Barriers in Interspecific Hybridization in Vegetable Breeding and Biotechnological Breakthroughs

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Introduction

Interspecific hybridization involves crossing individuals from different species within the same genus to combine desirable traits such as disease resistance, stress tolerance and improved yield. Interspecific hybridization plays a pivotal role in crop improvement by transferring desirable traits from wild relatives to cultivated species. While conventional breeding relies on intraspecific crosses, interspecific hybridization broadens the genetic pool, enabling the transfer of novel traits from wild relatives to cultivated crops (Prohens *et al.*, 2017).

Despite its potential, interspecific hybridization faces significant biological barriers, including prezygotic (before fertilization) and postzygotic (after fertilization) incompatibilities. Traditional breeding methods often fail to overcome these barriers, necessitating biotechnological interventions. Recent advances in biotechnology, including embryo rescue, somatic hybridization, chromosome doubling, CRISPR/Cas9-mediated gene editing and RNA interference (RNAi), have provided effective solutions to overcome these barriers. This review explores the challenges in interspecific hybridization and the techniques employed to circumvent them, with a focus on vegetable crops.

Barriers in Interspecific Hybridization

i) Prezygotic Barriers

Prezygotic barriers prevent fertilization between species, maintaining reproductive isolation. These include:

1. **Pollen-Pistil Incompatibility –** Pollen fails to germinate or the pollen tube cannot penetrate the ovule (e.g., in *Solanum* species).
2. **Mechanical Incompatibility –** Differences in floral morphology or pollinator specificity (e.g., long-styled vs. short-styled flowers in *Capsicum*).
3. **Gametic Incompatibility –** Molecular mismatches prevent sperm-egg fusion (e.g., *Brassica* spp.).

ii) Postzygotic Barriers

Postzygotic barriers affect hybrid viability and fertility after fertilization:

1. **Hybrid Inviability –** Embryo abortion due to genetic disharmony (e.g., wheat × rye crosses).
2. **Hybrid Sterility –** Meiotic irregularities lead to non-functional gametes (e.g., *Gossypium* cotton hybrids).
3. **Hybrid Breakdown –** F1 hybrids are viable, but F2 progeny are weak or sterile (e.g., intergeneric *Raphanobrassica*).

Fig 1. outlines plant reproductive stages and techniques to overcome hybridization barriers in each phase.

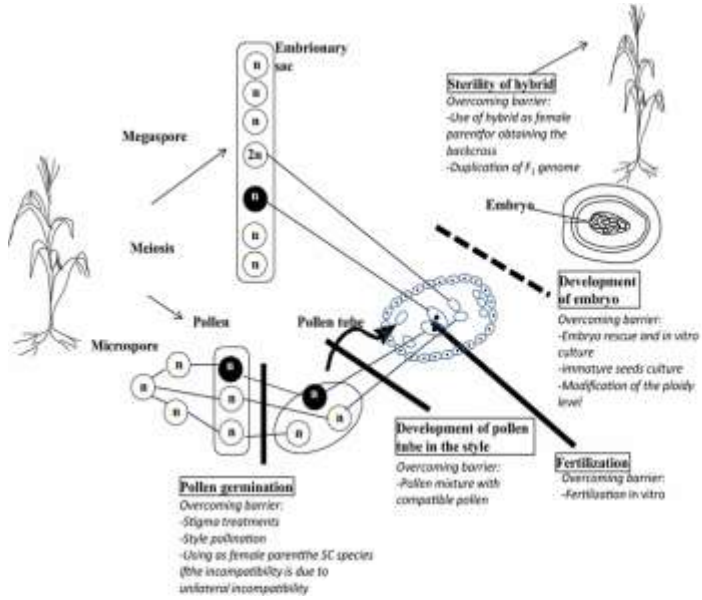

Fig. 1. Key Stages and Solutions in Interspecific Plant Hybridization (Prohens *et al.*, 2017)

Techniques to Overcome Hybridization Barriers

a) Embryo Rescue

Embryo rescue is an in vitro technique where hybrid embryos, which would normally abort due to interspecific incompatibility, are excised and cultured on artificial nutrient media to enable their development into viable plants.

Purpose in Interspecific Hybridization:

- **Overcomes Postzygotic Barriers:** Rescues embryos that fail to develop due to endosperm breakdown or parental genome incompatibility (e.g., in crosses between cultivated barley and wild *Hordeum* species).
- **Enables Wide Hybridization:** Facilitates gene transfer from wild relatives to crops when normal seed development is impaired (e.g., tomato × wild *Solanum* species for disease resistance).

- **Saves Rare Hybrids:** Preserves unique genetic combinations from abortive crosses (e.g., watermelon × citron hybrids for fungal resistance).

Applications:

- ✓ **Solanaceae**: Tomato (*Solanum lycopersicum*) × wild relatives for disease resistance.
- ✓ **Brassicaceae**: Cabbage (*B. oleracea*) × radish (*R. sativus*) to produce *Raphanobrassica*.

b) Somatic Hybridization

Somatic hybridization is a biotechnological technique that involves the fusion of protoplasts (plant cells without cell walls) from two different species to create hybrid cells. This method bypasses natural sexual reproduction barriers, enabling the combination of genomes from distantly related or sexually incompatible species.

Examples include disease-resistant citrus rootstocks and potato × tomato hybrids. While these somatic hybrids often require further chromosome manipulation for fertility, the technique provides unique opportunities for crop improvement by merging genomes that cannot hybridize naturally.

c) Chromosome Doubling

Chromosome doubling artificially duplicates a hybrid's chromosomes (e.g., using colchicine) to restore fertility. Interspecific hybrids often have sterile, mismatched chromosomes, but doubling creates homologous pairs for proper meiosis. Induced polyploidy restores fertility in sterile hybrid like,

- ✓ Triticale (wheat × rye hybrid) was stabilized by doubling chromosomes to form a fertile amphidiploid (allotetraploid) via colchicine treatment.
- ✓ Doubling helps create compatible intermediates bridge crosses in *Solanum tuberosum* using *S. acaule* as an intermediary.

d) Genetic and Molecular Approaches

Modern biotechnological tools are revolutionizing interspecific hybridization by overcoming reproductive barriers:

1. **CRISPR/Cas9 Gene Editing –** Precisely disables incompatibility genes (e.g., knockout of S-RNase in *Petunia* to bypass self-incompatibility).
2. **RNA Interference (RNAi) –** Silences specific genes causing pollen rejection (e.g., suppression of S-locus genes in apple and cherry for improved cross-compatibility).
3. **Transcriptomics & QTL Mapping –** Identifies key genes/QTLs regulating hybridization success, enabling marker-assisted breeding (e.g., compatibility loci in *Brassica* species).

These approaches accelerate trait introgression from wild relatives while maintaining genomic stability, offering targeted solutions to hybridization challenges in crop improvement.

Applications in Vegetable Breeding

1) Solanaceae Family

Interspecific hybridization has been extensively utilized in the *Solanaceae* family to enhance desirable traits in vegetable crops. For instance, crossing cultivated tomato (*Solanum lycopersicum*) with wild relatives such as *S. pimpinellifolium* has facilitated the introgression of resistance genes against *Fusarium* wilt, a devastating soil-borne disease. Similarly, hybridization between eggplant (*S. melongena*) and its wild relative *S. incanum* has led to improved drought tolerance, a critical trait in water-scarce regions. These crosses often require embryo rescue techniques to overcome postzygotic barriers, ensuring the survival of hybrid embryos that would otherwise abort under natural conditions.

2) Brassicaceae Family

In the *Brassicaceae* family, interspecific hybridization has been instrumental in improving both nutritional and agronomic traits. For example, crosses between broccoli (*Brassica oleracea* var. *italica*) and wild *Brassica* species have resulted in elevated glucosinolate content, enhancing natural pest resistance. Another notable example is *Raphanobrassica*, a synthetic hybrid between radish (*Raphanus sativus*) and cabbage (*B. oleracea*), developed through protoplast fusion. This hybrid exhibits a unique combination of root and leaf traits, making it a dual-purpose crop for both human consumption and livestock feed. Chromosome doubling techniques have been employed to restore fertility in such wide hybrids, enabling their use in breeding programs.

3) Cucurbitaceae Family

The *Cucurbitaceae* family has also benefited from interspecific hybridization, particularly in disease resistance breeding. A prominent example is the cross between cultivated cucumber (*Cucumis sativus*) and wild *Cucumis* species, which has introduced resistance to powdery mildew, a major fungal pathogen affecting cucumber production. These hybrids often exhibit enhanced vigor and resilience, though sterility issues may arise due to genomic incompatibilities. Biotechnological approaches such as somatic hybridization and CRISPR-mediated gene editing are increasingly being explored to stabilize these hybrids and introgress resistance genes without compromising yield or quality.

Challenges and Future Prospects

Despite advancements, interspecific hybridization faces:

- Regulatory hurdles for gene-edited crops.
- Unpredictable gene interactions in wide crosses.

Future research should focus on:

- High-throughput omics for identifying compatibility genes
- Synthetic biology to design hybrid-compatible pathways.

Conclusion

Interspecific hybridization is a powerful tool for vegetable breeding, but biological barriers limit its efficiency. Biotechnological innovations such as embryo rescue, somatic hybridization and CRISPR/Cas9 have revolutionized hybrid crop development. Continued integration of these techniques will drive the next generation of climate-resilient and nutritionally enhanced vegetables.

References

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