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Scope and opportunities of wide hybridization in fruit crops

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Abstract
Wild plant species closely related to domesticated crops have evolved into vital assets for enhancing the quality of fruit crops, fortifying their resilience against both biological and environmental challenges, and augmenting their genetic variation. Wide hybridization, which entails the complex cross-pollination of domesticated crop types with their wild counterparts or allied taxa, has assumed a central role in the arena of contemporary fruit crop improvement efforts. By overcoming the limitations of species borders, this ground-breaking method makes it possible for genetic material to move easily between different botanical lineages. The progeny's genotypic and phenotypic features undergo transformation as a result of this genetic alchemy. Nonetheless, prezygotic and postzygotic barriers, which typically thwart interbreeding, present formidable challenges when extensive hybridization systematically breaks down their defenses. The importance of wild plant species that are closely related to farmed crops cannot be emphasized in the quest to advance fruit crop development. These wild equivalents are a rich source of priceless qualities, promising to improve fruit crop quality, strengthen resistance to biotic and abiotic stresses, and increase the genetic variety necessary for sustainable agriculture recent endeavors aimed at enhancing fruit crops have experienced a fundamental shift in approach, with extensive hybridization emerging as a pivotal strategy. This novel strategy efficiently crosses traditional species boundaries through the complex merging of domesticated crop types with their wild relatives or kin taxa. Such genetic fusion has substantial effects since it ushers in a new age characterized by altered genotypes and phenotypes in the progeny.

Keywords: Wide, hybridization, fruit crops, importance

Introduction

Historically, techniques such as introduction, selection, or intraspecific hybridization have been employed to enhance the yields of perennial fruit crops. Unfortunately, this traditional method frequently leads to a decrease in genetic variety, which limits the ability of fruit crops to react to the constant changes in environmental conditions. A startling 75 percent of agricultural genetic variety has been covertly lost throughout the turbulent course of the twentieth century, according to the chronicles of history (Singh, 2017) [20]. Due to the absence of desired traits within existing species, breeders have been compelled to venture into the realm of advanced techniques, including mutation, polyploidization, and the enigmatic domain of recombinant DNA technology. Wild plant species that are related to crop plants have emerged as true lifelines in this period where the threat of climate change is pervasive and the necessity of increased genetic diversity is woven into the very fabric of agricultural success. In the context of food security and environmental sustainability in the twenty-first century, these botanical collaborators possess a treasure trove of indispensable attributes related to agronomy, quality, and their capacity to withstand both biological and environmental stressors. (Maxted *et al.*, 2006) [12]. Wide hybridization, a cutting-edge technique that involves orchestrating unions between crop wild relatives and consanguineous taxa, has therefore taken centre stage in recent fruit crop development projects. This groundbreaking approach transcends conventional species limitations, bestowing the ability to transfer genetic assets from one botanical lineage to another, resulting in substantial alterations in the genotypes and phenotypes of the progeny.

Challenges in Wide Crossings

The difficulties encountered in generating wide hybrids primarily stem from compatibility issues. These compatibility issues, serving as formidable impediments in wide hybridization, can be broadly categorized into two groups: prezygotic and postzygotic barriers. Prezygotic incompatibility, which manifests before fertilization, results in the inability to achieve successful crosses. Conversely, postzygotic incompatibility becomes apparent after fertilization, resulting in problems such as underdeveloped or nonviable hybrid seeds, sterility in F1 hybrids, and subsequent generations. Prezygotic incompatibilities involve factors such as the failure of pollen and stigma recognition, leading to unsuccessful pollen germination. Additionally, these barriers include pollen tube growth arrest within the stigma or stylar tissue and the inability of pollen tubes to successfully penetrate the ovule, ultimately resulting in unsuccessful pollination. On the other hand, postzygotic barriers present a diverse set of challenges Hybrid unviability can be attributed to abnormal endosperm development, which subsequently results in the abortion of the embryo. In certain cases, hybrid seedlings display lethality or sublethal abnormalities, further complicating the hybridization process. Hybrid sterility can emerge due to chromosomal or genic disparities, introducing additional layers of complexity .Furthermore, hybrid breakdown, a phenomenon observed in F2 or subsequent generations, involves a decline in the viability or fitness of hybrid offspring. These intricate barriers in wide crossings have been explored in the works of various researchers, such as Ladizinsky (1992) [8], Dickinson *et al*. (2012) [2], and Pershina and Trubacheeva (2017) [16]. Their contributions shed light on the multifaceted challenges and complexities faced by breeders as they navigate the intricate landscape of interspecific and intergeneric hybridization, striving to bridge the genetic chasm between species in the quest for innovative crop development. In intergeneric crosses involving *Duchesnea indica* and *Fragaria* × *ananassa*, a significant number of putative hybrids were successfully generated when D. indica served as the female parent. However, when it was employed as the male parent, only a meager yield of achenes and subsequent plants was obtained. To unravel the intricate breeding barriers at play in this scenario, an investigation into pollen-pistil compatibility relations was undertaken, employing fluorescence microscopy to dissect the dynamics of the cross. Among the numerous genotypic combinations examined, an astonishing 78.6 percent displayed incompatibility at the stigma level, with an additional 17.2 percent showing incompatibility at the first third of the style. A mere 3.6 percent of these combinations were found to be pollen-pistil compatible, yielding fruits complete with achenes. However, it is noteworthy that a portion of these achenes exhibited developmental anomalies, with seven failing to germinate and others yielding short-lived plants. In contrast, nine of the achenes produced from these compatible crosses gave rise to healthy, normal plants. This intricate examination of compatibility dynamics within intergeneric crosses, as elucidated by Marta *et al*. in 2004 [11], unveils the nuanced interplay between pollen and pistil compatibility in hybridization. These findings shed light on the complex mechanisms and barriers inherent to intergeneric breeding, offering valuable insights into the challenges and opportunities in bridging genetic divides between species for innovative crop development.

Methods for Overcoming the Obstacles

Approaches to Overcome Pre-Fertilization Obstacles Manipulating Chromosome Numbers

The endeavor to cross cultivated and wild species with differing ploidy levels poses significant hurdles. However, a valuable approach lies in the manipulation of chromosome numbers, a technique proven effective by Khush and Brar (1992) [7] and Pujar *et al*. (2017) [17]. By inducing chromosome duplication in one of the potential parent plants or the F1 offspring, the likelihood of successful wide hybridization is significantly enhanced. An illustrative example can be found in the effort to introduce a new aromatic dimension from a diploid wild strawberry, *Fragaria nilgerrensis*, into the cultivated octaploid strawberry, *F. x ananassa*. Initially, all lines resulting from inter-species hybridization were sterile. However, after subjecting the inter-species hybrid 'TN13' to colchicine treatment to double its chromosomes, a remarkable transformation occurred. Among the regenerated progeny, three distinct groups emerged: 15 plants did not produce flowers or fruits (Group I), 28 flowered but failed to set fruit (Group II), while 109 lines (Group III) exhibited both flowering and fruitful outcomes. Furthermore, an exceptional offspring identified as 'TN13-125,' characterized by a unique peach-like aroma, was singled out for future integration into breeding programs, as demonstrated by the work of Noguchi *et al*. in 2002.

Bridging Species Technique

In situations where direct crosses between two species, regardless of their ploidy levels, prove to be insurmountable challenges, a third species, known as the "bridging species," plays a crucial role in facilitating these complex crosses. Khush and Brar (1992) [7] and Pujar *et al*. (2017) [17] have described the effectiveness of this bridging species technique. For instance, in the pursuit of Papaya Ringspot Virus-Papaya (PRSV-P) resistance breeding in papaya (Carica papaya), initial crosses with the PRSV-P resistant species *Vasconcellea pubescens* resulted in sterile offspring plants. However, using V. parviflora, which is susceptible to PRSV-P, as a bridging species led to the production of hybrids with some degree of pollen fertility. Subsequent crosses between *V. pubescens* and *V. parviflora* produced fertile F1, F2, and F3 populations. Subsequent backcrosses to V. parviflora are facilitating the development of PRSV-P resistant V. parviflora, which can then be crossbred with cultivated *C. papaya*. This innovative use of V. parviflora as a bridging species simplifies the complex breeding process Introducing PRSV-P resistance from *V. pubescens* into cultivated papaya, as exemplified by O'Brien and Drew in 2009 [15].

Utilization of Nutrient Solutions and Growth Regulators Harnessing Growth Hormones and Nutrients

In the effort to overcome the hurdles of hybridization, researchers have discovered the crucial role of growth hormones and nutrients in stimulating pollen tube growth and embryo development. These essential elements also extend the receptivity of stigmas and prevent premature flower abscission, as elucidated by Khush and Brar (1992) [7] and Pujar *et al*. (2017) [17].

A concrete example of this approach can be found in the attempt to overcome the crossing barrier in intergeneric crosses between Carica papaya and *Vasconcellea cauliflora*. In this context, pollination was facilitated by applying sucrose solutions at varying concentrations (ranging from 1% to 5%) onto the stigmatic surface of the flowers. Remarkably, the application of a 5% sucrose concentration yielded the maximum viable seed set (13.73), significantly enhancing pollen germination. As the sucrose concentration decreased, its effectiveness in promoting pollen germination and pollen tube growth diminished, as evidenced by in vitro pollen germination studies conducted with and without sucrose. These findings underscore the efficacy of sucrose in fostering successful pollination, as demonstrated by Dinesh *et al.* in 2007 [3].

In another instance, to overcome the intergeneric hybridization barrier involving nine Carica papaya cultivars as female and *Vasconcellea cauliflora* as male, various nutrient combinations were explored. Among the combinations tested, sucrose (5%), sucrose (5%) + boron (0.5%), and sucrose (5%) + $CaCl_2$ (0.5%) emerged as particularly effective in enhancing fruit and seed set percentages, as highlighted by Jayavalli *et al*. in 2011 [6].

Utilization of Recognition Mentor Pollen

Sometimes, pollen grains of one species cannot germinate on the stigmas of another species, leading to incompatibility. To circumvent this issue, an ingenious technique involves mixing incompatible pollen grains with killed maternal pollen grains. When compatible pollen is treated with ethanol and combined with incompatible pollen for pollination, the proteinaceous recognition factors released from the walls of the killed compatible pollen grains serve to mask the rejection reaction of the recipient stigma. This, in turn, facilitates the germination of alien pollen grains, a process referred to as recognition or mentor pollen, as outlined by Khush and Brar (1992) [7].

For instance, Wenslaff and Lyrene (2000) [23] reported the use of mentor pollination in wide hybridization in blueberry. They conducted interspecific crosses between two diploid yellow leaf *Vaccinium elliottii* Chapmn. clones and pollen from the tetraploid southern highbush cultivar 'Misty' (*V. corymbosum* L). Typically, these interspecific crosses yield few hybrids due to a triploid block. However, when mentor pollen from *V. elliottii* was combined with *V. corymbosum* pollen, a significant increase in hybrid production was observed, underscoring the efficacy of this innovative approach.

Protoplast Fusion

In scenarios where the barriers to sexual hybridization are insurmountable, researchers have explored the technique of protoplast fusion, followed by the regeneration of somatic hybrids, as proposed by Khush and Brar (1992) [7].

A notable example of this approach can be found in the work of Ruiz *et al*. (2018) [19], where two somatic hybrids, SMC-58 and SMC-73, were successfully produced by fusing protoplasts derived from leaf mesophylls of *Citrus macrophylla* and Carrizo citrange (*Citrus sinensis x Poncirus trifoliata*). Genetic characterization of these somatic hybrids revealed allelic configurations corresponding to the combination of both parental genomes, exemplified by the JK-TAA15 SSR marker. This innovative technique of protoplast fusion offers a promising avenue for overcoming seemingly insurmountable hybridization barriers, as demonstrated by Ruiz *et al*. 2018 [19]

Strategies for Overcoming Post-Fertilization Barriers

Various techniques have been developed to address post-fertilization barriers in crop improvement. Among these, methods such as embryo rescue, backcrossing, and chromosome manipulation have proven to be particularly effective in annual crop plants. However, in the realm of fruit crop improvement, embryo rescue and backcrossing stand out as the most commonly employed approaches, as noted by Khush and Brar (1992) [7].

Embryo Rescue

Embryo abortion can occur at various developmental stages, depending on the genomic relationships of the parental species. Abortive embryos can be carefully...

Excerpt continues

Dissected from developing seeds, cultured within test tubes on nutrient-rich media, and nurtured into mature hybrid plants.

For instance, Tian *et al*. (2008) [24] conducted hybridization experiments using *V. vinifera* as female parents and wild Chinese Vitis spp. (*V. amurensis*) as male parents. In this endeavor, in-ovulo embryo rescue played a pivotal role in cultivating hybrid plants from seedless female parents. The choice of culture medium significantly impacted in vitro embryo formation, germination, and subsequent plant development. Among the mediums evaluated, the double-phase medium exhibited the highest embryo formation rate at 31.8%, surpassing both liquid (21.1%) and solid mediums (21.1%). In terms of embryo germination, the double-phase and liquid mediums yielded similar results, with percentages of 94.3% and 95.0%, respectively, outstripping the solid medium (73.7%). Notably, the double-phase medium also excelled in producing a higher percentage of total plants, standing at 23%, compared to 16% and 12% for the liquid and solid mediums, respectively.

Additionally, the duration of ovule culture was scrutinized for its effects on in vitro embryo formation, germination, and plant development. While culture duration exhibited minimal impact on embryo formation (ranging from 27.0% to 34.0%) and embryo germination (ranging from 70.4% to 91.2%), it wielded significant influence on plant development. Prolonged culture durations of 16 weeks resulted in a diminished plant development rate of 52.6%. The optimal ovule culture period was determined to be between 8 to 12 weeks, where the percentage of total plants ranged from 18% to 24%.

Applications

These techniques find applications in enhancing fruit quality and developing innovative solutions in agriculture. For example, Annona reticulata was crossbred with atemoya (*A. cherimola Mill. x A. squamosa* L.) to yield 250 trispecies hybrids. This endeavor aimed to preserve valuable genes from the three edible annonas while assessing the extent of variation in the progeny. Fruit traits such as shape, skin color, skin surface, total soluble solids (ranging from 17°B to 32°B), acidity (varying from 0.16% to 2.2%), and seed count per 100g of fruit (ranging from 3 to 49) exhibited significant diversity, offering opportunities for selecting desirable traits. Among the segregating population, slow-ripening genotypes were identified, requiring up to 12 days from harvesting to ripening, thus holding promise for extended fruit shelf life, as exemplified by Jalikop in 2010 [5].

In another application, the challenge of irregular fruit maturation within blueberry clusters was addressed through cluster harvesting. Miyashita *et al*. (2019) [13] explored the degree of parthenocarpy and the suitability of two interspecific hybrids of *Vaccinium corymbosum* and *Vaccinium virgatum* for cluster harvesting.The hybrids exhibited higher frequencies of parthenocarpy, with their fruit set and weight closely resembling those of pollinated fruits. Moreover, the pollinated fruits from these hybrids were seedless. These findings not only enhance the feasibility of cluster harvesting but also underscore the potential of parthenocarpic hybrids for breeding cultivars optimized for this harvesting method.

The uniformity of flowering and fruit maturation within the interspecific hybrids of *Vaccinium corymbosum* and *Vaccinium virgatum* was further assessed. These hybrids displayed relatively consistent fruit maturity within clusters, along with delayed fruit dropping. Comparative analysis between cluster and individual harvesting revealed significantly higher percentages of mature fruits within clusters for the two hybrids when compared to conventional cultivars, offering promising solutions for more efficient harvesting practices.

In sum, these innovative techniques have wide-ranging applications in fruit crop improvement, offering avenues for the preservation of valuable traits, enhanced fruit quality, and the development of novel agricultural solutions.

Enhancing Stress Tolerance in Fruit Crops: A Path to Sustainable Agriculture

Fruit cultivation is a vital component of global agriculture, providing essential nutrients and economic value to communities around the world. However, fruit crops face numerous challenges, including abiotic stressors like salinity and iron chlorosis, as well as biotic threats like viral infections and fungal diseases. In response, researchers have been employing innovative techniques to bolster the stress tolerance of these crops, offering the potential for increased yields and more resilient agriculture.

Abiotic Stress Tolerance

Salinity and Iron Chlorosis Tolerance in Citrus Hybrids

Citrus fruits, beloved for their refreshing flavors, are highly sensitive to salinity, a condition that occurs when soil accumulates high levels of salt. Furthermore, iron chlorosis, a common problem in alkaline soils, can severely affect citrus crops. The traditional rootstock, Carrizo citrange (CC), is renowned for its resistance to Citrus Tristeza Virus (CTV) but is sensitive to iron chlorosis. In contrast, C. macrophylla thrives in saline soils due to its ability to restrict ion transport to its aerial parts. Researchers embarked on an ambitious journey to combine the desirable traits of these two species and created somatic hybrids, namely SMC-58 and SMC-73 hybrids (Ruiz *et al*., 2018) [19]. The outcomes of these hybridization efforts are promising. The SMC-58 and SMC-73 hybrids exhibited substantial tolerance to iron chlorosis under experimental conditions that induce this stress. They displayed intermediate iron concentrations in their leaves, an indicator of resilience, falling between the levels of the resistant and susceptible parental species. When exposed to salinity stress, Carrizo citrange plants demonstrated sensitivity, experiencing lower dry weights compared to control plants. In stark contrast, C. macrophylla, a salt-tolerant species, maintained consistent growth in both salinity-stressed and control conditions. Interestingly, the SMC-73 hybrid showcased remarkable resilience to salinity stress, whereas SMC-58 displayed a moderate reduction in dry weight under salinity treatment compared to the control group.

Leaf symptoms resulting from salt toxicity were intense in Carrizo Citrange but mild in the somatic hybrids, with minimal leaf toxicity symptoms. This resistance was mirrored in the ion concentrations of sodium (Na^+), chloride (Cl^-), and potassium (K^+) in the leaves. Both somatic hybrids exhibited lower Cl^- exclusion capacity compared to the salt-tolerant parent C. macrophylla. Notably, SMC-58 demonstrated greater exclusion capacity than the salt-sensitive parent CC, while SMC-73 displayed similar exclusion capacity to CC. When it came to Na^+ exclusion, the behavior observed in SMC-58 resembled that of CC, while SMC-73 exhibited lower Na^+ accumulation in their leaves, suggesting superior tolerance compared to the sensitive parent CC (Ruiz *et al*., 2018) [19].

Biotic Stress Tolerance

Enhanced Resistance to Papaya Ringspot Virus (PRSV): In papaya cultivation, the Papaya Ringspot Virus (PRSV) has long been a menacing adversary, causing severe yield losses. Researchers embarked on a mission to fortify papaya against this virus by engaging in intergeneric hybridization between *Carica papaya* and *Vasconcellea cauliflora*. The objective was to transfer genes that confer PRSV resistance from one species to another. The second-generation (F2) progenies resulting from these crosses were subjected to rigorous evaluation under controlled conditions (Sudha *et al*., 2013) [25].

The results of this endeavor yielded substantial promise. Specific crosses, such as Pusa Nanha × *V. cauliflora*, demonstrated a remarkable percentage of disease-free seedlings. These hybrid plants exhibited varying levels of resistance when exposed to PRSV, with certain crosses showing minimal or delayed symptoms. In particular, CP 50 × *V. cauliflora* and Pusa Nanha × *V. cauliflora* displayed notable resistance, with little to no symptoms observed until late stages of growth. These findings underscore the potential of intergeneric hybridization as a strategy for breeding papaya cultivars with enhanced PRSV resistance. Such resistant cultivars hold the key to securing papaya production against the perils of PRSV (Sudha *et al*., 2013) [25].

Developing Disease-Resistant Seedless Grape Varieties

Grapes are renowned for their versatility, but they are not immune to diseases, with downy mildew being a significant threat. Researchers sought to create seedless grape varieties that could withstand this pathogen. They initiated crosses between stenospermocarpic *Vitis vinifera* varieties 'Flame Seedless' and 'Ruby Seedless,' known to be susceptible to downy mildew, and the disease-resistant 'Beichun' (*V. vinifera x V. amurensis*). This endeavor aimed to develop seedless grape cultivars with inherent resistance to downy mildew. Molecular markers were employed to identify the presence of the disease resistance gene S382-615 in the male parent 'Beichun' (Li *et al*., 2020) [9].

Two first-generation (F1) progenies from the 'Ruby Seedless' x 'Beichun' cross carried this disease resistance gene and exhibited resistance to downy mildew. This breakthrough holds tremendous potential for the grape industry, as it opens the door to breeding seedless grape cultivars that are naturally resilient to this destructive pathogen. Such cultivars not only simplify grape production but also reduce the reliance on chemical treatments for disease control (Li *et al*., 2020) [9].

In conclusion, the progress achieved in enhancing stress tolerance in fruit crops represents a significant step forward in the quest for sustainable agriculture. By mitigating the impact of both abiotic and biotic stressors, these innovations pave the way for higher crop yields, increased food security, and more resilient agriculture in the face of an ever-changing environment. The ongoing research in this field promises to deliver solutions that benefit both growers and consumers while reducing the ecological footprint of fruit production.

Conclusion: Broadening the Genetic Base of Fruit Crops through Wide Hybridization

Wide hybridization has emerged as a promising approach in fruit crop improvement, offering opportunities to enhance fruit quality and bolster tolerance to biotic and abiotic stresses. Several successful wide hybridization examples have demonstrated its potential in diversifying the genetic resources of fruit crops, leading to the development of novel cultivars with improved traits. Some prominent wide hybrids in the fruit industry include Kinnow mandarin (*Citrus nobilis x Citrus × deliciosa*), cultivated strawberry (*Fragaria chiloensis x Fragaria virginiana*), atemoya (*Annona cherimola x Annona squamosa*), and Arka Sahan custard apple (*A. atemoya x A. squamosa*).

Techniques such as chromosome doubling, bridging species, protoplast fusion, and embryo rescue have played pivotal roles in overcoming the challenges associated with wide hybridization, enabling the recovery of fertile progenies that exhibit desirable characteristics.

However, there remains a significant need for the identification, collection, and characterization of elite wild species. These resources can be efficiently utilized in breeding programs to broaden the genetic base of fruit crops further. The potential benefits of utilizing wild species in fruit crop improvement programs include increased disease resistance, enhanced stress tolerance, and the introduction of novel traits that can lead to more resilient and productive cultivars.

As we move forward in fruit crop research and breeding, the utilization of wide hybridization techniques, coupled with a deeper understanding of the genetic diversity offered by wild relatives, will be essential in meeting the evolving challenges of agriculture. This approach holds great promise for ensuring food security, sustainable fruit production, and the development of fruit varieties that can thrive in diverse environmental conditions.

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