless' x 'Beichun' also presented the 615bp specific band and were identified as resistant to downy mildew.

Conclusion

Wide hybridization has been proven promising in the fruit crop improvement programs, in terms of fruit quality and tolerance to biotic and abiotic stress tolerance. Some of the popular wide hybrids in fruit industry are Kinnow mandarin ('King' (*Citrus nobilis*) x 'Willow Leaf' (*Citrus* x *deliciosa*), cultivated strawberry (*F. chiloensis* x *F. virginiana*), atemoya (*Annona cherimola* x *A. squamosa*) and Arka Sahana custard apple (*A. atemoya* x *A. squamosa*). Techniques of chromosome doubling, bridging species, protoplast fusion and embryo rescue were highly beneficial in recovering fertile progenies. However, information on effective utilization of wild species in broadening the genetic base and developing cultivars in fruit crops is limited. Identification, collection and characterization of elite wild species and their efficient utilization in breeding program demands greater thrust.

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