Role of wide hybridization in crop improvement: A Review

Abstract

Wide hybridization in crop plants involves crossing two distantly related species to initiate desirable traits, such as disease resistance, into a cultivated crop. This technique expands the genetic diversity accessible for breeding programs, enhancing the adaptability and resilience of crops. Wide hybrids often surpass limitations of conventional breeding by incorporating unique genetic material. However, difficulties arise due to reproductive barriers and genomic incompatibilities between divergent species. Researchers employ techniques like embryo rescue and tissue culture to overcome these hurdles. Despite its potentiality, wide hybridization requires careful selection and extensive backcrossing to stabilize desired traits. The resulting crops may show improved resistance to pests, diseases, or environmental stressors, contributing to sustainable agriculture and food security. Continuous advancements in molecular biology and genomics facilitate the identification and transfer of specific genes, accelerating the development of wide hybrid varieties with enhanced agronomic traits.

Key word:wide hybridization, crop improvement, hybrid crops, Interspecific Hybridization, agronomic traits, germplasm

Introduction

"Hybridization has played a major role in the evolution of lineages. Hybridization between individuals from different species, belonging to the same genus or to different genera is termed as distant hybridisation or wide hybridization.When individuals from two distinct species of the same genus are crossed, it is known asinter-specific hybridization.When individuals being crossed belong tospecies from two different genera, it is referred as inter-generic hybridization.Wide hybridization comprises the exchange or modification of the genes due to crossing between species from distant gene pools. It is a unique tool to introduce useful traits in a variety of agricultural applications especially beneficial agronomical traits. It plays a substantial role in transferring traits of interest like disease and insect resistance, improved quality, early growth, dwarfism, increased yield, abiotic stress tolerance in crop plants besides bringing changes in the mode of reproduction as well.Wide hybridization involving crop wild relatives and related taxa has gained momentum in the recent fruit crop improvement programs"(**Anushma PL*et al*.,2020)**. Wide hybridization serves as a important tool in agricultural applications, facilitating the introduction of valuable traits that improve crop performance. Its importance extends beyond mere trait acquisition, as it also induces modifications in the reproductive mechanisms of the resulting hybrids.In the realm of crop improvement, wide hybridization stands out as a unique strategy, contributing to the sustainable enhancement of agronomic traits. By harnessing the genetic potential residing in distant gene pools, wide hybridization offers a pathway towards creating resilient and high-yielding crop varieties, thus addressing the evolving challenges in agriculture.Wide hybridization improves crop improvement programs by exchanging and modifying genes through crossbreeding different species. This dynamic process improves the momentum of recent agricultural advancements, enabling the introduction of beneficial traits that contribute to increased crop resilience, productivity, and adaptability in response to evolving environmental and economic challenges (**Patnaik *et al*.,2021).**

"In a study focusing on three cultivated species of *Ziziphus Mill.* (*Z. jujuba Mill., Z. mauritiana Lam., and Z. spina-christi (L.) Willd.*), comprising seventeen cultivars/genotypes, the mating system was explored. Phenological stage analysis revealed blooming overlap among the species during June. Intraspecific cross compatibility was assessed for *Z. jujuba* and *Z. mauritiana*, with interspecific crosses conducted over two flowering seasons. Following intraspecific hand cross-pollination, pollen tube formation and growth to the ovule 24 hours after pollination (HAP) were observed across all three species. Despite low seed set in intraspecific *Z. jujuba* crosses, histological examinations indicated normal embryo formation and endosperm development, suggesting double fertilization with subsequent embryo developmental arrest. In interspecific hand pollination, compatible crosses exhibited in vivo pollen grain germination, pollen tube formation, growth to the ovule 24 HAP, fruit, and seed set. Viable embryos were obtained from interspecific crosses, *including Z. mauritiana* × *Z. jujuba*, *Z. mauritiana* × *Z. spina-christi*, and *Z. spina-christi* × *Z. mauritiana*. Putative hybrids were germinated in vitro, with flow cytometric analysis suggesting true hybrid origin based on total 2C-DNA content, although these plantlets succumbed during the hardening-off phase. This study underscores the potential for gene flow among *Ziziphus* species, thus enhancing the prospect of using interspecific crosses for genetic crop enhancement" (**Asatryan, A., & Tel-Zur, N. 2014**)

Protoplast fusion, a technique merging the genetic material of *Brassica napus* and *Thlaspiperfoliatum*, both members of Brassicaceae, aimed to breed oil-rich crops with high levels of nervonic acid. Following fusion and selection via flow cytometry and cell sorting, 27 shoots were confirmed as hybrids via isoenzyme analysis. An additional 6 plants were identified as partial hybrids using *T. perfoliatum*-specific repetitive DNA sequences. Slot blot experiments revealed a wide range of copy numbers of repetitive DNA sequences in hybrids, ranging from 1-37% of *T. perfoliatum*'s value. Nuclear DNA content analysis unveiled both partial and symmetric hybrids. Despite challenges in rooting and greenhouse establishment, 19 plants reached maturity. Seeds from 15 plants were analyzed, with 5 hybrids exhibiting significantly higher nervonic acid levels than *B. napus*. This research underscores the potential of protoplast fusion in creating novel oil-rich crops with desired fatty acid profiles, albeit with challenges in hybrid establishment and maturation**(Fahleson*et al.*, 1994**).

Role of Wide Hybridisation

The green revolution in India has transformed the country from a food grain deficit to a surplus nation, but it has also resulted in reduced varietal diversity and increased uniformity in appearance and harvestable products. This has made agriculture more susceptible to natural calamities and the emergence of new pathogen races, leading to disease outbreaks and significant yield losses. The major role of wide hybridization in crop improvement are:

- Yield enhancement
- Improvement in quality
- Pest and disease resistance
- Abiotic and biotic stress resistance
- Development of new crop species

“Desired characters like resistance against pest and diseases, morphological characters and their responsible genes are mostly used in wild species, genera and species connected to the cultivars. It is used in primary and secondary gene by inter-specific and inter-generic hybridization”(**Patnaik*et al.*, 2021)**.

Wide hybridisation in amaranthus

The side effects of the green revolution include the emergence of new pathogen races and abiotic stresses, resulting in significant yield loss. Wide hybridization in amaranthusis suggested as a solution to address these challenges and improve agricultural productivity. Wide hybridization can introduce genetic diversity and enhance the resilience of crops to abiotic stresses, such as drought, flood, salinity, and high temperature. The utilization of wide hybridization techniques can lead to the progress of amaranthuswith improved yield and quality, contributing to the goal of feeding the growing population **(Pooja P. Gowda*et al.*,**

2020). Wide hybridization plays a crucial role in improving the adaptability of vegetable crops by introducing genetic diversity and enhancing their ability to withstand abiotic stresses and biotic pressures.

In a study involving 100 accessions of grain amaranth, comprising 50 from India and 50 from exotic sources, grown in a complete randomized block design, diverse characteristics were observed, with genotypes grouped into 10 clusters. Clusters I, VII, VIII, IX, and X displayed significant genetic distance from others. Correlation and path analysis underscored the importance of inflorescence length, number of leaves, and plant height in selecting high-yielding genotypes, irrespective of their geographic origin (**Rana *et al.,2005)*.**

Pseudo-xenic Effect of Allied *Annona* spp. Pollen in Hand Pollination of cv. 'Arka Sahan' [(*A. cherimola* × *A. squamosa*) × *A. squamosa*]

In the study investigating the efficacy of hand pollination in alleviating fruit set issues in Annonaceous fruits, it was observed that Annona species such as A. *squamosa* could significantly enhance fruit set and quality traits in cv. Arka Sahan. A total of 1080 flowers in 2003 and 3420 in 2004 were subjected to pollination with various Annona species pollen, alongside self-pollination. Results indicated significant effects of pollen source on various fruit traits, including fruit set, weight, size, and maturation period, with A. *squamosa* pollen demonstrating the highest fruit set and heaviest fruits. Moreover, fruits resulting from A. *squamosa* pollen exhibited quicker maturation and minimal weight loss upon ripening. Evaluation of fruit pulp quality revealed significant variations influenced by pollen source, particularly in total soluble solids and acidity. Additionally, seed count per 100g fruit varied with pollen source, with A. *squamosa* and A. *reticulata* pollen yielding higher seed counts and better fruit size and symmetry. The study underscores the potential for exploiting pseudo-xenia effects in fruit culture, emphasizing the practical significance of utilizing A. squamosa pollen for successful hand pollination in cv. Arka Sahan. (**S.H. Jalikop, 2007)**

First Fruiting Intergeneric Hybrids between *Citrus* and *Citropsis*

The first reported fruiting hybrids between Citrus L. and Citropsis have been achieved through conventional hybridization, utilizing Citrus wakonai and Citropsis. Despite the seeds being half the normal size, an impressive 90% germinated without embryo rescue techniques. After initial losses, 327 surviving hybrids were potted on after six months, showing vigorous growth on their own roots. Within two years, 35 hybrids flowered continuously, albeit pollen-sterile, with ovaries abscising shortly after petal fall. Nonetheless, at 25 months, two newly flowering hybrids exhibited fruit set, marking a significant milestone in the development of this unique germplasm with implications for future breeding endeavors. (**Malcolm W. Smith *et al.*, *2013)***

Interspecific hybridisation for improvement of Brassica crop

"In the Brassica genus, there are several crop species that allocate close relationships, such as rapeseed (*Brassica napus*), which is an ancestral hybrid between turnip (B. rapa) and cabbage (*B. oleracea*).Mustard species like *B. juncea*, *B. carinata*, and *B. nigra* also share genomes with the Brassica crop species.The Brassica genus offers potential for crop advancement through inter-specific hybridization due to the close relationships between crop species and their wild relatives. Embryo rescue techniques have been effectively used to relocate useful traits between Brassica species, such as triazine resistance from B. napus to B. oleracea.Natural hybridization between distant relatives in the Brassica genus is rare, emphasizing the importance of conducting controlled hybridizations"**(Elvis Katche*et al.*, 2019).**

Utilization of wild relatives of Sorghum in wide hybridisation

"Sorghum, with its diverse species, provides significant genetic and genomic variation, making it a suitable candidate for wide hybridization.Traits such as resistance to sorghum midge, shootfly, and spotted stem borer have been documented in many sorghum species, including those outside the section Eusorghum .The Inhibition of Alien Pollen gene has been used to produce inter-specific hybrids with species from sections Chaetosorghum, Parasorghum, and Stiposorghum, overcoming pre-fertilization barriers.Post-fertilization barriers can be eliminated through embryo rescue techniques and the use of 2n gametes, allowing for gene transfer between species"**(George L. Hodnett*et al.*,2020)**.

Wide hybridization in food legumes

"Inter-specific hybridization has been used to relocate foliar and viral disease resistance genes into inter-specific derivatives of groundnut.Inter-specific derivatives of pigeonpea obtained through crossing *Cajanusplatycarpus* with *C. cajan* have resistance to Phytophthora blight disease, a trait inherited from the wild species”**(Nalini Mallikarjuna,2003).**

Genetic analysis of segregating generations of irradiated interspecific hybrids in Okra (*Abelmoschus spp.*)

A study conducted at the Department of Plant Breeding and Genetics, College of Agriculture, Vellayani, during 1996-1997 aimed to assess variability in F2M2 and F3M3 generations resulting from hybridization and hybrid irradiation between *A. esculentus* and *A. manihot*, with a focus on isolating high-yielding, yellow vein mosaic disease (YVM) resistant lines. In F2M2, irradiated treatments showed late flowering, increased leaf and flower numbers, and enhanced fruit set, with maximum effects observed at 20 kR. Irradiation correlated with higher YVM and fruit borer incidence but also yielded some high-yielding, disease-resistant plants at 30 kR. In F3M3, irradiation led to late flowering, increased leaf and flower numbers, and higher fruit yield per plant, particularly at 40 kR. Notably, promising recombinants resembling the cultivated parent, resistant to YVM, were isolated from 30 kR, suggesting its suitability for developing disease-resistant okra varieties. High heritability and genetic advance were observed in multiple traits across irradiated treatments, indicating potential for further breeding efforts. Future work entails evaluating promising lines for additional generations to confirm superiority and disease resistance for varietal development (**John, S. 1997**).

Interspecific Somatic Hybridisation in Aspergillus

Clear evidence of interspecific somatic hybrids production has been demonstrated through fusions within the 'nidulans' group of Aspergillus species. Kevei and Peberdy (in press) documented the formation of heterokaryons or hybrids resulting from nuclear fusion among five species: *A. nidulans, A. rugulosus, A. quadrilineatus, A. violaceus*, and *A. nidulans var. echinulatus*, except for specific combinations. This laboratory also confirmed somatic hybridization between *A. quadrilineatus* and *A. nidulans var. echinulatus*, indicating the potential for hybridization across all pairwise combinations within the mentioned species. Additional observations revealed somatic hybridization between *A. nidulans* and *A. nidulans var. aeristatus, A. nidulans var. latus*, and two unidentified 'nidulans' group species, suggesting their inclusion as potential hybridizing species. However, species like *A. stellatus, A. unguis*, and both mating types of *A. heterothallus* from the 'nidulans' taxonomic group did not form somatic hybrids with each other or with the aforementioned species. Attempts to produce interspecific hybrids among members of other taxonomic species groups have been reported with varied success. For instance, fusion attempts within the 'niger' group yielded heterokaryons only between *A. niger* and *A. awamori*, while attempts in the 'flavus' group failed to hybridize *A. flavus* and *A. parasiticus*, despite their close taxonomic relationship. Further studies are warranted to establish the extent of successful somatic hybridization across different taxonomic groups (**Croft, J. H., & Dales, R. B. G. 1983**).

Interspecific Hybridisation of white clover

White clover (*Trifolium repens*) thrives in moist, fertile soils of temperate regions but exhibits limited genetic variation for adaptation to semi-arid, infertile soils. Despite apparent genetic isolation, 11 other taxa can be artificially hybridized, broadening the gene pool. These taxa encompass annuals to resilient perennials adapted to stress environments, offering potential traits for breeding resilient white clover varieties. Secondary, tertiary, and quaternary gene pools are delineated, with a review of interspecific hybrids achieved thus far. Breeding programs integrating traits from *T. nigrescens* and 4x *T. ambiguum* are highlighted, alongside schemes utilizing *T. uniflorum, T. occidentale, T. pallescens*, 2x *T. ambiguum,* and 6x *T. ambiguum*. Interspecific hybrid breeding holds promise for developing resilient perennial clovers suited to seasonally dry, infertile grasslands worldwide (**Williams, W. M. 2014**).

Utilisation of wild Cicer in chickpea improvement

Efforts to enhance the yield and quality of cultivated chickpea (*Cicer arietinum L.*) face limitations due to low intraspecific genetic diversity. Hybridization with 'wild' relatives within the Cicer genus, comprising 43 species, offers a pathway to augment genetic diversity. Screening efforts have primarily focused on the eight annual species sharing growth habits and chromosome numbers with *C. arietinum*. These screenings have revealed morphological traits and resistance to various stresses valuable for chickpea improvement programs. Detailed analyses, including protein and DNA studies, karyotyping, and crossability assessments, have initiated the elucidation of relationships among annual *Cicer* species. However, perennial species have received scant attention due to challenges in collection, propagation, and evaluation (**Croser*et al.*, 2003**).

Interspecific crossing among the new world lupin species for *Lupinusmutabilis* crop improvement

L. mutabilis stands out for its potential as a high-protein and oil legume and shares a close phylogenetic relationship with other *lupin* species in western North and South America, many of which exhibit a chromosome number of 2n=48. Recognizing the potential of genetic variation in these species, efforts have been made to initiate an introgression program through a series of crosses. Successful hybrids have been obtained between L. mutabilis and species such as *L. mexicanus*, *L. arizonicus*, and *L. tomentosus*, indicating a moderate level of compatibility. While some cross combinations did not require embryo rescue, others did, suggesting varying degrees of compatibility. Ongoing efforts involve further crossing of additional species with *L. mutabilis*, with intermediate morphological characters observed in hybrids. Tripping and backcrossing strategies are being employed to enhance hybrid fertility, with the aim of advancing BC1 progeny for potential utilization in the crop improvement of *L. mutabilis* (**Clements *et al., 2008*)**.

Breaking the intergeneric crossing barrier in papaya using sucrose treatment

In a breeding program aimed at developing progenies resistant to 'papaya ringspot virus' (PRSV), *Carica papaya* var. Surya and *Vasconcelleacauliflora* were utilized, despite earlier demonstrations of cross incompatibility between these genera. To overcome this barrier, sucrose was employed. Pollen from *V. cauliflora* was collected and pollination conducted by applying sucrose solutions at concentrations ranging from 1% to 5% onto the stigmatic surface of the flower. The highest viable seed set (13.73) was achieved with a 5% sucrose concentration, indicating the efficacy of sucrose in breaking the intergeneric barrier by enhancing pollen germination. Subsequent studies revealed a significant decrease in the effect of sucrose as concentration levels decreased. Pollen germination assays conducted with and without sucrose further confirmed its role in enhancing pollen germination and pollen tube growth. Additionally, an intervarietal hybridization between varieties Surya and Pusa Dwarf yielded a fruit set rate of 91.7% with 300 viable seeds per fruit. Hybridity of the progenies was verified using ISSR primers, which identified male-specific bands present in progenies but absent in the female parent, providing further evidence of successful hybridization (**Dinesh*et al.*, 2007**)

Conclusion

In conclusion, wide hybridization holds significant promise as the next horizon for crop improvement. This approach, which involves crossing distantly related species, has the capacity to unlock valuable genetic diversity and introduce novel traits that can improve crop resilience, yield, and nutritional content. As global challenges such as climate change and population growth intensify, the ability to utilize the genetic potential offered by wide hybridization becomes increasingly essential. While there are obstacles and intricacies associated with this technique, ongoing research and technological advancements are laying the way for its successful implementation. Adopting wide hybridization as a tool for crop improvement highlights a proactive and forward-thinking strategy in ensuring food security and sustainability in the face of evolving agricultural landscapes.

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