Breeding approaches for quality improvement in fruit crops: strategies and achievements

Abstract

As we know that fruit plays important role in the daily human diet for healthy living and is also a commercial commodity in trade and processing industries. The primary factor that customers use to determine whether or not a fruit is acceptable is its quality like appearance, shape, size, colour and taste, etc. Success in a breeding program depends upon the overall acceptability of fruit quality because most of the developed varieties having desired traits like resistance to biotic and abiotic stresses could not be commercialized and are not in commercial cultivation owing to their poor-quality traits. Therefore, the development of cultivars with desirable quality attributes in fruit crops is challenging. Quality improvement in fruit crops is restricted by several factors such as long juvenility, tall stature, environmental stress and high heterozygosity. Quality traits in fruit crops are polygenic and governed by many genes which makesit difficult to improve particular desirable traits. Many attempts have been made to enhance the qualitative characteristics of annual crops, although perennial fruit crops neatly overlook this issue. Accordingly, the use of both combined conventional and modern breeding techniques could in overcoming these problems. Biotechnological and molecular approaches like marker-assisted selection, transgenics, genomic editing, genomics cis-genics and candidate gene offer precision and reliability to reduce the breeding cycle and are also advantageous when dealing with tedious fruit crops. The challenges with fruit breeding and the state of various breeding techniques for enhancing fruit quality in fruit trees will be the main topics of this review.

Keywords: MAS, Fruit quality, Resistance, Genomics, Shelf life

Introduction

“Fruit breeding programs have a wide range of specific aimsregarding abiotic and biotic stress resistance, tree architecture, precocity, and productivity, with a common objective to develop high-quality fruit” (Kader, 2002).Fruit quality has a different meaning for different fruit species consisting of diverse attributes (Callahan, M. A. 2003). “For instance, in some species, the crisp texture is much more acceptable than the soft vice versa.Few fruits require a balance of acidity and sweetness for taste,whereas,for others, it is simply defined by the degree of sweetness” (Kader, 2002). “However, all commercial cultivar releases must have deliciouspalatable fruits. It does not matter whether thetree is a disease or insect resistant or highly productive,but if the quality of the fruit is not acceptable, it would be failed in the commercial market” (Ray, P.K. 2002).

“It is difficult to define the accurate definition of fruit quality as it varies according to the taste or requirement of the consumer, so the simple definition of fruit quality is: Whatever the consumer desires” (Barritt, 2001; Elia, 2001; Kupferman, 2002). “Since the nature of people is different, their desires and ideas of quality are different hence breeders need to provide numerous alternativeforms to meet the market desire. Quality may also refer to aspects like colour, size, nutrients, shelf life, suitability for processing, texture, taste, and sweetness" (Kader, 2002). "However, breeding for quality improvement in fruit crops as they are perennial in nature is hampered by several limitations including the large size of the plant, long juvenile phase, and environmental problems (e.g., fruit drops due to natural calamities). Besides, fruit quality is a polygenic trait, which is quantitatively inherited and thus making the breeding program complicated in the quality improvement of fruit crops. Any property of an individual showing heritable variation is known as a character or trait. A trait that defines some aspect ofproduce quality is quality traits. Each crop has a specific and often somewhat completely different set of quality traits. Fruits play a significant role in the nutritiousdiet (Wargovich, 2000) as it has the potential to provide all the essential elements or compoundswhich are generally deficient in agronomical crops such asfibre, vitamins, minerals, proteins, fats, and carbohydrates which are perfect for curing nutritional disorders".

Bottlenecks in fruit trees breeding and quality improvements

"To date, continuous attempts have been made to improve the quality traits of staple crops, whereas very fewer efforts have been made tothe improvement of fruit trees as compared to agronomical crops. In the case of fruits, breeding objectives are mainly focused on tree architecture, precociousness, yield, resistance against biotic, abiotic stresses, and physiological disorders (Ray, P.K. 2002); however, very little attention has been paid to maintaining the quality of fruit".There are many constraints in the breeding of fruit crops such as consumers usually prefer the local or indigenous varieties as compared to improved varieties thereby makingthe breeding program a failure. For example, in the case of mango, many improved selections and hybrids have been developed, which are regular and heavy bearers and are also free from the problem of spongy tissue, however, local farmers tend to prefer Alphonso mango, though it is the shy and alternate bearer, and suffers from the spongy tissue.

"The major constraint in breeding is the large size and perennial behaviourof the fruit trees which makes the breeding program difficult especially while carrying out important operations such as emasculation, bagging, tagging, data recording, harvesting, etc. The majority of fruit trees are propagated through the asexual mean of reproduction, which bears flowers and fruit normally after 5 years of age (Arias et al., 2012), whereas those derived from seedlings take more than 10 years, thereby prolonging the breeding cycle". "Breeders have to wait for a long time to get the result. In addition, after the long wait, still, the chance of getting undesired results is there and which forced the breeder to be least interested. For example, an apple-breeding program that was undertaken by Dresden-Pillnitz, Germany screened 52,000 seedlings for 26 years and eventually, only three varieties were released at the commercial level" (Myles, 2013). "Knowledge of genetics is very meagre in the case of tropical fruits as compared to temperate fruits" (Arias et al., 2012). "Breeding fruit trees are even made complicated by reproductive biology (diurnal flowering in avocado), polyembryony (zygotic and nucellar seedlings in mango), sexual types (dioeciousness in papaya) and apomixes (obligate apomixes in garcinia)" (Arias et al., 2012). In addition to this, environmental problems and natural calamities often result in huge commercial and breeding losses due to flower drops, fruit drops, pest and disease infestations etc.

"Fruit quality improvement needs strong genetic knowledge about its inheritance and variation. Quality characters are inherited quantitatively and regulated by multiple genes.Fruit trees are heterozygous crops, and a mass population is required for screening to identify the promising genotypes for the breeding of quality fruits" (Keniset al., 2008). "The biotechnological approaches provide a precise, reliable, and easy way for addressing some of the problems encountered during conventional breeding. Molecular approaches like marker-assisted selection, candidate genes, genomics (Murovec and Bohanec, 2012), transgenic, and cis-genicshave shown to be advantageous in terms of time, effort and patience required while dealing with the cumbersome crops".There is a negative correlation between yield and quality of fruits. When quality is enhanced by breeding by applying breeding methods, it affects the total yield of crop plants for instance, if quality is increases yield decreases and vice-versa.

Source of fruit quality traits

1. A cultivated variety: most preferred source.
2. A germplasm lines.
3. A spontaneous or induced mutant.
4. A wild relative.
5. A transgene.

Fruit quality traits

Appearance

"The characteristics that affect appearance are primarily size and colour. During consumer surveys on peaches and apples, it was found that the bigger size is more demanding with bright and clear colour and consumers are willing to pay enough more to have it" (Kupferman, 2002).

1. Size and shape

"Fruit size has a large genetic component, thus selecting for larger fruit is relatively straightforward. Fruit size is a function of cell number, cell volume, and cell density" (Janick and Moore, 1996).

2. Colour

"This trait is an important aspect of appearance. The overall colour of fruit is reflected by the colour of the outer pericarp and the flesh colour. Pigments responsible for the colours are various modifications of anthocyanins, lycopene, and carotenoids. Predictingcolours is difficult because small modifications or combinations of pigments result in unpredictable colours. Due to that fruit depicts different shades of colour. The de-greening process during ripening exposes the colours in both the pericarp and the flesh" (Winkel-Shirley, 2001). "The de-greening process is the breakdown of chlorophylls, which is usually done by ethylene. The other pigments are no longer masked by chlorophyll and the fruit 'colours. One of the potential problems of some modern cultivars is that brightly coloured blush in the pericarp has been selected that appears before ripening" (Janick and Moore, 1996). This in itself masks the chlorophylls thereby negating the de-greening as an indication of ripeness. For example,the orange colour of mango is due to Beta-carotene and the red and purple colour of Grapes, Pomegranate, Blackberries and Blueberriesaredue to Anthocyanin.

Table 1: Pigments responsible for fruit colouration

Colour	Pigments	Example

Orange	Beta-carotene	Mango, Pineapple
Red-purple	Anthocyanins	Grape, Pomegranate, raspberry
Orange	Caricaxanthin	Papaya
Red	Lycopene	Papaya, Guava var.Arka Kiran
Orange-yellow	Flavonoids	Peach, Papaya, Orange, Tangerine
Yellow-green	Lutein & Zeaxanthin	Avocado
Green	Chlorophyll	Guava
Yellow	Xanthophylls	Guava

Source: Singh, J. 2002; Ray, P.K. 2002

Taste

"The most important aspect of fruit quality is taste. The fruit may be the most desirable looking, but if it doesn't taste good the consumer will not buy it again. Consumer preference is for higher sweetness, more intense flavors, and firm fruit that soften before consumption" (Kader, 2002).

1. **Sweetness**

"Major fruit's TSS ranges from 9–20° Brix (Refractometry measure of soluble solids) when ripe. Brix is highly correlated with the amount of sugar contained in the juice. The levels of sucrose, fructose and glucose are what determines sweetness; however, the level of acidity affects the perception of sweetness such that fruit with high sugar and moderate levels of acid will be perceived to be as sweet as fruit with moderate levels of sugar and low acid"(Janick and Moore, 1996). "The acid levels are primarily based on the concentration of malic or citric acid. Generally, the acid present in fruits is malic acid, tartaric acid, etc. For example, new cultivar development in peach has concentrated on high sugar with low acid to fill a niche in the Asian market" (Baldwin, 2002). The fact that sugar accumulation occurs before final ripening makes it easier to harvest at a time with high sugar.

2. **Flavour and aroma**

"Flavour and aroma are determined by a combination of volatiles. There are three main pathways for volatile production; cleavage of lipids followed by alcohol dehydrogenase activity to yield short-chain aldehydes and alcohols, the shikimic acid pathway, and the degradation of terpenoids. Interestingly the colour pigments are also derived from these pathways, anthocyanins from the shikimic acid pathway and β-carotene and lycopene from the degradation of terpenoids" (Baldwin, 2002). "As fruit ripens there are hundreds of volatiles detected, but only some above threshold levels that taste panels can detect. Of those, a few have been shown to determine the characteristic aroma/flavour of particular fruits. For example,p-hydroxyphenylbutan (raspberry), cinnamate derivatives (strawberry), cyanidin-3-rutinoside (litchi), decadienoate esters (pear), γ-decalactone, and linalool (peach), Benzaldehyde (Almond), 2-methyl butyrate (Apple) citral (orange), Isopentyl acetate (Banana),etc" (Kumar and Ellis, 2001).

Table 2: Aroma compoundsin fruits

Fruits	**Compound**
Apple-Ripe	Ethyle-2 methylebutyrate
Apple-Green	Hexanal, 2 Hexanal
Banana-Green	2 Hexanal
Banana –Ripe	Eugenol

Banana-overripe	Isopentanol
Grapefruit	Nootakatone
Grape	Methyle Anthranilate
Lemon	Citral
Orange	Valencene
Raspberry	1-(p-hydroxyphenyl)-3-butanone

Source: Singh, J. 2002; Ray, P.K. 2002

3. Texture

"The texture of the fruit flesh is based on how cells shear in the chewing process, the mouth feels. Texture ranges from crisp to melting and all the stages inbetween. In melting texture, swelling and softening of the cell wall are evident, but in crisp texture, cell wall swell is not observed during ripening. Three enzymes, polygalacturonase (PG), the β-subunit of PG, and pectin methylesterase (PME) have been associated with texture determination. Their substrate is the homogalacturonans or pectin located primarily in the middle lamella of cell walls"(Redgwell andFischer, 2002).

Keeping quality

Fruit can be harvested at various times concerning their peak quality and that time is dependent on the desired texture, the handling process, and the shelf-life of each commodity. Some fruits (non-climacteric such as blueberry,Grape, and strawberry) are harvested eaten ripe and then stored. Climacteric fruit such as Mango,peach, or apricot is harvested at earlier stages for the fruit to withstand the handling. This fruit will finish ripening during storage and transport. The rate at which the fruit ripens and softens determines when it must be harvested to withstand handling and arrive to the consumer either in the process of ripening or eating ripe. These aspects can be modified postharvest but there is also a large genetic component that can be taken into account in a breeding program.

1. **Softening**

"Softening is attributed to the disruption of the cellulose/xyloglucan cell. Numerous enzymes have been postulated to be involved including β-galactosidase, expansin (EXP), pectate lyase (PEL), endo-(2-4) β-D-glucanase (EGase), and xyloglucan endotransglycosylase (XET)" (Brummell and Harpster, 2001). "For example: pectate lyase in strawberries resulted in significantly firmer fruitimplying they do have a role in softening" (Smithet al., 2002). Polyuronides are depolymerized to a very small size during ripening in avocado.Matrix glycans become highly depolymerized in strawberries but not in avocado.

2. **Control of ripening**.

"The expression of quality traits normally is co-ordinately regulated and peaks at ripening. Breeders have been selected for early expression of some of these traits such as skin blush, but the texture, softening flavour development, reduction of acid and phenolic compounds, and colour development peak at the ripe stage" (Seymour and Manning,2002). "Sugar accumulation takes place prior to the ripest stage. The problem with harvesting fruit at the peak of quality and ripeness is that the fruit at that stage has practically no shelf life. The fruit needs to be picked before peak ripe, at a stage that combines the maximum development of desirable traits and the maximum shelf life.For example: in climacteric fruit, the increase in the amount of ethylene synthesized triggers final ripening.Non-climacteric fruit does not increase ethylene with ripening" (Seymour and Manning, 2002).

Many of the genes involved in those ripening traits are under the control of ethylene. It is unclear whether or not low levels of ethylene in non-climacteric fruit isenough to induce those ripening-related genes or if there are other mechanisms to control ripening. Such as the discovery of a MADS-box transcription factor as the gene responsible for early ripening in non-climacteric strawberries (Vrebalovet al., 2002).

Nutritional Quality traits

“Fruitsaregoodsourcesoffiber,minerals, vitamins, and some beneficial phytochemicals such as carotenoids, phenolics, and glucosinolates.Fruits are a major source of both “macro” nutrientssuchasfiberandcarbohydrates,and“micro”nutrients such as Vitamin C, B complex (thiamin, riboflavin, B6, niacin, folate), A, E, minerals, and the lesser-studied polyphenolics, carotenoids, and glucosinolates. Nutrients may be classified as either water or lipid soluble—meaning they dissolve in water or a lipid medium” (Wargovich, 2000). “Water soluble nutrients include Vitamin C, B complex, polyphenolics, and glucosinolates. Fat-soluble nutrients include Vitamin A, D, E, K and other carotenoids such as lycopeneand β-carotene.VitaminCisoneofthemostsensitive vitamins,beingdegradedrelativelyquicklybyexposuretoheat, light, and oxygen(VitaminCisunstable)” (Oguntibejuet al., 2013). For this reason, it is often used as an index of the Nutrients Department of Health and Human Services and the degradation.

Table 3: Nutritive value of fruits

Nutrition	**Fruits/100gm**
Vitamin A (β-carotene)	Mango (4800IU) > Papaya (2020IU)
Vitamin B_1	Cashew nut (630mg)> Walnut (450mg)
Vitamin B_2	Bael (1191mg) > Papaya (250mg)
Vitamin C	Barbados cherry (1000-4000mg)>Aonla (600mg)>Guava(199mg)
Carbohydrate	Raisins (77.3%)>Dry Apricot (72.2%)
Protein	Cashew nut (21.2%)>Almond (20.8%)
Fat	Pecan nut (70.4%)>Walnut (64.5%)
Fibre	Fig >Guava (6.9%)
Calcium	Litchi (0.21%)
Phosphorus	Almond (0.49%)
Potassium	Banana
Iron	Dry Karonda (39.1%)
Calorific value	Walnut (687mg)>Almond (655mg)>cashew (596mg)

Source: Singh, J. 2002; Ray, P.K. 2002

Breeding methods for fruit quality

Breeding for fruit quality can require extended periods, particularly fortree fruits since fruit evaluation cannot be done until the tree is mature and fruiting and the progeny will be in the field for several years before the first evaluations can be done. Secondly, a balance must be achieved to produce beautiful fruit that has the desirable taste and adequate shelf life to get that fruit to the consumer still beautiful with desirable taste. This task requires the combination of multiple complex traits and precise evaluation.General steps involved in the breeding of fruit crops are depicted in fig.1.

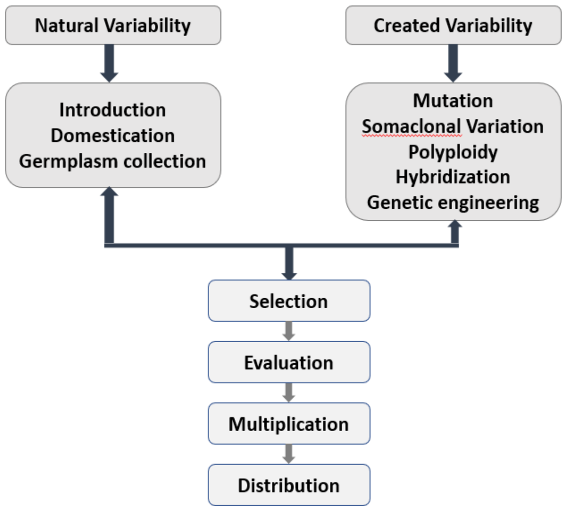

Fig.1 General steps of fruit breeding program.

Domestication:

"Domestication is the process of bringing wild species under human cultivation.The process of domestication started when man started superior plants for use" (Simmonds, 1979). Domestication of plants is the change of ideotype to adapt them better to manmade environments (Harlan 1971). It is the first step in the development of cultivated plants. Most of the crops were domesticated by prehistoric man under domestication and the crop species have changed considerably. Examples, Date palm, Olive, Grape, Almond, Fig, and Pomegranate.

Plant Introduction:

"Plant introduction consists of taking a genotype or a group of genotypes of plants into new environments where they were not being grown before. The introduction may involve new varieties of a crop already grown in the area, wild relatives of the crop species or a totally new crop species. Mostly materials are introduced from other countries or continents" (Singh et al., 2005). Butthe movement of crop varieties s from one environment into another within a country is alsoan introduction.

Table 4: Important introductions in different fruit crops

Fruit	**Cultivar**	**Country**
Mango	Tommy Atkins, Sensation, Haden-Coloured varieties	USA, Florida
	Sweet	Thailand
	Carabao- Regular bearer	Philippines

Banana	Lady finger (Resistant to Bunchy top)	Australia
	Grand Naine	France
Citrus	Torocco	USA
	Sunramon	Peru
	Kinnow	USA
	Grapefruit	California and Florida
Grape	Thompson Seedless, Perlette, Beauty Seedless	USA
	KIshmishBeli, KishmishChorni	USSR
Guava	Beaumont G-135	Australia
Pomegranate	Wonderful	USA
Apple	Red Spur, Oregon Spur	Italy
	Prima, Sir Prize, Jonafree Liberty, Priscilla- Scab resistant	USA
	Vance Delicious, Top Red, Royal Red	USA

Source: Ray, P.K. 2002.

Selection:

"Selection is basic to any crop improvement. Isolation of desirable plant types fromthe population is known as selection. It is one of the two fundamental steps of any breeding program viz., 1. creation of variation and 2 Selection. There are two agencies involvedin carrying out the selection: one is nature itself (natural selection) and the other is man(artificial selection). Though both may complement each other in some cases, they are mostlyopposite in direction since their aims are different under the two conditions (nature anddomestication). The effectiveness of selection primarily depends upon the degree to whichphenotype reflects the genotype" (Ray, P.K., 2002) It involves three basic principles: 1) it works on already existing germplasm, 2) it acts only through

heritable alterations and 3) it works by favouringsome individuals over others in reproduction.

Table 5: Some important varieties with improved fruit quality traits developed through selection

Fruit crop	Varieties	Method of breeding/Parents	Quality traits improved
Aonla	Kanchan (NA-4)	Selection	Suitable for processing
	Krishna (NA-5)	Selection	Suitable for processing
	Goma Aishwariya	Selection	Suitable for processing and export
Cherry	CITH-Cherry-2	Selection	Bold, attractive
Guava	Allahabad Safeda	Selection from Allahabad	White soft pulp, Sweet
	ArkaMridula	Selection From Allahabad Safeda	Soft seeded sweet, good pectin content, Keeping quality
	L-49	Selection from Allahabad Safead	Highest vitamin C
	Lalit	-do-	Red colour pulp
	Try (G)-1	Selection	Off season, drought, sodicity tolerance
Jackfruit	PLR (J)-2	Selection from	good quality, fetch more price

		Pathirakkotai Local	due to attractive characters and good keeping quality
Litchi	Rose Scented	Selection	Rose Scented, moderately juicy, soft and white flesh
Mandarin	Nagpur mandarin	Selection	Major position in mandarin markets, sweet, juicy and saffron coloured segments
Mango	Alphanso	Selection	Popular variety in domestic and export markets, yellow pleasant pulp, good keeping and processing quality
	Dashehari	Selection	Good keeping and table quality
	Pusa Surya	Selection from Eldon	Apricot yellow peel colour
	Langra	Selection	Turpentine flavour,
Papaya	CO-5	Selection	Cultivated mainly for papain production
	Coorg Honey Dew	Selection from Honey Dew	Gynodioecious
Sapota	Kirthabharti	Selection	Pulp is very sweet. Good for transportation to distant places
Walnut	CITH Walnut-1	Selection	Export purpose, bold nuts
Pomegranate	Ganesh	Selection from Alandi	Very soft seed,
	G-137	Selection from Ganesh	Soft seeded
Apple	Granny Smityh	Sel. From Lady Hamilton	
	Cameo	Sel. From Block of Red	--
Peach	Sharbati	Selection	Good flavour

Source: An individual selection and its traits taken from individual institute's website from where these varieties are developed and released and Department of Agriculture and Cooperation, 2012.

Table 6: Important selection in different fruits from pantnagar

Fruit crop	**Cultivar**	**Parents**
Mango	Pant Chandra	Seedling selection
	Pant Sindhuri	Clonal selection
Guava	Pant Prabhat	---
Papaya	Pant Papaya-1,2 & 3	----
Bael	Pant Urvashi, Pant sujata, Pant Aparna, Pant Shivani	---
Peach	Pant Peach-1	Seedling sel. From Sharbati
Pear	Pant Pear-3	---
Plum	Fla-12	Exotic type
	Pant Plum-1	---
Aonla	Pant Aonla-1	Seedling selection
Karonda	Pant Manohar, Pant Sudarshan, Pant Suvarna	Clonal selection
Jackfruit	Pant Mahima, Pant Garima	Clonal selection
Lemon	Pant Lemon-1	Clonal sel. from Kagazi Kalan

Source: Individual variety is taken from GBPUAT, Pantnagar Website (www.gbpuat.ac.in).

Hybridization

"Hybridization involves the crossing of desired parents and a further selection of progenies. These desired parents are generally obtained after an appropriate screening of the natural populations and crop wild relatives being conserved under in-situ and ex-situ conditions hence, germplasm conservation is the most important step, particularly for the utilization of wild species in breeding programs" (Sharma et al., 2015). "It helps in the selection of elite parents for the crossing and development of superior varieties. Conventional breeding involving approaches like hybridization, bridge crossing, distant hybridization, sibmating, half-sib mating, etc. had been advantageous in framing breeding strategies for various crops and perennial fruit crops. Also, an admirable approach like doubled haploids could overcome some of the breeding limitations and is extremely useful for genetic studies such as gene mapping and genomics" (Murovec and Bohanec, 2012). "However, suitable technology for the development of superior fruit quality has to be adopted. Conventional breeding has contributed tofruit quality improvement with quite a good number of fruit trees. Many improved varieties have evolved through inter-specific hybridization or polyploidization or a combination of both" (Jalikop, 2015).

Objectives:

- To create genetic variation.
- Transfer of one or few qualitative characters.
- To develop biotic and abiotic resistant varieties.

Types of hybridization

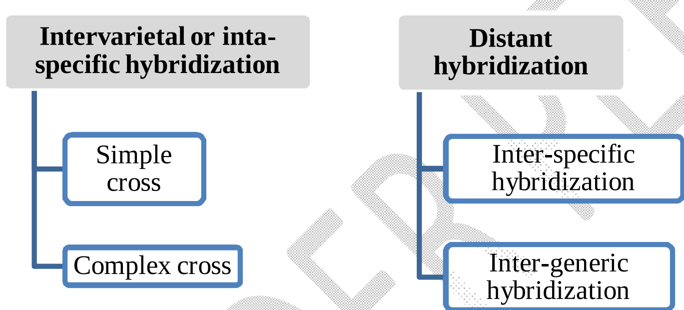

Table 7: Some important varieties with improved fruit quality traits developed through hybridization breeding approaches

Fruit crop	**Varieties**	**Method of breeding/Parents**	**Quality traits improved**
Mango	Amrapali	Dashehari x Neelum	Fibreless, excellent taste, high carotenoids
	ArkaAnmol	Alphonso x Janardhan Pasand	Attractive skin colour, free from spongy tissue, good keeping quality, good sugar/acid blend
	Sindhu	Ratna x Alphonso	Seedless
	Konkan Ruchi	Neelum x Alphonso	Suitable for pickle making
	Arka Puneet	Alphanso x Banganpalli	Free from spongy tissue

Papaya	Arka Surya	Sunrise Solo x Pink Flesh Sweet	Red pulp, sweet
	CO-4	CO-1 x Washington	Purple pigment Variety
Pomegranate	Phule Arakta	Ganesh x Gul-e-shah Red	Dark red aril
	Amlidana	Ganesh x Nana	Suitablr for anardana
	Mridula	Ganesh x Gulshah Red	Rose Pink, blood red aril, red rind, soft seeded
	Ruby	Ganesh x Kabul x Yercaud x Gulshah Rose Pink	The rind is pinkish yellow to reddish yellow. Fruit contains red and bold aril. It is soft seeded variety.
Lime	Rasraj	Kagzi Lime x Nepali Round	Good quality, juicy
Guava	ArkaAmulya	Allahabad Safeda x triploids	Round, firm, white fleshed, soft seeded, good keeping quality
Sapota	PKM-3	Guthi x Cricket Ball	Cluster Bearing habit
	Hisar Surkha	Apple Colour x BanarsiSurkha	Pink flesh
	ArkaKiran	Kamasari x purple local	Soft, yellow peel
Custard apple	ArkaSahan	*A. Atemoya x A. Squomosa*	Waxy skin, pleasant aroma
Apple	Ambred	Red Delicious x Ambri 157	Crisp, aromatic, juicy, shelf life
	Honeycrisp	MN447 x Northern Spy x unknown	Red peel, Rich flavor

Source: Individual hybrid is taken from their respective institutional website from where these varieties are developed and released

Table 8: Inter-generic hybrids of citrus

Crop	Parent	Name of hybrid
Citrange	*C.sinensis* x *P. trifoliata*	Troyer, Morton, Carrizo, Rusk
Citrumelo	*C.paradisii* x *P.trifoliata*	Swingle
Lemonimes	*C.limon* x *C.aurantifolia*	Parrine
Tangor	*C.reticulata* x *C.sinensis*	Temple, Clementine, Monreal
Tangerin	Robinson x Osceola	Sunbrust
Tangelo	*C.reticulata* x *C.paradisii*	Orlando, Sampson, Minneola

Source: Individual hybrid is taken from their respective institutional website from where these varieties are developed and released

Mutation breeding:

"Mutations are the heritable changes in the DNA sequence that are not derived from genetic segregation or recombination" (VanHarten 1998). "The occurring mutations might be spontaneous or induced. The majority of mutation studies were concentrated upon annual crops, mainly flowers and ornamental crops and special attention is required for attempting mutation in woody perennial crops. Induced mutations are highly effective in enhancing natural genetic resources, and have significantly assisted in developing improved fruit cultivars. Many commercial varieties have evolved mainly from spontaneous mutations and chance seedlings. Bud mutations are a valuable source of variation (Usman et al., 2001), which could result in variants having characteristics, including good fruit quality". "Spontaneous mutants are reported in citrus (Liu et al., 2007) and mango" (Medina 1997). For example, the variety Rosica has been reported in mango, which is the bud mutant of the Rosado-de–lca variety and has large and good quality fruits.

Mutagens

A. Physical mutagens

1. Ionizing radiations

(a) Particulate radiations- α-rays, fast neutrons, thermal neutrons.

(b) Non-Particulate radiations- X-rays, γ-rays.

2. non-ionizing radiations- UV rays.

B. Chemical mutagens.

1. Alkylating agents – Sulphur mustard, EMS (Ethyl Methyl Sulphonate), Ethylene Imine.

2. Acridine dyes – Acridine orange, ethidium bromide, acriflavine.

3. Base analogues – 5- bromouracil, 5-chlorouracil.

4. Others – Nitric acid, hydroxyl amine.

Steps in mutation

- Well defined objective of the program.
- Selection of a variety of treatments.
- Part of a plant treated- seed, pollen grain, bud, cutting.
- Dose of mutagen- LD50
- Treatment- method and treatment.

Table 9: Important varieties with improved fruit quality traits developed through Mutation Breeding

Fruit crops	Cultivar	Year	Mutagens	Improved fruit traits
Mango	Rosica	1966	Spontaneous mutation	Large and good quality
Apple	Golden Haidegg	1986	γ-rays	Fruit size
	McIntosh 8F-2-32	1970	γ-rays	Skin colour
	Senbatsu-Fuji-2-Kei	1985	γ-rays	Fruit colour
Grapefruit	Rio Red	1984	thN	Fruit colour
	Star Ruby	1970	thN	Seedless
Indian jujube	Mahong	1986	thN	Round, pink rose sweeter taste
Loquat	Shiro-mogi	1981	γ-rays	Fruit size
Orange	Xuegan 9-12-1	1983	γ-rays	Seedless

	Hongju 420	1986	γ-rays	Seedless
	Eureka 22	1987	X-rays	Fruit set, fruit quality
	Valencia 2	1987	X-rays	Fruit quality
Peach	Magnif	1968	γ-rays	Large, red skin
	Plovdiv	1981	γ-rays	Large, fruit quality
Sweet cherry	Lapins	1983	X-rays	Larger size, firmer
	Compact Lambert	1964	X-rays	Compact growth
	Ferrovia spur	1992	X-rays	Dwarfness
Pear	Fuxiangyanghongdli	1983	γ-rays	Eating, cooking quality
Almond	Supernova	1987	γ-rays	Late maturity
Fig	Bol	1979	γ-rays	Not specified
Banana	Novaria	1993	γ-rays	Earliness
	Al-beely	2007	γ-rays	High yield
Japanese pear	Gold Nijisseiki	1993	γ-rays	Disease resistance
Papaya	PusaNanha	1986	γ-rays	Dwarfness
Plum	Spurdente-Ferco	1988	γ-rays	Earliness
Pomegranate	Karabakh	1979	γ-rays	--
Sea buckthorn	zyriank			

Source:(Predieri, 2001)

Table 10: Other important varieties developed through mutation breeding

Fruit crop	Variety	Parents	Nature of mutation
Mango	Rosica	Rosa-do-delca (Peru)	Natural
	Davis Haden	Haden	Natural
Grape	Marvel Seedless	Delight	Induced
Banana	High Gate	Gros Michel	Natural
	Motta Poovan	Poovan	Natural
	Krishna Vazahi	Virupakshi	Natural
Orange	Washington Novel	Navel Orange	Natural
Grape fruit	Foster	Walter	Natural
	Red Blush	Thompson	Natural
	Thompson	Marsh Seedless	Natural
	Star Ruby	Hudson	Induced

Source: Ray, P.K. 2002

Polyploidy breeding

“Polyploidy refers to the presence of more than two complete sets of chromosomes per cell nucleus, which has been considered a ubiquitous phenomenon in plant evolution and diversification” (Ray, P.K. 2002). “A polyploid individual arising within one or between populations of a single species is denominated autopolyploid, while the term allopolyploid refers to individuals of hybrid origin. Allopolyploids are often divided into two sub-classes: true and segmental allopolyploids. The formation of true allopolyploids involves hybridization between distantly related species. In this case, the divergent chromosome complements do not pair with each other, resulting in the formation of bivalents during meiosis and a disomic inheritance pattern. On the other hand, segmental allopolyploids originate from hybridization between closely related species with partially differentiated genomes" (Shukla and Shukla, 2004). Therefore, segmental allopolyploids may undergo univalent, bivalent and/or multivalent pairingduring meiosis, being considered intermediate types between true allopolyploids and auto-polyploids. Now day Colchicine @ 0.2-0.8% is mostly used for the induction of polyploids.

Morphological and cytological aspects of polyploids

1. Slow growth and delayed flowering.
2. Increased flesh weight, but reduced dry weight.
3. Increase in size but the decrease in the number of leaves, flowers, and fruits.
4. Larger cell size.
5. Stomatal gourd cell larger (stomata count/unit area lesser).
6. Variation in ploidy from species to species.

Application of polyploidy

- Quality improvement: e.g.,Seedlessness
- Direct use as new var. or species.
- Inter-specific gene transfer.
- Widening genetic base of existing allopolyploids.
- Tracking the origin of natural allopolyploids.

Table 11: Varieties developed through polyploidy breeding in fruit crops

Ploidy level	**Autopolyploidy type**	**Crop**
3x	Auto triploid	Banana, Apple, Tahiti lime
4x	Auto tetraploid	Aonla, beal, Litchi, Phalsa, Jackfruit, grapes, Ber (cv. Umran)
6x	Auto hexaploid	Kiwifruit, Persimmon
8x	Auto octaploid	Ber (cv. Gola, Illaichi)
	Allo-polyploidy types	**Crop**
	Amphidiploids/Allo-tetraploid	Mango
	Allo-hexaploid	European plum
	Allo-octaploid	Strawberry
Aneuploidy		
	Aneuploid-82	PusaSrijan (Guava dwarf rootstock)

Source: Ray. P.K. 2002.

Table 12: Ploidy level of varieties and their origin in different fruit crops

Fruit crop	**Common interest**	**Polyploidy level**	**Origin**	**Reference**
Banana	Edible fruit	Autopolyploidy/ allopolyploidy	Synthetic/natural	Silvaet al.,(2001)
Grape	Edible fruit	Autopolyploidy/ allopolyploidy	Synthetic/natural	Motosugiet al.,(2002)

Apple	Edible fruit	Autopolyploidy/allopolyploidy	Synthetic/natural	Janick and Moore, 1996
Strawberry	Edible fruit	Allopolyploidy	Natural	Whitaker(2011)
Kiwifruit	Edible fruit	Autopolyploidy	Natural	Hopping(1994)
Tahiti Lime	Edible fruit	Allopolyploid	Natural	Morton(1987)
Plum	Edible fruit	Allopolyploid	Natural	BennettandLeitch(1995)

Source: Ray. P.K. 2002.

Somatic hybridization

“Somatic hybridization involves the fusion of partial and complete genome exchange, which might result in the development of novel varieties” (Loredana et al., 2012). “That means a combination of the nuclear, chloroplast and mitochondrial genomes in a novel arrangement (Singh and Rajam 2009) might aid in the development of novel varieties with desirable fruit quality traits”. “Since the transgenic approaches are limited by social barriers, these approaches might be useful to surpass this limitation in the development of new cultivars particularly having improved polygenic fruit quality.Somatic hybridization has widely been attempted for developing inter-specific/inter-generic crosses in the Citrus group, to overcome problems like sexual incompatibility, polyembryony and pollen or ovule sterility” (Singh and Rajam 2009). Loredana et al., (2012) developed “a hybrid and two cybrids by protoplast fusion of sweet orange (Citrus sinensis L. Osbeck) and lemon (*C. limon* L. Burm.). These cybrids exhibited improved essential oil composition, responsible for imparting aroma in citrus”.

Molecular Approaches

“The work related to molecular biology in the case of perennial crops is very scarce as compared to annuals” (Pena and Seguin 2001). “Molecular approaches have proved to overcome some of the breeding problems in fruit trees. Prediction of colour, taste, shelf-lifebehaviour, texture, and nutrition characteristics by detection of marker genes before the tree even starts to bear fruit would be of much practical utility. Germplasm maintenance and evaluation, an integral part of conventional breeding, is more expensive in terms of time, labour, money and other inputs” (Myles, 2013). “The approaches of genomics and marker-assisted selection (MAS) are more advantageous, particularly in woody perennials, including fruit crops” (Kumar et al.,2006). “Besides, advanced technologies in genetic transformation have been proven to shorten the juvenile phases of the tree, resistance to biotic stresses, and phytoremediation in perennial trees” (Pena and Seguin 2001). Some of the major molecular based technologies and significant attempts made for fruit quality improvement are discussed hereunder.

Marker assisted selection and QTLs (Quantitative traits loci)

The term QTL was first coined by Gelderman(1975). “The markers tightly linked with the trait of interest are very informative in utilizing them in the identification and further selection process. Molecular markers have been in vogue for identifying the trait of interest, SSR and SNPs being the most preferred. QTLs provide this information with manifold applications in breeding for complex fruit quality traits.The close linkage with the other quality parameters is advantageous when both are of superior and acceptable traits by breeders. However, if one is not acceptable in hybrids then it becomes difficult to segregate them apart in the progeny. To know this, series of crosses between cultivars with superior and poor traits are made and their population information provides QTL, which could be used as markers in MAS. Such analyses for fruit texture were conducted by Longhiet al., (2012), wherein the located QTLs by SSR and SNP markers. The most common method of QTL is interval mapping”. For examples:

1. Costa et al., (2005) in their study, utilized gene-specific molecular markers (ACS genes) for studying their effects on ethylene production and shelf life in apple and could position them on a

linkage map. They reported the marker for the identification of apple cultivars having a good shelf life.

2. Huan et al., (2012) developed SSR and AFLP markers linked to major gene loci involved in the fruit shape index of apples (*Malus domestica*).It was also reported that the fruit shape is controlled by five genes.
3. Davey et al., (2006) identified QTLs related to ascorbic acid in fruit skin and flesh in apple.

Candidate genes approach and source to sink interaction in fruits

According to the concept that a molecular polymorphism is directly connected to phenotype, the candidate gene technique includes breeding in reverse, going from the gene to the trait of interest (Pflieger et al., 2001). The candidate gene approach for understanding the signalling mechanism and biosynthetic pathway might be a useful approach because fruit quality is directly proportional to the metabolites present in it. Flavonoids, antioxidants, phenolics, active ingredients, pigments, etc. give the goodflavour, taste and colourto the fruits. Secondary metabolites are produced using particular or common metabolic pathways. Enzymes that are connected to these pathways are encoded by structural and regulatory genes. Source-to-sink relation is very complicatedfor improving the traits (Nookarajuet al., 2010). Biosynthetic pathways of pigments have been extensively studied in fruit crops like apple (Honda et al., 2002), peach(Li et al., 2012), sweet cherry(Wei et al., 2015), litchi (Zan-weiet al., 2011), mangosteen (Palapolet al., 2009) and citrus (Kato et al., 2004). For examples:

1. Anthocyanin-related gene expressions in fruit pericarp of many cultivars of litchi (Zan-weiet al., 2011).
2. The integrated approach of metabolomics and transcriptomics related to mango fruit peel colouration.
3. Candidate genes involved in sugar and organic acid metabolism in apple and 12 candidate genes were allocated to 4 linkage groups of the peach genome (Etienne et al., 2002).
4. Candidate genes are involved in taste development in the citrusfruit (Kato et al., 2004).
5. Candidate genes are responsible for the texture, ethylene production Costa et al., (2005), sugar and organic acid content (Etienne et al., 2002) and polyphenols (Chagneet al., 2012) in apple have been studied.

Genomics approach:

Genomics includes study of whole genetic information related to particular fruit crop. The term genomics werecoined by Dr.T. Roderick. It can be used forselection of better parents and for hybrid development. Genomic selection (GS) is a type of marker-assisted selection in which genetic markers available on the whole genome expresses its phenotypic variation Myles, (2013). The application of genomics and its function in battlingfew of the breeding difficulties has also been studied in apple and grapes by Myles (2013). The Apple Breed Data Base offers easy admittance for the documentation of molecular markers linked to fruit quality traits (e.g., skin colour, shape, or taste) (Antofieet al., 2007). Genomic analysis relatid to produce quality at developmental stages has been assessed in fruit trees like bayberry, citrus, apple and peach (Zhu et al., 2013; Cer-cos et al., 2006; Terolet al., 2008;).

Transgenic approach

Transgenic or genetically alteredfruit crops involve introduction ofdesired genes (Dias, J.S. and Ortiz, R. 2014)principally for the development of elite varieties with improved quality traits. *Agro-*

bacterium-mediated transformation, fruit quality gene was successfully transferred through protoplast transformation with the retrieval of transgenic plants in citrus (Guo et al., 2005).The first transgenic plant was developed is Tobacco in 1983.

Table 13: Transgenic varieties in fruit crops

Fruit crop	**Variety**	**Trait**	**Year**	**References**
Papaya	Rainbow, Sunup	Rainbow, Sunup Resistant to papaya ring spot virus	1998	Gonsalves et al., (1998)
Grapes	Pinot Noir			Franks et al., (1998)
Banana	DH-Pahang	Hepatitis B	-	Kumar et al., (2006)
Plum	Honey Sweet	Resistant to Plum Pox Virus (PPV)	2009	Scorzaet al., (1994): Richard, H. (2014)
Apple	Arctic Apple	Resistant to browning	2015	Murata et al., (2001)
	Artic golden delicious	Resistant to browning		
	Artic granny smith	Resistant to browning		
Strawberry	Apel	fruit firmness	-	Jimenez-Bermudez et al., (2002)

Table 14: Transgenic fruit crops developed by *Agro-bacterium* mediated transformation

Species	**Traits**	**Plasmid**	**Transgenes**	**References**
Orange				
Citrus sinensis	Method optimization	*pGA482GG*	*gusA, nptII*	Oliveira et al., 2009
Citrus aurantifolia	Resistance to virus	*pBin19-sgfp*	*nptII, sgfp, p23*	Fagoagaet al., 2006
Poncirustrifoliate	Enhanced salt tolerance	*pBin438*	*nptII, AhBADH*Fu.et.all., 2011	
Papaya				
Carica papaya	Resistance to *PRSV pRPTW*	*pRPTW*	*PRSV* replicase gene, neo	Chen et al.,2001
Pomegranate				
Punica granatum	Method optimization	*pBIN19-sgfp*	*nptII, gfp*	Terakamiet al., 2007
Fragaria spp. (Strawberry)	Modulation of fruit softening	*pBI121*	antisense of endo-*b1*,4-glucanase	Lee & Kim,2011

Table 15: Permits and notifications of transgenic fruits approved

Crop	**Trait**	**Genes**
Apple	Reduced polyphenol oxidase	PPO suppression transgene, *nptII*
	Non-browning reduced polyphenol oxidase	Polyphenol oxidase antisense, *PGAS1, PGAS2*
Banana	Bunchy top resistance	Replicase associated protein,

		replicase inverted repeat, nptII
Grape rootstock	Grapevine fan leafneo virus resistance	Coat protein gene,heat shock 90 homologous genes, nptII
Grapefruit	Aphid resistance	Agglutin coat protein, *GUS*.
Papaya	Female to male (or) hermaphrodite	*EST116*, *ESTS*, *FSH11*, *FSH19*

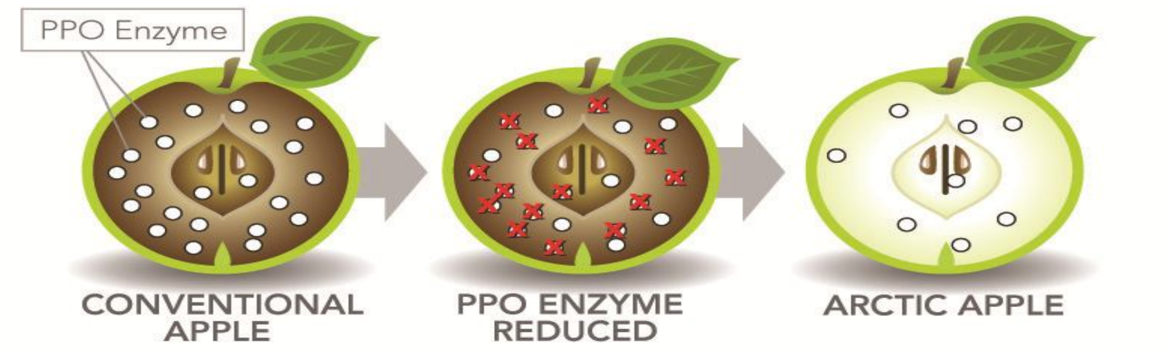

Figure 2: Pro-enzyme induced apple

Problems and prospects

The intake of fresh market fruits in the United Nationlike stone fruits and apple,has been stable or decreasing (Perez et al., 2001). Surveys propose that, primary reason is that the consumers are not pleased with quality offruits(Stockwin, 1996). Our present strategy for quality as breeders has been to choose subtle alterations in a high-quality cultivar to fill a specific niche. A little alteration that will solve an issue in the consumer market may be a different ripening duration, a change in colour, or a greater sugar to acid ratio. Although this breeding strategy has had great success, it hasn't necessarily improved customer access to fruit of the utmost quality. Our understanding of the factors influencing fruit quality has improved from the use of molecular approaches to the study of fruit ripening (Giovannoni, 2001)yet, it is not sufficient to make molecular selection and modification at practical level as a primary approach to improve quality traits for consumers. Long juvenile phases, prolonged gestation periods, plant architecture and inadequate planting material are the key issues with fruit breeding programmes aimed at enhancing the qualitative attributes of fruit crops.

Breeders may have to think outside the box, at least until we have a better knowledge of fruit quality traits. Fruit quality traitsare defined by cultivars that may perhaps be centuries old such as 'Montmorency' cherry (17th century), 'Bing' cherry (1800s), 'Golden Delicious', 'Delicious', 'Granny Smith', and 'Jonathan' apples (18th and 19th century) (Sun et al., 2011), 'Chinese Cling' peach (18th century), 'Belle of Georgia', and 'Elberta' peach (1850s), 'Bartlett' and 'Bosc' Pear (18th century), Smooth Cayenne pineapple (1819) and 'Fuerte' avocado (1911), all are very dominant germplasm in breeding programs. One strategy for breeding for better fruit quality is to diligently screen for better fruit quality and not be deterred by poor appearance (Janick and Moore, 1996). Perhaps we should have to select quality attributes as per future thrust to improve. More varieties of small, firm fruit, like the recently released "Pixie Crunch" apple, or fruit that can be consumed without creating a mess, like the Zee Sweet series of peaches, nectarines, and pluots, which can be consumed while still crisp due to their high sugar and low acid ratios, may be included in successful releases. In present, fruit processing industry is growing and now major challenges for fruit breeder is to improve the variety in way that have good flavour, texture and taste after cut into the pieces and lightly processed.

Conclusions

Fruit crops need integrated techniques that combine traditional and non-conventional methods for breeding varieties with positive characteristics since they are constrained by a variety of restrictions compared to short-lived and seasonal crops. As polygenes are in control of controlling fruit quality, integration becomes essential for achieving the desired improvement. The biotechnological approaches deliveranaccurate, reliable and easy way for breeding fruit trees for improvement of fruit quality. Also, it lessens the time, effort and patience of the breeder, when dealing with breeding related activities of fruit trees. These methods might aid in the development of high-quality fruits to fulfil both domestic and global fruit demand.

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