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Breeding approaches in fruit crops improvement

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Abstract
Fruits are an important part of human diets and consumption of adequate quantity of fruits as well as foods enriched in nutraceuticals and bioactive compounds is considered necessary for a healthy human life. The first step in plant breeding is to identify suitable genotypes having the desired genes among existing genotypes and to create one if it is not present in nature. Breeding improvement in perennial fruit crops is affected by a number of limitations like, long juvenile phase, heterozygosity, large size of the plant, sterility and environmental problems like flower and fruit drops. Breeding in fruit crops is essential requirement for enhance the quality fruit production and decrease the incidence of biotic and abiotic stress. However, improvement of perennial fruit crops is mainly depending on traditional approaches of introduction, selection, mutation and hybridization by using the cultivated genotypes of a species. Biotechnological approaches give precision and they are thought to shorten the breeding cycle in fruit crops. Recently biotechnology techniques have been employed including *in-vitro* propagation, embryo rescue, recombinant DNA technology, protoplast fusion, somatic hybrid, somaclonal variation, transgenics and marker-assisted selection. In this article, we will discuss general conventional and non-conventional approaches in breeding for improvement of fruit crops.

Keywords: Breeding, perennial fruit, *in-vitro*, hybridization, mutation

Introduction

Plant breeding is involved with the development of cultivars that are suited to specific environmental and production practices, as well as to fulfil food, fibre and feed requirements. Fruit crops supply humans with a various type of nutritious foods. Fruit breeding refers to the process of improving the genetics of fruit crops by diverse approaches such as selection, hybridization, mutation, induction, and molecular techniques (Janick *et al*., 2012) [8]. However, breeding for quality improvement in perennial fruit crops is affected by a number of limitations like, long juvenile phase, large size of the plant, and environmental problems e.g., flower & fruit drops (Karanjalker and Begane, 2015). A balanced approach that combines traditional and non-conventional breeding approaches could assist to solve this problem. Biotechnological approaches give precision and dependability, and they are thought to shorten the breeding cycle in long juvenility crops. When dealing with cumbersome crops, the efficacy of procedures such as marker assisted selection, genomics, candidate gene, transgenics, and cisgenics has been demonstrated to be beneficial. DNA sequence-specific modification has become a powerful tool as molecular biology has progressed. Therefore, developing genetic resources with a wide range of desired qualities will be crucial for improving fruit products. Breeding approaches required for improve the quality fruit production and reduce the incidence of biotic and abiotic stress.

Domestication: Plant domestication is the process of genetically altering a wild species for human use (Gaut *et al*., 2015) [4]. Fruit tree domestication and cultivation began after grain agriculture was established, most likely in the fourth millennium B.C. While the history of domestication was similar in many fruit plants, certain fruits were domesticated before others. This was due to ease of vegetative propagation (banana, fig, grape, date, Olive) and polyembryony (citrus, jamun, mango). Conservation of trees, vines and walling in preceded vegetative propagation. Date palms, olives, grapes, almonds, figs, and pomegranates appear to be the earliest fruits crops to be domesticated. Fruit domestication entails a combination of events that include species selection, recurrent selection of elite clones, and vegetative propagation, as well as horticultural technology like irrigation in dry climates, training and pruning, pollination in date palms, and storage and processing technology. Major alteration under domestication includes hermaphroditism in grape, self-fertility (sour cherry, peach), lower resin in mango, elimination of bitterness in almond, and thornlessness (many crops).

Significant modification also includes polyembryony, parthenocarpy or seedlessness in banana and citrus.

Introduction: Taking a genotype or a group of genotypes in to a new place or environment where they were not grown previously. Plant introduction may be of two types' primary introduction and secondary introduction. Primary introduction when the introduced crop or variety is well suited to the new environment, it is directly grown or cultivated without any alteration in the original genotype. Secondary introduction the introduced variety may be subjected to selection to isolate a superior variety or it may be used in hybridization programme to transfer some useful traits.

Selection: Thomas Andrew Knight (1759–1838) was the first to improve fruits by genetic recombination resulting from clonal cross-pollination. Traditional plant breeding is effective because it is evolutionary. It is based on the constant selection of superior phenotypes from genetically diverse populations produced through sexual recombination (Janick, 2012) [8]. The isolation of elite selections, along with large plantings, produced an environment in which mass and recurrent selection could occur spontaneously. Prerequisite for selection is variation must be present in the population and variation must be heritable.

Hybridization: Hybridization involves the matting of desirable parents and the subsequent selection of offspring. There are two types: intergeneric (matting between two genera in the same family) and interspecific (matting between two species in the same genera). Hybridization is a critical step in producing beneficial variety in crop breeding populations. Desirable traits such as resistance to biotic and abiotic stress in various environments, metabolite composition, or morphological traits, as well as their responsible genes, are frequently found only in related species, wild species, and genera of cultivars, but are mostly absent in cultivated species (Pujar *et al*., 2017) [17]. These desirable parents are usually obtained after suitable screening of natural populations and crop wild relatives being conserved under *in-situ* and *ex-situ* environment condition and so germplasm conservation is the most critical phase, especially when using wild species in breeding programmes (Sharma *et al*., 2015). Wide hybridization removes the species barrier to gene transfer, allowing the genome of one species to be transferred to another, resulting in changes in genotypes and phenotypes of the progenies (Anushma *et al*., 2021) [1]. Traditional breeding techniques such as hybridization, distant hybridization, bridge crossing, sib mating, and half sib mating etc. have proven to be effective in developing breeding methods for a variety of crops, including perennial fruit crops.

Steps in hybridization: Selection of parent's → Evaluation of parent's → Emasculation → Bagging → Tagging → Pollination → Harvesting and storage of F1 seeds

The goal is to artificially produce a variable population for the purpose of selection types with the desired combination of characters, combining the desired characters into a single individual, and exploiting and using hybrid variations (Nayak *et al*., 2020) [15]. The major problems in hybridization are high heterozygosity, long juvenile phase, lack of information related to inheritance pattern of traits, cross and self-incompatibility and requirement of large area for seedling evaluation. Some of the important fruit varieties developed through conventional breeding approaches have been listed in Table 1.

Table 1: Important varieties developed through conventional breeding approaches

Fruit crop	Variety	Methods	Important trait
Mango	Pusa Surya	Introduction	Attractive apricot yellow peel colour
	Chausa	Selection	Highly sweet in test
	Amrapali	Hybrid (Dashehari x Neelum)	Regular bearing, late-maturing cultivar, dwarfing and rich in vitamin A.
	Pusa Arunima	Hybrid (Amrapali x Sensation)	Suitable for both domestic and international markets.
Papaya	CO-1	Selection	Dwarfing, orange flesh colour
	Arka Surya	Hybrid (Sunrise Solo x Pink Fleshed Sweet)	The pulp is deep pink and firm with 13-14°Brix TSS and good keeping quality.
Guava	Beaumont	Introduction	Prone to stylar end rot
	L-49	Selection	Fruit slightly acidic flavour, attractive aroma, with many seeds, and good keeping quality.
	Arka Amulya	Hybrid (Allahabad Safeda x Triploid).	Good keeping quality and white pulp
	Arka Kiran	Hybrid (Kamsari X Purple Local)	Pulp is deep pink, high lycopene content
Citrus	Pramalini, Vikram (Lime)	Selection	Cluster bearing
	Rasraj (Lime)	Hybrid (Kagzi Lime x Nepali Round Lemon)	Good quality, juicy
Banana	Lady Finger	Introduction	Resistant to bunchy top virus
	Udhayam	Selection	Field tolerance to wilt and nematodes
	FHIA- 01 (AAAB)	Hybrid (Prata Ana × SH-3142)	Resistance to black sigatoka, races 1 and 4 of *Fusarium* wilt and nematode (*Radopholus similis*)
	SH-3640	Hybrid (Prata Ana × SH-3393)	Resistance to black Sigatoka, partial resistance to yellow Sigatoka and ripe fruits present very good taste
Apple	Lal Ambri	Hybrid (Red Delicious x Ambri)	Yield and fruit quality
	Pusa Amartara Pride	Hybrid (Royal Delicious X Prima)	Resistant to powdery mildew and apple scab
	Northern Spy	Selection	Resistant to woolly apple aphid
	Golden Delicious	Selection	
Litchi	Early Bedana, Dehra	Selection	Early maturity

	Rose		
	Swarna Roopa	Selection	Highly resistant to cracking
	Sabour Madhu	Hybrid (Purbi x Bedana)	Higher number of fruit and high pulp content
	Sabour Priya	Hybrid (Purbi x Bedana)	Better fruit quality
Ber	Thar Bhubhraj	Selection	Early maturing
	Thar Sevika	Hybrid (Seb x Katha)	Resistant to fruit fly, superior fruit quality and early maturity
Pomegranate	Ganesh	Selection	Arils bold, pink and soft seeded
	Mridula	Hybrid (Ganesh X Gul-e- Shah Red)	Suitable for processing
Sapota	Cricket Ball	Selection	Largest sized fruits which are round in shape
	CO-1	Hybrid (Cricket Ball x Oval)	Oval shaped fruit

Polyploidy: Polyploidy is a phenomenon caused by meiotic abnormalities in the production of spindle fibres. There are two types of polyploidy: euploidy and aneuploidy. It can happen naturally or be created through artificial (Sahoo and Kaluram, 2019). Various characteristics of polyploid plants include larger leaves, thicker and stouter stems, delayed precocity, disease resistance, larger fruit size, and more instance leaf and fruit colour. It will aid in the development of fruit varieties that are biotic and abiotic resistance, seedlessness etc. By avoiding some of the problems associated with traditional sexual hybridization, such as sexual incompatibility, nucellar embryogenesis, and male or female sterility, this strategy can help with conventional breeding, gene transfer, and cultivar development (Grosser and Gmitter 1990) [5]. Autopolyploid play a significant role in fruit production, particularly seedless fruits such as watermelon, guava, and grape, which are produced by triploid. Multiplication of a single genome (autopolyploid example- Banana) or a mixture of two or more divergent genomes produces polyploidy (allopolyploid example- Mango, kiwi). Induced chromosomal modification is referred to as polyploidy breeding (Chen and Ni, 2006) [2].

Mutation: Mutations are heritable changes in an organism's genetic material and, its characteristics that are not acquired by genetic segregation or recombination (Harten, 1998) [20]. The history of plant mutation may be traced back to 300 BC, when accounts of mutant crops in China were first reported (Kharkwal *et al*., 2012) [11]. Spontaneous mutation happens with a frequency of 10^{-6}. Bud sports are more common in fruit crops, where spontaneous bud mutation is more widespread because there are so many natural bud sports in citrus, grapes, and other fruits (Lamo *et al*., 2017) [12]. The highest mutation rates seen in doses that cause 25 to 50 per cent lethality in mutant plants. Fruit crop improvement is limited by perennial nature, long juvenile period, heterozygosity, sexual incompatibilities, and other factors. Mutagens are the agents that cause mutations. In terms of the time of occurrence and the gene in which they occur, spontaneously occurring mutations are extremely rare and random events. The initial step in plant breeding is to find suitable genotypes with the desired genes among existing genotypes, or to develop one if none exist. Mutations are the primary cause of variety in nature, and plant breeding would be impossible without them. In this context, the primary goal of mutation-based breeding is to create and improve well-adapted plant varieties by changing one or two major features to boost production or quality (Oladosu *et al*., 2015). Mutagenesis is the process by which chemical, physical, or biological agents create heritable changes in an organism's genetic information that are not caused through genetic segregation or genetic recombination. In the case of fruits, the mainly emphasized are tree architecture, precocious, yield, resistance to biotic and abiotic stress, and physiological disorders; nevertheless, fruit quality has received very little attention. Although better cultivars with essential features like as biotic and abiotic stress tolerance have been generated through breeding programmes. Some of the important fruit varieties developed through mutation and polyploidy breeding approach have been listed in Table 2.

Table 2: Important varieties developed through mutation and polyploidy breeding methods in fruit crops

Fruit crop	Variety	Method	Important trait
Papaya	Pusa Nanha	Mutation (Gamma)	Dwarfing
Apple	Golden Haidegg	Mutation (Gamma)	Fruit size
	Blackjoin BA 2 520	Mutation (Gamma)	Fruit colour
Grapefruit	Star Ruby	Mutation	Seedless
	Rio Red	Mutation	Fruit colour
	Melogold and Oroblanco	Polyploidy	Seedless
Orange	Eureka 22 INTA	Mutation (X-rays)	Fruit set, quality
Pummelo	Pusa Seedless Pummelo-1	Mutation	Seedless
Sweet Cherry	Lapins	Mutation (X-rays)	Larger size, firmness
Banana	FATOM 1	Mutation	Early flowering
Grape	Perle	Polyploidy	Cold hardy
Guava	Pusa Srijan	Polyploidy	Dwarfing
Pineapple	Cabezona	Polyploidy	Larger seedless fruit
Ber	Gola	Polyploidy	Early maturing

Biotechnological method: The biotechnological revolution is based upon novel genetic strategies obtain from molecular techniques including, protoplast fusion, *in-vitro* propagation, embryo rescue, recombinant DNA technology and marker-assisted selection. By boosting selection effectiveness with molecular markers and transgenic technology, which allows individual genes from multiple sources to be inserted without disrupting unique genetic combinations, advances in molecular genetics may overcome some of the constraints of traditional fruit breeding.

Molecular approaches: Some of the problems with fruit tree breeding have been solved using molecular approaches. The ability to predict colour, shelf-life behaviour, and taste, texture, and nutrition qualities by detecting marker genes before the tree bears fruit would be extremely useful. Germplasm preservation and evaluation, which is an important aspect of traditional breeding, costs more in terms of labour, money, time and other resources (Myles, 2013) [14]. Furthermore, advanced genetic transformation technologies have been shown to shorten the juvenile phases of trees, improve biotic stress resistance, and phytoremediation in perennial trees (Pena and Seguin, 2001) [16].

Plant tissue culture: Plant tissue culture refers to the growing of seeds, plants, and plant parts (organs, tissues, single cells, protoplasts and embryos) *in-vitro* on nutritional media under aseptic conditions. Induction of callus and micropropagation, plant regeneration, somatic embryogenesis, meristem culture, somaclonal variation, embryo culture, anther culture, protoplast culture, secondary metabolite production and cryopreservation are only a few of the specialised fields covered. Micropropagation, embryo, somaclonal variation, and protoplast and anther cultures are among those with direct crop enhancement applications (Gosal *et al*., 2010).

Genome editing: Genome editing means site-directed insertion/deletion or replacement at a specific locus in an organism's genome (Zhang *et al*., 2017) [22]. Mainly three types of genome editing tools are available like ZFNs (Zinc Finger Nucleases), TALENs (Transcription Activator-like Effector Nucleases) and CRISPR/Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats) (Tiwari *et al*., 2020) [10]. These engineered endonucleases have enabled genome editing in various biological systems (Xu *et al*., 2019) [21]. In some highly heterozygous and clonally propagated horticultural species, including as banana and potato, removing foreign DNA fragments (transferred T-DNAs) to obtain transgene-free edited plants remains problematic. One option is to make a large number of transformants and then screen them for transgene-free mutants in a high-throughput manner (Chen *et al*., 2018) [3].

Transgenic approach: Transgenic or genetically engineered crops involve bringing of beneficial traits, especially for the development of elite varieties with increased fruit quality. The genetic transformation in woody perennials trees has been studied by Pena and Seguin (2001) [16]. Fruit quality gene was efficiently conveyed by protoplast transformation with recovery of transgenic plants in citrus, in addition to Agrobacterium-mediated transformation (Guo *et al*., 2005). Flavr Savr tomato, the first genetically modified fruit product, was legalised in 1992. In transgenic tomato, a gene that causes pectin solubilisation was downregulated, resulting in delayed fruit softening and a longer shelf-life. Several other fruit crops with genetically modified features have acquired regulatory permission for commercialization in various regions of the world. Papaya, apple, plum, and pineapple are among them. The majority of transgenic fruits were created to boost agronomic production by giving pest or disease resistance as well as a delayed ripening (Lobato-Gomez *et al*., 2021) [13]. Some of the important fruit varieties developed through modern breeding approaches have been listed in Table 3.

Table 3: Important varieties developed through modern breeding approaches in fruit crops

Fruit crop	Variety	Method	Important trait
Grape	Pusa Purple Seedless	Embryo rescue	Extra early maturing
Papaya	Rainbow and Sunup	Transgenic approach	Resistant to papaya ring spot virus
Apple	Arctic apple	RNAi approach	Resistant to browning
Pineapple	Pinkglow	RNAi technology	Distinct pink colour, accumulation of lycopene at levels of up to 200 mg/g. Flowering senescence trait that prevents the undesirable early flowering of pineapple plants.
Citrus	Flhorag1	Somatic hybrid	Highly tolerant to iron deficiency
Peach	Somaclones S156 and S122	Somaclonal variation	Resistant to leaf spot, moderately resistant to canker.

Conclusion
Fruit crops, unlike short-lived and seasonal crops, are confined by a variety of constraints so, required integrated approaches using conventional and modern approaches for breeding varieties with desirable attributes. Biotechnological techniques give accuracy, consistency, and are thought to reduce the breeding cycle in long juvenility crops. When dealing with cumbersome crops, the efficacy of approaches such as marker assisted selection, genomics, candidate gene, transgenics has been demonstrated to be beneficial.

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