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Genetic improvement of medicinal plant Phyllanthus emblica L. (V. Nelli) as a potential cash crop

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RUNNING TITLE: Genetic improvement of Phyllanthus emblica: A review

Abstract

Phyllanthus emblica L. (V. Nelli) is an important constituent of indigenous medicine and a commercially important species which provides fresh fruits. All parts of the tree are used in medicine and but drupe is the predominantly used plant part. Pharmacological studies have indicated that fruit extracts show antioxidant, anticarcinogenic, antitumor, antigenotoxic and anti-inflammatory activities which are attributed to the phenolic content in drupes. P. emblica germplasm is diverse in terms of drupe traits. Yet this diversity has not been studied genetically to date. Also drupes of different trees show a marked variation in astringency. If the underlying genes and genetics of the mentioned diversities can be molecularly characterized, that information could be used to breed superior varieties through Marker Assisted Selection. In India few molecular studies have been conducted and as a result of them, species specific microsatellite DNA markers are available which can be successfully utilized in diversity analysis. Therefore molecular characterization of the P. emblica germplasm in Sri Lanka using approaches such as microsatellite markers is a key to open up dozens of avenues that strengthen the value, utilization and germplasm conservation ultimately promoting this species from its' underutilized status.

Keywords: *Phyllanthus emblica*, Medicinal Plant, Amla, Cash Crop, Marker Assisted Selection

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Introduction

Phyllanthus emblica (V. Nelli) is commercially and medicinally important species. In traditional medicine, it is known as one of "the best rejuvenating herbs" [1]. All parts of the tree are used as medicinally important products [2] and the drupe plays the central role in medicine. It is also a part of multicomponent drugs in *Ayurveda.* Ethnopharmocological studies indicated that 17 countries use *P. emblica* as an indigenous medicinal remedy [3]. There is a market demand for fresh drupes because it relieves fatigue, increases the appetite and also acts as a good purgative [1]. The current pharmacological studies indicated that drupes show antioxidant and antiproliferative activities attributed to the phenolic compounds in the drupes [4] highlighting the importance of *P. emblica.*

Morphological diversity of *P. emblica* can be seen in relation to the drupe and stone traits. Drupe traits include drupe height, weight, width, mesocarp thickness and color of the epicarp while stone traits include stone height, weight and width. Drupe weight showed highest genetic variation, heritability and thereby a big potential for genetic gain in a diversity study conducted in India [5]. Generally *P. emblica* germplasm in India was characterized into different genotypes based on the drupe shape.

Molecular characterization of the *P. emblica* germplasm opens up new avenues to the prospect of the species [2]. In India, there have been studies on DNA fingerprinting of medicinally important species including *P. emblica.* But there too, the underlying genetics of drupe trait diversity has not been studied extensively. If the underlying genetics of drupe size and drupe development of *P. emblica* can be studied it will aid molecular breeding techniques that will facilitate the development of an ideotype cultivar for the market. This will uplift P. emblica from its' underutilized status and will also help to establish it as a medicinally important cash crop. This review summarizes the potentials, current molecular findings and possible future directions for the improvement of *P. emblica.*

Historical uses in indigenous medicine

Indigenous medicine has a growing demand as an alternative therapeutic need [6]. Professional medical systems like Chinese, Ayurvedic and Unani have many inscriptions where *P. emblica* has been used as a medicinal remedy. Inscriptions indicated that *P. emblica* was used in Sri Lankan indigenous medicine even far back than 350 AD. In ancient literature, it is referred to as *"Ambulu"* [7]. One of the most influential ancient inscriptions on Ayurveda, "The Sarartha Sangrahaya" by King Budhadhasa also indicated the uses of *P. emblica.*

It is one of the main constituents of Amalaki Rasayanaya, Brungamalaka Thailaya, Dhathri loha, Shyawanaprashawalehaya and Thripala Choornaya coming under Sinhala Ayurvedic medicine [7].

The entire plant; leaves, bark, stem, drupes, seeds, flowers and roots both in dried and fresh form, has its' applications in Sri Lankan indigenous medicine. Different plant parts have different medicinal properties making it useful in treating an array of ailments (Table 1).

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Table 1: *Medicinal properties of P. emblica plant parts*

Plant part	Medicinal Properties	Ailments
Unripe drupes	Diuretic, laxative, purgative	Hemorrhages, diarrhea,dysentery
Ripe drupes	Purgative, diuretic. aperients	Dysentery, diarrhea, cholera, biliary disorders
Dried drupes	Astringent	Hemorrhages, diarrhea,dysentery
Leaves	Diuretic	Chronic diarrhea
Root and bark	Astringent, emetic	Urinary diseases, antidiarrheic, leucorrhea, gas trointestinal disorders

Sources: [1,7]

Economic value

P. emblica has a high economic value because of its medicinal and nutritive richness. Therefore it is used to produce cosmetics, commercial beverages, confectionaries and medicinally important value added products. Fruit itself has a high demand as a fresh fruit in the market. In modern era where there is a boost in "global herbal market", *P. emblica* has a high potential as a cash crop in Sri Lanka. In India alone 10,000 tons of *P. emblica* drupes are commercially utilized per annum [8].

***P. emblica* fruit industry**

Although all parts of the tree are utilized for range of products, drupe is the most significant part due to high nutritive and therapeutic values. According to *Ayurveda*, the fruits are astringent, bitter, pungent, sour and sweet. Drupe can be used both in dried and fresh forms. The dried fruits are used as a part of multicomponent drugs such as *thripala* in *Ayurveda* [1]. The fresh fruits are one of the richest sources of Vitamin C (Table 2) with antioxidant properties [2]. The fresh fruits are eaten for general fatigue, weakness and poor appetite [1] making it appealable as a fresh fruit in the market.

It has a growing demand in the market both as fresh fruit and as a constituent of *P. emblica* based value added products. Because it is a fruit crop that has not been commercially exploited in Sri Lanka, it is important to establish diversity structure for *P. emblica* germplasm to enhance the effective use of *P. emblica* in fruit industry at global level.

Table 2 : Nutritional properties of *P. emblica* drupes

Chemical component	Percentage (%)
Carbohydrates	14.1000
Fibre	3.4000
Mineral matter	0.7000
Vitamin C	0.6000
Protein	0.5000
Fat	0.1000
Calcium	0.0500
Phosphorus	0.0200
Iron	0.0012
Nicotinic acid	0.0002

Source: [9]

Value added products based on *P. emblica*

Although *P. emblica* is a fruit crop, it has other uses such as dye, firewood, green manure, medicine and tannins [2]. Thus every part of the tree is commercially valuable and is used to develop different value added products (Table 3).

Table 3 : Value added products from different plant parts

Plant part	Products
Mature drupe	Candy, pickle, jam, jelly, juice, syrup and dye
Dried drupe	Tablets and amla powder
Wood	Boxes and furniture
Bark	Dye
Leaves	Dye and oil

Source: [2]

In order to sustain the production of medicinal value added products, each year Sri Lanka imports 50,000 kg of dried *P. emblica* fruits spending over one million rupees [2], which signifies a demand that surpasses the current crop output of *P. embilca*. This problem needs to be addressed by establishing *P. emblica* as a fruit crop in Sri Lanka. In India it has a larger market as a value added product, where it is used to produce amla preserves, candy, chutney, jam, jelly, juice, oil, ointments, pickle and tablets [2]. Thus it has well established itself as a fruit crop in India.

Nutraceutical properties

P. emblica is rich in minerals and is regarded as one of the richest source of Vitamin C with 200-900 mg per 100 g of edible portion [1]. In addition drupes are rich in carbohydrate, fibre and iron (Table 2). But the drupes predominantly comprise of water [1] making it a good source of water along with other nutrients.

Current pharmacological studies indicate that fruit extracts show antioxidant, anticarcinogenic, antitumour, antigenotoxic, antiinflammatory activities, supporting its traditional uses. *P. emblica* fruit extracts have proven abilities to inhibit the activity of "Activator Proteins" that induce the activity and transcription of the Human Papillomavirus causing tumourgenecity of human cervical cancer [10]. Cytotoxic effects of *P. emblica* on cancer are found to involve with the induction of apoptosis in cancer cells lines and it is effective against six human cancer cell lines, A549 (lung), HepG2 (liver), HeLa (cervical), MDA-MB-231 (breast), SK-OV3 (ovarian) and SW620 (colorectal) [11]. Therefore it has a great potential as a pharmaceutical that could even be the cure to the third most common cancer type in women; cervical cancer.

Taxonomy of *Phyllanthus emblica*

P. emblica is a deciduous tree of family Phyllanthaceae widely distributed in the tropics and the subtropics. The genus comprises about 700 species [2] and family Phyllanthaceae is among the largest flowering plant families.

Nomenclature of *P. emblica*;

Regnum	Plantae
Clade	Angiospermae
Clade	Rosids
Order	Malpigiales
Family	Phyllanthaceae
Genus	*Phyllathus* L.
Species	*Phyllanthus emblica* L.

The accepted name for the species is *Phyllanthus emblica* Linnaeus, Sp. Pl. 2: 982.1753 and also designated by the synonym *Emblica officinalis* Gaertn [12]. It is known by a number of vernacular names and the most famous include *Amlan* (Hindi), *Amalki* (Sanskrit), Indian Gooseberry (English) and *Nelli* (Sinhala).

Distribution and origin of *Phyllanthus emblica*

The Indian subcontinent comprises a rich diversity of medicinal plants that are used in various indigenous medicinal practices. Among those species, *P. emblica* is distributed through India; Sri Lanka to eastern Asia [12]. This is also the geographic area of origin. It also extends in geographic region into western hemisphere [2] where it has been long neglected but now is in the limelight as a medicinal fruit crop.

Morphological characterization of *Phyllanthus emblica*

Morphological diversity of *P. emblica* can be seen in relation to the drupe and stone traits. Drupe traits include drupe height, weight, width, mesocarp thickness and color of the epicarp while stone traits include stone height, weight and stone width. Drupe weight showed highest genotypic variation, heritability and potential for genetic gain in a diversity study conducted in India [5]. Generally *P. emblica* germplasm of India was characterized into different genotypes based on the drupe shape (Table 4).

Table 4 : Different *P. emblica* phenotypes and drupe characteristics

Phenotype	Drupe characteristics
Banarasi	Large, conical and triangular at apex
Krishna	Large, conical and triangular with papillate base
NA-9	Large, flat and round
NA-10	Flattened, round and moderate in size
Francis	Flattened, oblong and medium in size
NA-6	Small to medium, flattened and oblong shaped
NA-7	Medium to large, conical in shape with pappilate apex at maturity appears flattened and oval shaped
Chakaiya	Small to medium in size and flattened and round in shape
Kanchan	Small to medium in size and flattened oblong in shape

Source: [13]

In Sri Lanka the diversity of *P. emblica* germplasm is yet to be explored and has not been mapped to date. However, according to the general perception in Sri Lanka, *P. emblica* germplasm is distinguished in to two categories; small drupe bearing *P. emblica* (*"behethnelli"* i.e. medicinal *P. emblica*) and large drupe bearing *P. emblica* (*"rata nelli"* i.e. exotic *P. emblica*). *"Beheth nelli"* is used in *Ayurvedic* preparations and while *"rata nelli"* is commercially important as a fresh fruit (Pers. Comm.).

In Sri Lanka, apart from the drupe size variations, it has been observed that the color of the epicarp also shows a moderately higher diversity, with epicarp color ranging from green to yellowish green. This diversity in terms of color of the epicarp could be due to the genetic differences or differences in microclimatic conditions. Therefore this diversity is in dire need of scientific investigation.

Biochemical characterization of *Phyllanthus emblica*

P. emblica is rich in Vitamin C, alkaloids and phenolic compounds [14]. It is traditionally consumed as a fresh fruit for its rich source of Vitamin C. However the recent studies indicated that *P. emblica* is more vital in terms of its antioxidant activity [15] than the nutraceutical activity.

Vitamin C

The concentration of Vitamin C in *P. emblica* drupes is 160 times higher than in apple [16].

Vitamin C accounts for 45 – 70% of the antioxidant activity of *P. emblica* [17]. In India, the concentration of Vitamin C differed considerably among *P. emblica* according to its area of origin. This variation of vitamin C was 873 ± 342 mg/ 100 g / gram of pulp [13]. The fruit ascorbic acid content shows quantitative inheritance and the quantitatively trait loci for tomato ascorbic acid content variations has been mapped [18]. But the underlying genetics of this variation in *P. emblica* is not known.

Phenolic compounds

The phenolic compounds in *P. emblica* accounted for its strong radical scavenging activity, chelation of Fe^{2+} and inhibition of lipid peroxidation [4]. The phenolic compounds of *P. emblica* includes tannins, gallic acid, ellagic acid, mucic acid 1,4-lactone 3-*O*-gallate, isocorilagin, chebulanin, chebulagic acid, mallotusinin, monogalloylglucose, digalloylglucose, putranjivain A, galloyl-*HHDP*-glucose and elaeocarpusin [4,19].

Phenolic compounds have the ability to inhibit the survival of MCF-7 human cancer cells [4]. In addition, fruit extracts of *P. emblica* showed mosquitocidal activity where mainly phenolic compounds increased the mortality of *Anopheles stephensi*, which is a Malaria vector, [20]. It is also suggested that the antioxidant activity could be a result of a synergistic effect of several phenolic compounds in the *P. emblica* drupe.

Astringency and phenolic compounds

Astringency is the most dominant taste of *P. emblica* drupes [21]. The concentration of tannins in drupe is directly proportionate to the astringency of the drupe [22]. *P. emblica* showed a significant variation in drupe bitterness. The juicier and less bitter drupes harvested from China were low in ellagitannins [19]. This diversity in bitterness is seen among the Sri Lankan *P. emblica* germplasm as well.

Furthermore the drupe phenolic content and antioxidant activity was attributed to the genotype of the plant [23]. Yet it is not known whether an environment played a role in diversity of bitterness. Therefore the diversity in terms of bitterness could be an outcome of genetics coming in to play.

Amino acid content

Even though fruit species are not a good supplement of protein, it has been found that the protein content in *P. emblica* is 2.7 times than in species as apple (Table 5). Therefore the low energy value of the drupe is due to lower carbohydrate content [16].

Table 5 : Amino acid composition in *P. emblica* drupes

Amino acid	mg/ 100 g of drupe fresh weight
Alanine	24.0
Arginine	17.6
Aspartic acid	36.1
Glutamic acid	132.0
Glycine	19.8
Histidine	13.3
Isoleucine	12.0
Leucine	21.9
Lysine	23.6
Methionine	7.2
Phenylalanine	13.3
Proline	65.2
Serine	18.3
Threonine	13.5
Tyrosine	11.6
Valine	15.9

Source: [16]

Genetics and Genomics of *Phyllanthus emblica*

If the underlying genes and genetics of the fruit size variation can be molecularly characterized for fresh fruit crops like *P. embilica* such information can be used to develop superior varieties and to employ germplasm conservation measures. DNA fingerprinting can be used to accurately identify the genomic regions (QTLs) associated with agronomical traits (i.e. drupe size), medicinal traits (i.e. bitterness) and pharmacognostic traits (i.e. ascorbic acid content) in *P. emblica* germplasm.

Molecular characterization of the *P. emblica* germplasm

Molecular characterization of the *P. emblica* germplasm opens up new avenues to the prospect of the species [2]. In India, there have been studies on DNA fingerprinting of medicinally important species including *P. emblica*. But there too, the underlying genetics of diversity in drupe traits has not been studied extensively.

DNA fingerprinting

In India using seven different cultivars of *P. emblica*, a Sequence Characterized Amplified Region (SCAR) marker has been developed based on a Randomly Amplified Polymorphic DNA (RAPD) amplicon (Table 6). It has the ability to verify the presence of *P. emblica* in multi-formulated drugs [24] Similar DNA fingerprinting based herbal product authentication has been conducted for bamboo [25] and ginger [26] based products. In addition, species specific microsatellite markers which are polymorphic (Table 7) have been developed with the aim of using in diversity and population genetic studies of *P. emblica* [27].

Table 6: Species specific DNA markers for *P. emblica*

Marker type	Primer name	Sequence(5'à3')	Primer annealing temperature in PCR (^{0}C)
SCAR	D1	CAG ATC TCG TGT AAA AAG CGT TG	55
	D2	TGC AGT GAA TTC CAA GTG TTT C	
RAPD	OPA16	AGCCAGCGAA	36

Source: [24]

Table 7 : Species specific microsatellite markers for *P. emblica*

Marker name	Primer name	Primer Sequence (5'→3')	Primer annealing temperature in PCR (°C)
*Phyll*7	*Phyll*7F	CGGGAAAGAGAAACGAAATG	67
	*Phyll*7R	GCATCAGGTGGACTTCTTGG	
*Phyll*13	*Phyll*13F	AAGATCCGGCTTTAAAACTTTG	65
	*Phyll*13R	GCTAGCACTCTTCCTTCTTGC	
*Phyll*31	*Phyll*31F	AACTGGTGACTCCCCTTTACTC	50
	*Phyll*31R	TCCTTGGCTGAATTTTGGAG	
*Phyll*53	*Phyll*53F	CTTTCTCCAGCCACCAAATG	52
	*Phyll*53R	GTTGGTGGGTTTTCAACCTG	
*Phyll*68	*Phyll*68F	CAGGGACATTACACGGACAAC	53
	*Phyll*68R	CAGCCTAAGACAACTCTCATTTACC	
*Phyll*12	*Phyll*112F	TCGCTTTTATTTTCTTCAGTTCC	50
	*Phyll*112R	AAACCCACTGAGCATGAACC	

Source: [27]

DNA barcoding

DNA barcoding is an emerging trend which uses a short DNA sequence for species identification [28]. When barcoding flowering plants commonly used loci include *trnH-psbA* intergenic spacer, *matK, rbcL and trnL-F* [28]. Mitochondrial and plastid DNA markers still remain as the most easily assessable sequences for DNA barcoding. But it has been found that when dealing with plant barcoding multiple genetic loci might be necessary. Current trend requires multiple, low-copy nuclear genomic markers with sufficient genetic variability and PCR-reliability which will aid detecting hybrids and help correct species delimitation [29].

By attempting to barcode *P. emblica* germplasm in Sri Lanka it will help to establish any speciation of the germplasm in relation to drupe traits. The knowledge obtained will greatly aid in future germplasm conservation measures. Genomics is important to understand the genes of underlying traits and play a central role in crop improvement [30]. According to our knowledge there have not been any studies on the genomics of drupe size variation or any other traits in *P. emblica* in Sri Lanka. As it has been highlighted earlier *P. emblica* is a potential cash fruit crop that can establish well in the global market. Once the underlying genes have been identified they can be used to breed genetically superior varieties (i.e. *P. emblica* trees bearing larger and bitterer drupes which are equally important in fresh fruit market and in global herbal market). Thus studying thegenomics will be the foundation for the next level of breeding programs designed towards crop improvement.

Cytogenetic behaviour

The cytogenetic behavior of *P. emblica* is one of the least studied areas. The genus *Phyllanthus* is predominantly polyploidy and $X = [31]$. In *P. emblica* 2n= 94 – 104, a very high chromosome number. But still this involves lot of controversy and requires further cytogenetic analysis to establish the ploidy level [31].

When drupe traits and genetic diversity is combined there is a strong positive correlation between drupe cell size and ploidy in tomato. The drupe weight is also significantly correlated with the cell size and ploidy [32]. Considering the above facts, it is possible that the drupe size variation in *P. emblica* is also due to a similar phenomenon. Therefore in order to unravel the secret behind the diversity of *P. emblica* germplasm in relation to drupe traits, it is important to have a deeper look into its cytogenetic behavior.

Current knowledge on *Phyllanthus emblica* germplasm in Sri Lanka

Unlike in neighboring India where it is studied in much detail very little information on the *P. emblica* germplasm is available in Sri Lanka. Therefore *P. emblica* is still characterized as an underutilized fruit crop in Sri Lanka. Although any diversity mapping research has not been conducted so far, there have been research studies that were aimed at accessing the phytochemical properties of *P. emblica* that further substantiate the higher antioxidant activity of the drupe extracts [33].

Evidences points out that *P. emblica* germplasm is gradually eroding due to destructive harvesting [2] and cutting down of small fruited trees from home gardens (Pers. comm.). The potential to establish it as a fruit crop is greatly hindered due to lack of knowledge on the diversity, proper planting material and proper harvesting methods and storage [2]. Due to these flaws, Sri Lanka has to import dried drupes even to sustain the annual production of *Ayurvedic* remedies. Thus the *P. emblica* germplasm in Sri Lanka is in dire need of accurate diversity mapping as the basic step in large scale crop improvement programs.

Establishing the correct diversity structure will facilitate to promote *P. emblica* from its understudied status. Once the diversity has been mapped it opens up the avenue to crop development based on breeding methods. In turn it will increase the production of *P. emblica* based value added products. In an era where the world is prompted to "*Go Green*", studies in this line will certainly strengthen the Sri Lankan agricultural economy, bringing in extra revenue.

Potential advancements through molecular characterization of *P. emblica*

The diversity of the *P. emblica* germplasm can be seen in terms of drupe characteristics and the drupe is the most commercially important part of the tree. The diversity in relation to traits can be mapped based as QTL using molecular markers (*E.g.* AFLP, RFLP, SSR and SNP) (Table 8). The QTL will then provide foundation to dissect the genetic basis of traits and expedite the process of selection in plant breeding.

Table 8 : Example cases where markers assisted to dissect genes/QTL for important crop traits

Species	Trait	Gene/QTL	Marker
Barley	Leaf rust	*Rphq 6*	AFLP
Rice	Deep roots	QTLs on chromosomes 1, 2, 3 ,7 and 9	RFLP anSSR
Rice	Submergence tolerance	*Sub1* QTL	SSR
Wheat	Powdery mildew	*22 Pm* gene	AFLP

Source: [34]

Most of the world crops such as oil palms (35) have been subjected to molecular characterization with the aim of developing genetically superior verities. This breeding methodology is named marker assisted selection (MAS). Many of the commercial species are now using MAS in order to produce genetically improved varieties all over the world (Table 9).

Table 9 : Few of the many institutes carrying out marker assisted selection in breeding

Institute	Country	Crop Species
IRRI	The Philippines	Rice
University of Guelph	Canada	Bean
CIMMYT	Mexico	Wheat and Maize
University of Adelaide	Australia	Wheat
Michigan State University	United States of America	Soybean, potato and dry beans
Cornell University	United States of America	Grapes
University of California , Davis	United States of America	Cattle and wheat
University of British Columbia	Canada	Lavender
The New Zealand Institute for Plant and Food Research	New Zealand	Kiwi fruit and Potato

Source: [33]

Potential as a cash crop

Currently in Sri Lanka *P. emblica* is utilized only in *Ayurvedic* remedies, cosmetics and beverage industry. Yet it has a high potential as fresh fruit that can even beat the market for apple in terms of its high nutraceutical value. But this requires an ideotype that can sustain itself in the global market.

Genetically superior varieties can be developed through breeding techniques. But conventional breeding will be time consuming for these kinds of perennial herbs [35]. Various molecular biological techniques are available today for detection of genetic variability and for establishing genetic similarity relationships in crosses. Thus molecular characterization of *P. emblica* germplasm aids molecular breeding techniques aimed at developing an ideotype for the market (Figure 1).

In Sri Lanka our research team has conducted research to dissect the underlying genes and alleles related to drupe size. There, an allele at 176 bp of the marker *Phyll* 112 [27] showed the highest significant association ($P<0.001$ and $P<0.0001$) with commercially important traits such as drupe height, width, weight and mesocarp thickness [36]. These alleles are linked to putative QTLs that can be used in future breeding programmes in breeding superior varieties.

Once an ideo type is developed, it has an equal potential to conquer the fresh fruit market as well as the indigenous medical industry. It can be used to develop value added products locally without depending on imported *P. emblica* drupes. Therefore molecular characterization of the *P. emblica* germplasm is a key that opens up dozens of avenues that strengthen its value, utilization and also germplasm conservation.

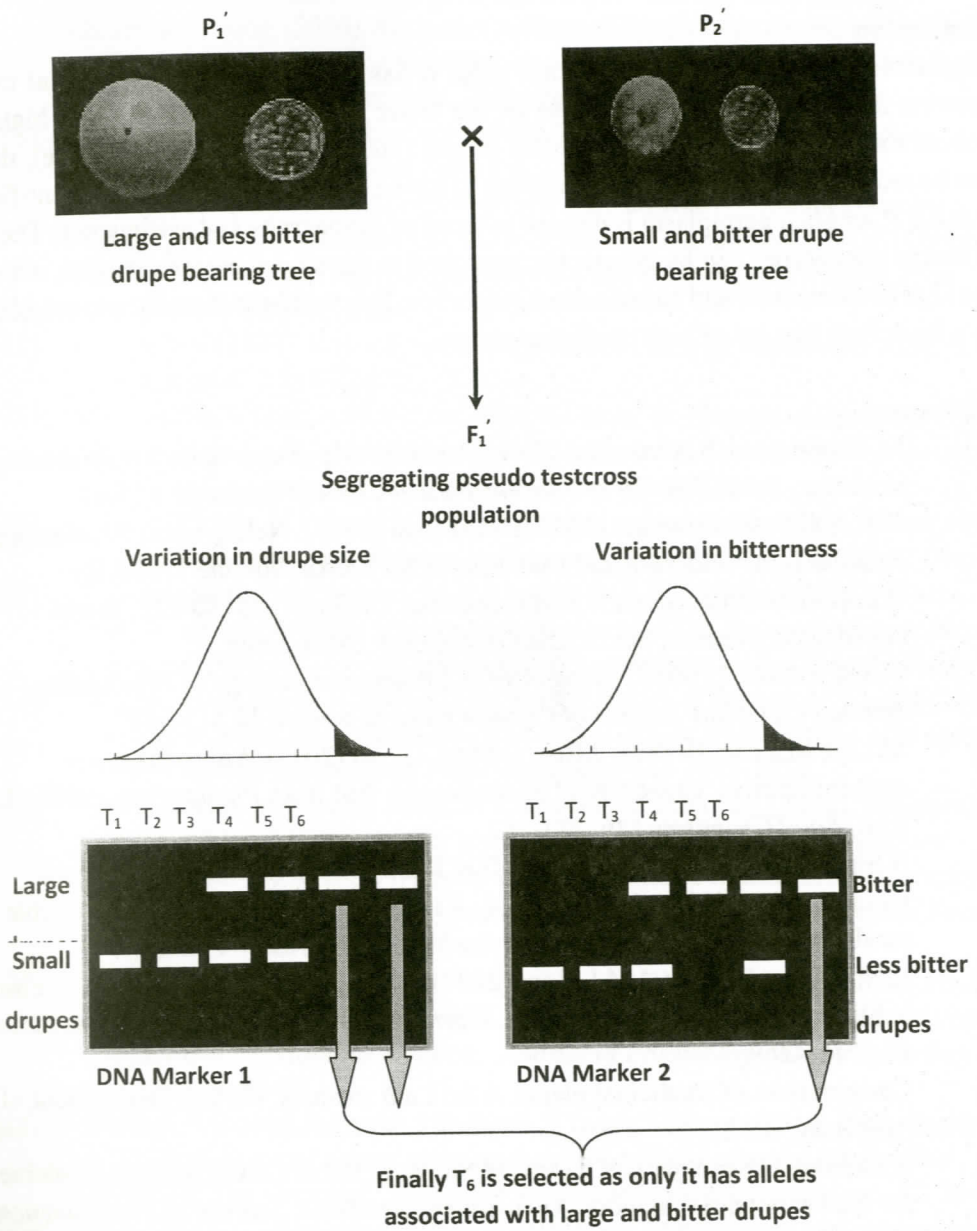

Figure 1 : Marker assisted selection in developing an ideotype for *P. emblica*. P_1' and P_2' are not true homozygotes and F1' is not heterozygous instead a pseudo test crosses population with mixtures of genotypes. In F_1' drupe size and bitterness are distributed quantitatively. T is indicates trees in F_1' population. The marker alleles that are linked with drupe size and bitterness are used for the selection of large and bitter druped plants in breeding and selection of seedlings for new establishments.

Conclusion

Phyllanthus emblica is a potential cash crop. It has a high therapeutic potential that answers number of ailments. Because of the lower carbohydrate content and higher concentration of Vitamin C in *P. emblica* drupes (160 times higher than in apple), this can be recommended for daily consumption to have a healthy life. It has been identified that the *P. emblica* germplasm is diverse in terms of drupe traits and astringency. These different phenotypes can be genetically exploited to develop superior varieties, which are high in therapeutic and nutraceutical properties. This will help *P. emblica* to establish as a fresh fruit species of high medicinal value.

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