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Recent Advances in Annona Breeding: A Review

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ABSTRACT

The Annonaceae family comprises about 120 genera and more than 2000 species. The genus Annona is the most economically important one, containing 120 species. The opportunity for breeding improvement in the annona is significant and challenging. There is a lot more species are available but certain inherent constraints are involved like: long juvenility, higher levels of heterozygosity and large area requirement for assessment of hybrids. On the other hand, wide range of diversity and ease of vegetative hybrid propagation are the advantages for the breeders. There are very few man made commercially important hybrids. Its development is mostly based on the selection of clones were made for fruit quality only. Requirements of a good cultivar involve: dwarfness, precocity and profuse bearing, attractive, good sized and quality fruit, absence of physiological disorders, disease and pest resistance and improved shelf life etc. Comprehensive knowledge about the phenology and advanced techniques for hybridization have been quite helpful to overcome the problems like poor eating and keeping quality. The development of the genetic markers has further reduced the uncertainty in breeding of annona and maintaining the hybrid populations in a better way.

Key words: *Annona, Heterozygosity, Breeding, Juvenility.*

INTRODUCTION

The Annonaceae family comprises about 120 genera and more than 2000 species. The genus Annona is the most economically important one, containing 120 species[19]. The relatively wider adaptability of the trees and a good consumer preference for their fruits make the commercial cultivation of these species attractive. Among the edible annonas, cherimoya *(Annona cherimola* Mill.), sugar apple (*A. squamosa* L.) and the hybrid between the two, atemoya, are commercially significant[40] and economically important fruits in several tropical and sub-tropical countries. And also some other species are ramphal (*Annona reticulata*) soursop (*Annona muricata*) and pond apple (*Annona glabra*). The sugar apple species name '*squamosa*' refers to the knobby appearance of the fruit. Sugar apple is a small tropical tree originating in the New World tropics, probably Central America. Sugar apple is the mostly grown annona species.

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The fruit is frequently found in village markets but has not shown much potential for large commercial cultivation due to the small fruit size, frequent cracking at maturity and poor shelf life. The perishable nature and supply shortages make marketing localized or air shipment essential. Commercial cultivation of cherimoya occurs in Chile, Ecuador, Columbia, Australia, Spain and California[28], However, the cultivation of atemoya is mostly limited to Australia and Florida[12]. Another allied species bullock's heart, which possesses a number of traits that would be desirable in other edible annonas, is grown on a limited scale in Australia, Africa, India, Peru and South-East Asia[19].

The improvement of annona rather any crop needs to explore new recombinants primarily by means of exploiting the breeding methodologies. Diversity or heterogeneity is the main character desired for breeding either natural or manmade. It is required to have vast genetic pool to get new combinations of desired nature and developing new hybrids. Breeding has yet to play its role in the development of this crop as it has not been effectively manipulated in the distant past. Now, the scientists have developed certain hybrids of annona. To go for such strategies, a comprehensive knowledge of the different annona species, physiology of the reproductive parts, their breeding behaviour and cytological information of the crop is needed. This will eventually help to improve annona production status.

Description of the important Annona species

1. Cherimola - *Annona cherimola* P. Mill.

The name cherimoya derives from the Quechua name "chirimuya", which means "cold seeds"[27]. It is a small, erect, deciduous tree. It has simple, alternate, leaves are ovate-lanceolate to elliptical in shape, 10 to 25 cm long, glabrous on the ventral surface and pubescent dorsally, with leaf shedding in the spring. The single, protogynous, mild fragrant flower emerges from the leaf axils, and possesses a short peduncle. Flowering occurs once in a year and it starts when the tree is 3 to 4 years old. Anthesis starts in the early morning and it takes 8h to attain complete opening. The fruit is normally heart-shaped, conical, oval or somewhat irregular in form due to irregular pollination. Fruits measure 7.5 to 12.5 cm in length and weigh from 200 to 700 g. The fruit surface is smooth in some varieties; in others, it is covered with small conical protuberances over the carpels. The fruit rind is delicate and thin, and is greenish-yellow when ripe[29]. The white, subacid flesh has a fragrant, delicate flavor. The fruit has numerous seeds (21 to 41 seeds/fruit), which are 1.5 to 2.0 cm in length and approximately 1.0 cm in width[42].

2. Sugar apple – *Annona squamosa* L.

The sugar apple tree is deciduous and much smaller than the soursop, reaching a maximum of 6.0 m in height, with many lateral branches. The stems present lenticels, while the young shoots are pubescent and the oldest are smooth. The leaves are oblong-elliptical in form, measuring 5 to 17 cm in length and 2 to 7 cm in width, with an obtuse or acuminate apex[35]. The flowers measure 2.0 to 2.5 cm in length and are much smaller than soursop flowers, being similar in size and form to those of cherimoya. Pollen germination is low and may influence final fruit set, which varies from 5.4% to 5.6%[51]. The fruit is rounded, heart-shaped, ovate or conical, 5 to 7.5 cm in diameter, 6 to 10 cm in length and weighing 120 to 330 g. The white, custard-like pulp has a pleasant sweet-sour flavour. The fruit contains 35 to 45 black seeds, each 1.5 to 2.0 cm in length and 0.6 to 0.8 cm in width[50].

3. Atemoya – *Annona atemoya* L.

The tree is highly vigorous, fast-growing, 25 to 30 feet tall. The branches typically drooping and the lowest touching the ground. The leaves are deciduous, alternate, elliptical, leathery and

less hairy than those of the cherimoya. The flowers are long-stalked, triangular, yellow, 6 cm long and 4-5 cm wide[15]. The fruit is conical or heart-shaped, weighs more than 1.00kg. The rind is thick and composed of fused areoles more prominent and angular than those of the sugar apple, with tips that are rounded or slightly upturned; firm, pliable, and indehiscent. Pulp is fragrant, snowy-white, fine texture, almost solid, not conspicuously divided into segments, with fewer seeds than the sugar apple; sweet and subacid at the same time and resembling the cherimoya in flavor. The seeds are cylindrical, dark brown or black in colour[13].

4. Bullock's heart / Ramphal - *Annona reticulata* L.

The tree reaches 6.0 to 7.5 m in height, with many lateral branches; stems are cylindrical, with lenticels and very short coffee-coloured hairs. It is considered the most vigorous of the annonas. The fruit is considered to be of inferior quality. It can be distinguished from cherimoya by its long, narrow, glabrous leaves, from sugar apple by its solid, compact fruit, as well as its larger leaves, and from *A. glabra* by its small, dark brown seeds[42]. The leaves are oblong-lanceolate and dark-green, measuring 25 to 30 cm in length and 7 cm wide, with 10 to 20 vein pairs and a pubescent petiole. Flowers are similar in form to those of sugar apple, except that they are grouped in a short inflorescence with 2 to 10 flowers, with pedicels measuring 1.5 to 3.0 cm in length. This species also presents inefficient natural pollination and poor fruit set[13].

Fruits weigh from 0.1 to 1.0 kg and are commonly heart-shaped, but may be conical, ovate or irregular in form, and 10 to 12 cm in length. They are coriaceous and have a reddish-yellow surface colour, with impressed lines (around 5 to 6 angled areoles) above the carpels[42]. The flesh is milk-white and sweet, although insipid in flavour, being considered the least tasty of the cultivated annonas. There are commonly more than 40 oblong, dark coffee-coloured seeds per fruit.

5. Soursop - *Annona muricata* L.

The soursop has an erect growth habit with a high canopy height-to-diameter ratio, although it tends to be low-branching and bushy, with upturned limbs. It is a small, slender, evergreen tree, 4 to 8 m tall when fully mature. The stems are rounded, rough and not pubescent, with a dark-brown colour. The leaves have short petioles, and are oblong-ovate to cylindrical, 14 to 16 cm in length and 5 to 7 cm in width[42]. The flowers of soursop are much larger than the other species, being 3.2 to 3.8 cm in length. The flowers start to open in the early morning and complete anthesis takes approximately 6h, depending on the climate. Soursop produces an ovate, conical or heart-shaped fruit, that is dark green when unripe and a slightly lighter green when ripe. The rind has many short, fleshy, pointed carpel protuberances and is popularly regarded as 'spiny'. The soursop has the largest fruit in the genus, weighing from 0.9 to 10 kg, and averaging 4 kg[41]. The flavour is more acid and less sweet .The fruit has 127 to 170 seeds, scattered throughout the pulp. They are toxic. Seed size varies from 1 to 2 cm in length and from 0.33 to 0.59 g in weight, with a black colour soon after harvest, but becoming dark-brown later[42].

6. Pond apple - *Annona glabra* L

The tree vigorous, commonly known by Alligator apple, Monkey apple and originated in south eastern America and Mexico, the flowers are born in current season growth and flower length measures about 2-3 cm, the fruits are oblong or spherical in shape and weight about 300-700 gm, the peculiar characters of pond apple are the flesh is sweet-scented flavour; resemble like papaya and pineapple flavour. This species is mainly used for the drought tolerance and coloured flesh[42].

Reprodcutive Physiology

Floral biology:

Flowering in most of the annona species appeared in different flushes although April and September were observed to be major bloom periods under tropical humid eastern coastal region of India. Flowers were terminal or axillary and borne solitary, paired or in multiflowered fascicles either on new branches, older branches or on the main trunk as cauliflorous nature of bearing[25]. The flowers are hermaphrodite and dichogamous in nature. The soursop flowers are found to be protoandrous and other species are protogynous in nature[34].

The anthesis takes place in sugar apple flowers at morning 5:30 am to 6:30 am and anther dehiscence at 12 pm to 2 pm of next day of the anthesis and stigma receptivity start a day prior to anthesis and continue upto day after anthesis but found maximum receptivity at the day of anthesis[25]. In cherimoya the anthesis takes place at 7 am to 9 am in the morning, anther dehiscence at 12 pm to 4 pm of next day of the anthesis and maximum receptivity found at the day of anthesis. Atemoya flowers are borne on current season growth, the peak anthesis of flowers at afternoon 2 pm to 4 pm then the anther dehiscence accurse after a day of anthesis at 12 pm to 2 pm and the stigma receptivity start a day prior to anthesis and continue upto day after anthesis but found maximum receptivity at the day of anthesis. The anthesis takes place in reticulata flowers at morning 6:00 am to 8:00 am and anther dehiscence at 12 pm to 2 pm of next day of the anthesis and stigma receptivity start a day prior to anthesis and continue upto day after anthesis but found maximum receptivity at the day of anthesis[34]. In muricata the flowers exhibited protoandrous dichogamy, the peak anthesis occurs in the afternoon 2:00 pm to evening 8:00 pm, anther dehiscence at 4 am – 8 am and stigma receptivity start after completion of male phase. In glabra the anthesis takes place at 7 am to 9 am in the morning, anther dehiscence occurs at 12 pm to 4 pm of next day of the anthesis and maximum receptivity found at the day of anthesis[34].

Pollination:

The flowers are hermaphrodite and dichogamous in nature, both the male and female flowers are borne in the same flower[25], but the female part matures before the male part (protogyny) in most of the species except pond apple (protoandry), therefore the annonas possess highly cross pollination[47]. Natural pollination in annona mainly carried by only through insects, such as beetles (canthrophily), nitidulid beetles (*Carpophilos domidiatus* and *C. hemipterous*) play a major role in pollination of sugar apple[25], insects rarely visits the annona flowers because the flowers are not attractive and have low mild fragrance to attracts the insects, which suggests that canthorophylous pollination plays only a secondary role in the annona species. Because pollen grains occur in clumps and are somewhat sticky, wind pollination is not common[13]. Although dichogamy and the low population density of pollinators insects are important limiting factors to successful natural pollination, the effect of these factors may result in fertilization failure of all or several ovules, resulting in small or asymmetrical fruits, which obviously affects the yield, quality and commercialization of annona fruits[49].

Hand pollination is the only certain strategy to ensure commercial production, since it guarantees significantly higher production and better fruit quality than open/natural pollination in annona[18,47]. Hand pollination improves fruit set and substantially boosts fruit yield per tree by increasing the fruit size and number, the major yield components[43]. Hand pollination is normally carried out before 8.00 am using a small brush. Pollen can be collected in the morning

between 5.00 and 8.00 am from fully open flowers, when the sacs have turned from white to cream. The collected pollen is used to pollinate half-open flowers whose pistils are already receptive[18].

Pollinizers

Several agents have been credited as pollinators of annona. Nitidulid beetles, *Carpophilos domidiahus* and *Carpophilos hemipterous* were identified as pollinators in terms of visitation frequency, pollination potential score and pollination efficiency. However, *Carpophilos hemipterous* was relatively effective pollinator than *Carpophilos domidiahus*. Sugar apple clearly exhibit obligate specialization by filtering only *Carpophilos hemipterous* and *Carpophilos domidiahus* as pollinators and thereby explicitly indicating canthrophilous pollination syndrome[25].

Cytogenetics of annona

Understanding cytology of a crop is useful in planning breeding programme. The chromosome numbers of cherimoya, reticulata, soursop and sugar apple are 2n = 14 to 16[10,33]. Although there is some variation in chromosome number, they are all diploids, 2n = 2x. This slight variation in chromosome number may explain the ease or difficulty of interspecific hybridisation and warrants further work to determine if intra-specific variation also exists. *A. glabra*, are found to be tetraploid[3].

Perspective of breeding in Annona

The opportunity for breeding improvement in the annona is significant and challenging. There is a lot more species wealth available but certain inherent constraints are involved like: long juvenility, high heterozygosity and large area requirement for assessment of hybrids. On the other hand, wide range of diversity and ease of vegetative hybrid propagation are the advantages for the breeders. There are very few man made commercially important hybrids. Annona development is mostly based on the selection of clones were made for fruit quality only. Seedling screening from known mother plants is another way of selection for better lines. Modern age requirements of a good cultivar involve: dwarfness, precocity, profuse and regular bearing, attractive, good sized and quality fruit, absence of physiological disorders, disease and pest resistance and improved shelf life etc. Now, more comprehensive knowledge about the phenology, advanced techniques for hybridization is available. Many environmental and physiological factors related to the undesirable character of annona varieties (susceptibility to diseases and pests, poor eating and keeping quality, etc., are closely controlled by genes. To overcome these, plant breeding can play an important role and work should be done in three directions as introduction, selection and hybridization. Hybrid populations can be managed in a better way and the development of the genetic markers has further reduced the uncertainty in breeding of annona.

Breeding objectives

The first objective is the determination of the specific characteristics that are important for the new cultivar - the ideo-type[42]. General and very important objectives of breeding in annona are; Precocity in bearing, higher in percentage of flower and fruit set, have good shelf life of fruits and yield, prefer symmetrical shape and good size fruits, prefer fruits without carpel projections that withstand bruising during transportation, resistance to common pests and diseases of annona and other traits includes attractive skin colour, high pulp recovery, low number of seeds and good flavour. These characteristics may help a breeder to select parental groups to be used in an annona breeding programme, in order to obtain desirable progenies. These progenies may not have all desirable characteristics, but at least will have those most important to growers, retailers and consumers.

Table 1: Main characteristics of cherimoya, soursop and custard apple ideo-types (adult plants)[42]

	Characteristics		**Cherimoya**	**Soursop**	**Custard apple**
PLANT	Vegetative vigor		Medium – low	Medium – low	Medium – low
	Yield		> 30kg/tree/yr	> 60kg/tree/yr	> 20kg/tree/yr
	Bearing		Regular	Regular	Regular
	Rootstock/scion compatibility		High	High	High
	Flower number		Abundant (>150 flowers/tree)	Abundant (>100 flowers/tree)	Abundant (>180 flowers/tree)
	Pollen fertility		High (> 76%)	High (> 76%)	High (> 76%)
Fruit					
	Size/ weight	Industry	Large/>650g	Large/>2.5kg	Large/>400g
		fresh consumption	Medium/300-600g	Small/800g	Medium/300-400g
	Shape		Heart	Conical	Round to Heart
	Fruit set (Natural)		High (>27%)	High (>26%)	High (>25%)
	Skin		Smooth	Short protuberances	Tuberculate
	Pulp		Sweet, fiberless	Sub-acid, low fibre	Sweet, low fibre
	Flavor		Delicate	Sub-acid flavor	Pleasant acidulous
	Seed number/ 100g of pulp		Low/<6 seeds	Low/10-30 seeds	Low/<10 seeds
	Shelf life		Long (> 10 days)	Long (> 5 days)	Long (> 5 days)

Breeding Strategies

According to Jordan and Botti[21], there is great scope in edible annonas for gene transfer across species boundaries, for widening the genetic base and for developing strategic breeding reserves. Gene exchange among annonas should result in interesting novel recombinants, with the popular atemoya being the most esteemed one currently available. Additionally, annona species vary in their climatic adaptation. In the tropics, cherimoya grows well at higher altitudes and sugar apple at lower altitudes, while the interspecific hybrid, atemoya adaptation is intermediate between its parents[44,46]. *A. reticulata* flourishes well in costal lowlands. This adaptation suggests that it may be possible to break the geographical barriers of annonas through interspecific hybridization and construct new annonas suitable to wider or new climatic zones.

Most *Annona* species and cultivars differ in environmental adaptation, productivity and fruit quality. Therefore, different conventional methods can be used in their breeding. According to Fehr[9], there are three requirements for the development of an asexually propagated cultivar: a) a suitable

source of genetic variability b) evaluation of individuals from the population c) asexual multiplication of a new cultivar for commercial use.

Selection and cultivar development

Introduction of superior genotypes and/or cultivars to establish a germplasm collection is, basically, the first requirement of any breeding programme[15]. This can be complemented by the introduction into the collection of some wild *Annona* species with useful genes, mainly for resistance to diseases. All accessions require comprehensive characterization and documentation, followed by evaluation and selection. Several types of populations can be developed by hybridization, from which superior clones are selected[42].

India and Taiwan have selected a few named cultivars of sugar apple from seedling population that are propagated vegetatively. In Cuba, researchers developed 'Cuban Seedless', which is a seedless cultivar with medium-sized fruits, and another cultivar with low fibre content that is very important for the commercial market[33].

Table 2: Some selections and cultivars of sugar apple that are currently grown in various countries[42]

Cultivar/Selection	Country of origin	Cultivar/Selection	Country of origin
IPA selection	Brazil	Leone	Italy
Libby	USA	Madeira	Portugal
Lisa		Mateus 1	
Ott		Negrito	Spain
Red sugar apple		Reretai	New Zealand
Noi	Thailand	Abd El-Razik	Egypt
Molate	Philippines	Cuban Seedless	Cuba
Lobo		Balanagar	India
Mossman	Australia	Mammoth	
Whaley		Red Sitaphal	

Table 3: Selected cultivars of atemoya[42]

Cultivars	Country of origin
Maroochy Gold, KJ Pinks, Pink's Mammoth	Australia
African Pride	Southern Africa
Bradley, Page	Florida, USA
Gefner, Kabri, Malalai	Israel

Table 4: Selected cultivars of cherimoya[42]

Cultivars	Country of origin
Andrews, Kempsey, Mossman	Australia
Bays, Booth, Libby, Lisa, White	California, USA
Bronceada, Concha Lisa	Chile
Burton's Wonder	New Zealand
Campa, Cristalino	Spain
E-8	Ecuador

Some of the new selections of sugar apple are developed by different institutions in India; Central Horticultural Experimental Station (ICAR-IIHR), Bhubaneshwar, develop a new clonal selection and named as Arka Neelchal Vikram, which bears good quality fruits with high yield (>15 kg/tree/year), the shelf life upto 5-6 days after maturity and have good taste with high TSS (24^{O}B). Regional Research Station (TNAU) – Aruppukottai, Tamilnadu, develop another sugar apple selection; APK-1, which gives more yield, bears good quality fruits and more suitable for South India. Another selection release from Australian custard apple growers association (ACAGA) in Australia, the variety belong to atemoya, which is named as Tropic Sun, which is semi dwarf and good for high density planting, more suitable for tropical and sub-tropical regions of the world with sweet and good quality fruits.

Although institutions did work on the release of varieties in annona also some farmers in the India has been developed some varieties of the sugar apple. Shri Navnath Malhari Kaspate, he is the farmer from Solapur district of Maharastra, he develop four varieties; Annona-2, NMK-1, NMK-2 and NMK-3 (IHC, 2014), among these NMK-1 gain lot of importance in south India and commonly called as golden, because of its colour and quality of the fruits. Impact of these varieties; The productivity of existing varieties with traditional cultivation is about 10 to 12 ton / ha. Whereas the productivity of new selections is about 15-19 ton/ha, with 70-80% Grade-1 fruits. Due to good quality fruits production the market rates are 120-150% more over existing varieties. The benefit-cost Ratio is 4.6. Near about 700-800 farmers from 9-10 district of Maharashtra cultivators got the benefit of Custard Apple production technology & new cultivars and diversified their cropping pattern towards dry land fruit farming.

Due to the efforts of Mr. N.M. Kaspate and Krishi Vigyan Kendra, Solapur the area under Custard Apple increased to 800 ha. In Solapur district and approximately 450 ha, in adjoining districts during last 8-10 years (IHC, 2014).

Some cultivars were identified in India based on colour of the fruit , its origin and plant part: Yellow custard apple named after the colour of the fruit; Cultivars like Kakarlapahad, Mahaboobnagar, Local Hyderabad and Saharanpur Local named after the place of origin; White stemmed custard apple named after the part of plant.

Hybridization

Wester[52] was the first scientist to realize the possibilities for genetic improvement of annonas and initiated breeding programmes in Florida and in the Philippines, due to some problems during the breeding programme no hybrids has been developed on that time[42]. Globally, interspecific hybridization in annonas is limited to four research groups, one each in Australia, America, Israel and India[24,54].

Several types of populations can be developed by hybridization, from which superior clones are selected and released as new hybrid after evaluation[11], breeding in annona has been neglected and only a few hybrids have been developed in the past, due to lack of breeding programmes and clear strategies. Australian Custard Apple Growers Association started breeding programme with the objective of developing high quality seedless hybrids of atemoya[14,39]. In this breeding programme, first they produce tetraploids, either through gamma irradiation or colchicine, and crossed these to diploids to produce seedless triploids. Other potentially useful methods of obtaining seedlessness in annonas, a) progeny from cross between diploid x diploid with small size seeds, b) producing triploids from diploid x tetraploid and tetraploid x diploid crossing, c) protoplast fusion of diploids and haploids, d) crossing of low seeded parents, e) development of self-incompatible parthenocarpic hybrids and f) endosperm culture to develop triploids[42].

Girwani *et al*[16]., also developed certain sugar apple hybrids at Sangareddy, India, and revealed that All the hybrids varied in fruit shape (round, conical and cordate), fruit colour

(yellowish green, grayish green, light green and red), pulp colour (creamy white, light pink and white), areole shape (tuberculate, hexagonal) and texture (course, soft and meaty). Time of fruit maturity varied from September 2nd fortnight and lasted till the end of December. Hybrid-1 (17/4 Atemoya × Balanagar) was earliest to mature. Maximum fruit weight (250 g) was recorded in Hybrid-1 (17/4 Atemoya × Balanagar) followed by Hybrid-6 (15/3 Red Sitaphal × Atemoya) (225 g) and Hybrid-4 (1/6 British Guinea × Atemoya) (220 g). Among all the hybrids, maximum number of fruits per tree (94) was recorded in Hybrid-1 (17/4 Atemoya × Balanagar) followed by Hybrid-6 (15/3 Red Sitaphal × Atemoya) (67). The TSS ranged from 22 to 28°B and was found to be maximum in Hybrid-2 (15/2 Red Sitaphal × Pond apple), while the seed content per fruit was also minimum (20) in this hybrid. Based on overall performance the Hybrid-1 (17/4 Atemoya × Balanagar) and Hybrid-3 (15/3 Red Sitaphal × Atemoya) and Hybrid-2 (15/2 Red Sitaphal × Pond apple) were found to be excellent in quality with pleasant aroma, smooth pulp texture with less seed, good sugar acid blend and shelf life.

Crosses have also been made between four different species; *A. cherimola, A. squamosa, A. reticulata, A. diversifolia* and *A. atemoya*. To date none of the progeny of these crosses has produced commercial cultivars. In florida approximately 3000 seedling progeny[14], mainly interspecific crosses, have been planted out in Zill orchards near Boynton beach. To date, none of the progeny of these crosses has produced commercial cultivars. The studies conducted in Serdang, Selangor, Malaysia, involved six selected species of *Annonaceae* family, namely: *Annona muricata*, *A. squamosa*, *A. glabra*, *A. montana*, *A. rollinia*, and *A. reticulata*. Hybridizations were attempted between the species and also their reciprocal crosses. Although fruits are formed between some species, seeds were not viable except for those produced in two hybridizations: *Annona muricata* (female) x *Annona montana* (male) and *Rollinia mucosa* (female) x *Annona muricata* (male). The former F1 hybrids produced plants that flowered but set no fruit. The latter F1 hybrids produced fruit similar to Rollinia[31]. Out of 35 germplasm of custard apple evaluated the varieties viz; Atemoya X Balanagar (highest fruit weight - 278.9g), Y. Palli – 12 (highest pulp weight – 148g), NLD – 8 (maximum T.S.S – 25.9), Balanagar SR (more number of fruits/tree – 51.4) and Ramaphal (highest yield/tree – 15.7) can be recommendable for scarce rainfall zone under rainfed conditions in poor fertile soils[4]. Among the 22 accessions, notable variations were observed in tree shape, trunk colour, trunk ramification, colour of young branches, leaf blade shape, pulp colour and taste. Generally, soursop genotypes showed good adaptation to the specific soil and climate conditions of these regions and great variability was found among them[30]. There is a growing awareness particularly about the anti cancer properties of this plant among the people and the consumption of this fruit and leaves by the cancer-affected patients is on the increase. So, it has become necessary to document the trees available in Tamil Nadu and variations them to exploit the potential of the trees[30].

In India breeding work was initiated in 1989 at the Indian Institute of Horticultural Research, Bangalore on evolving a less seeded custard apple cultivar with higher proportion of good quality edible pulp and better shelf life[20]. More than 2000 inter and intra-species *Annona* hybrids F_2 backcrosses were developed and evaluated which resulted in the release of a hybrid by the name 'Arka Sahan'. It is an outstanding recombinant isolated from the family of 'Island Gem' (atemoya) and 'Mammoth' (custard apple). However, for improving the fruit-set, fruit-size and fruit-shape of this rare interspecific recombinant pollination with *Annona squamosa* pollen was found to be most effective[18]. Fruits of 'Arka Sahan' are remarkable for scarce small seeds (9/100 g fruit weight), sweetness (>32°B) and slow ripening (5-6 days). Nutritionally, 100 g pulp of 'Arka Sahan' contains 2.49 g crude protein, 42.29 mg phosphorus and 225 mg

calcium compared to 1.33 g, 17.05 mg and 159 mg respectively, in common custard apple.

After success of Arka Sahan the *A. atemoya* x *A. reticulata* was another cross combination that has attracted interest as *A. reticulata* carries genes for uniform heart-shaped fruits with a stout stalk and smooth and attractive skin colour as well as having a robust root system and compact tree habit (Council of Scientific and Industrial Research 1985). Jalikop[19] studied that *A. reticulata* is potentially a valuable source of a variety of novel traits. However, the F_1 trihybrids exhibited reduced fertility with pronounced undesirable traits of *A. reticulata* and the survival of F_2 trees was poor, with those that did survive being extremely weak and failing to flower or set fruit. Consequently, backcrossing may be more suitable approach than advancing trihybrid generation for transferring desirable genes from *A. reticulata*. Backcrossing F_1 progeny of [(*A. cherimola* x *A. squamosa*) x *A. reticulata*] to *A. squamosa*, a highly fertile species, is expected to yield productive introgressants in which the contribution of the *A. squamosa* genome will be extended from 25 to 62.5% in the F_1BC_1 generation[19]. The genetics is described herein based on the expressions of certain tree and fruit traits in the progeny.

Biotechnology

The tissue culture in annona is mainly used for the micropropogation, maintains of germplasm collection, used for the embryo rescue technique, preparation of explants for transformation of genes, enhancement of variability by somaclonal variation and production of haploid plants[42].

The vegetative propagation of these species present problems of different degree, being its sexual propagation of limited agronomic value due to the high degree of heterozygosis of these species and preventing their propagation by seed.

Among these Annonaceae, in cherimoya (*Annona cherimola*), the most exploited annona, the morphogenetic capacity is extremely low, showing minimal level of rooting making almost impossible the use of classic vegetative propagation methods, because the induction rate of adventitious roots from scions obtained from adult specimens with agronomic interest is null. This low morphogenetic capacity is the general trend for most of other annona species even if it happens in different degrees depending on the species. In order to fix the Annonaceae vegetative propagation problems, *in vitro* methods have been applied. But unfortunately the limited funds and human resources devoted to this subject never reach the necessary volume to obtain quick and substantial advances.

The in vitro tissue culture methods of micropropagation can be applied successfully to cherimoya and other annona species to overcome these problems. Most of the protocols of micropropagation and regeneration were developed using the cultivar Fino de Jete, which is the major cultivar in Spain. First it is developed the method to micropropagate the juvenile material of cherimoya[5], and later it was optimized a protocol to micropropagate adult cherimoya genotypes selected by outstanding agronomical traits[37] and further it was improved the process through micrografting[38]. At the present time we are involved in inducing and obtaining new elite genotypes, as part of a breeding program for the cherimoya and other annonas, using and optimizing different methodologies *in vitro*: a) Adventitious organogenesis and regeneration from cellular cultures[6], b) Ploidy manipulation of the cherimoya, to obtain haploid, tetraploid and triploid plants (seedless), c) Genetic transformation, for the genes introduction to control the postharvest processes and the genes introduction to provide resistance to pathogen and insects and d) Micropropagation and regeneration of other wild Annona or related species such as: *Annona senegalensis*, *A. scleroderma*, *A. montana*, *A. reticulata*, *A. glabra*, *A. diversifolia* and *Rollinia* sp.

The first haploid plants induced by anther culture in fruit trees were reported in sugar apple. Aim of the work is to development of seedless fruits. The

researchers obtained callus differentiation, and formation of roots and shoots from sugar apple endosperm, but a complete plantlet was not obtained[32].

Genetic transformation

The preliminary steps in the cherimoya genetic transformation via *Agrobacterium* have been done to increases the shelf life, to change post-harvest characteristics and to provide pest and disease resistances. For other *Annona* species there are no advanced studies[7].

Molecular markers

In annona the molecular markers are mainly used for germplasm characterization, hybridity confirmation, identification of parents of the progeny/hybrid and diversity analysis of annona. There are limited reports found on exploitation of molecular markers for diversity analysis in annonas. Few of these markers are random amplified polymorphic marker (RAPD) marker[1,48], amplified fragment length polymorphism (AFLP) markers[45,53] and simple sequence repeat (SSR) markers[8,26]. On the other hand, information regarding nutritional value is of utmost important to select desired genotype for domestication in area of adaptation. Very little information is available on proximate analysis of annona fruits[2,23,36]. During analysis, a set of 11 RAPD primers yielded a total of 152 bands with 80.01 % polymorphism and 12 SSR primers produced 39 amplicons. The dendrogram discriminated all the annona genotypes suggesting that significant genetic diversity was present among the genotypes[17]. Proximate fruit composition study of nine fruiting genotypes of annona revealed that *A. squamosa* possessed significantly higher amount of most of the studied biochemicals which gives an opportunity to fruit breeders to improve the other annona species. Likewise, *A. muricata* being rich in seed oil content can be exploited in oil industries[17].

Mutation

There is very limited work has been done in annona for development of mutant varieties but the natural mutants are obtained; Brazilian seedless and Thai seedless (Ts), Thai seedless, produces fully seedless normal-sized fruits and provides the potential to introduce this feature into annona fruit production. Jorge *et al*[22]., determined the developmental cause of seedlessness in Ts and, using information from studies on Arabidopsis, showed that the mutant is associated with the deletion of the *A. squamosa* INO (inner no outer) gene. A unique case of elucidation of the function of a gene through forward genetics. A potentially agronomically useful manifestation of the particular mutation is the production of seedless fruits. The determination of the molecular basis of the Ts mutant can aid introgression of the trait into commercially desirable varieties by providing a method for identification of homozygous mutant seedless plants. The likely conservation of the function of ino in the genetic regulatory pathway of ovule development across angiosperms broadens the implications of these results[22].

CONCLUSION

Immense prospects exist to develop desirable cultivars of annonaceous fruits by conventional breeding techniques, there is greater scope to exploit inter-specific variation than intra-species variation, edible *annona* species exhibit morphological affinity to one another but each one is unique and distinct for fruit shape, size, skin surface, pulp colour, texture, flavour and taste, some species like *annona squamosa* exhibit narrow genetic variation. However, several interesting traits are available in one or the other allied *Annona* species, because there are no serious barriers in inter-specific hybridization between *A. squamosa*, *A. cherimola*, and *A. reticulata* gene transfer across species boundaries is possible, gene exchange among them should result in interesting novel recombinants at times most esteemed ones like the popular atemoya.

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