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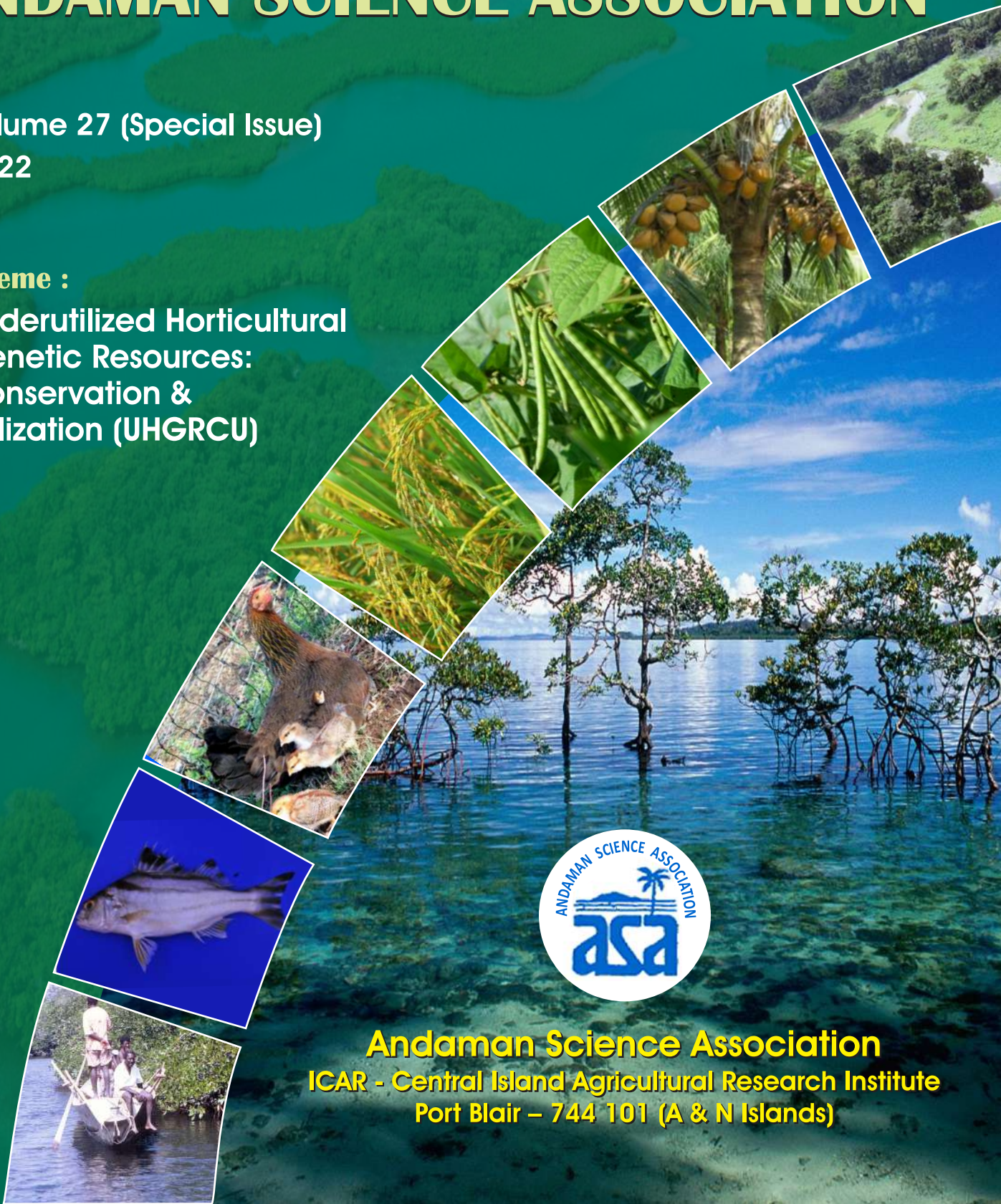
Underutilized Horticultural Genetic Resources: Conservation & Utilization (UHGRCU)



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About Andaman Science Association (ASA)

Andaman and Nicobar Islands which are situated at Latitude 6°- 14° N and Longitude 92°-94° E, consists of 836 green emerald islands, islets and rocks. Almost all the islands are oriented in the north-south direction like an arc and stretch over a length of about 1912 km. Most of the islands are originated due to volcanic eruption in the sea, while few of them originated by coral reef formation. All the islands are biological and geological paradise having indigenous biota of flora & fauna and geological wonders which are yet to be unearthed. To conduct research in these small and fragile islands, there are several scientific organizations that have been doing focused research in different areas. However, there was no platform for them to present their research works which had local importance. Andaman Science Association (ASA) was established in 1984 at CARI, Port Blair with an objective to promote research and development in the unique Island ecosystem. Journal of the Andaman Science Association, published by ASA, is a unique journal that provides platform for those associated with tropical and island agriculture, horticulture, fisheries, animal science, oceanographic and marine biological studies, disaster management, issues related to science and technology, environment, forest etc. ASA intermittently organizes National and International seminars, conferences, knowledge sharing meets etc.

- Dr. Eaknath B. Chakurkar
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Editorial

Underutilized Horticultural Genetic Resources: Conservation and Utilization (UHGRCU)

India is bestowed with rich plant diversity including the diversity of important horticultural species. Many of these species, though have potential, are mostly grown regionally and are poorly studied. Further, landraces and wild relatives of commercially cultivated species are fast disappearing due to several reasons. Lack of awareness about these genetic resources coupled with limited traditional preferences for them pose specific challenges in their conservation and utilization. Creation of awareness about their nutritional and ecological importance is the foremost requirement to attract the attention of researchers/ stakeholders on these issues. The present Special Issue deals with articles representing various research aspects of underutilized horticultural genetic resources.

As the special issue is being launched after the immensely successful National Conference on Underutilized Horticultural Genetic Resources: Conservation and Utilization (NCUHGR-2022), a succinct event report has been included for the benefit of the general readers. Invited article on kokum (*Garcinia indica*) is a success story of conservation and sustainable utilization of a threatened species through systematic interventions. An overview of research efforts made for crop improvement in indigenous vegetables of Andaman and Nicobar Islands has been presented. The article on interspecific diversity of fagaceous nuts from Meghalaya creates awareness about this largely lesser known group of temperate nuts. Intraspecific natural diversity of *Artocarpus heterophyllus* from Tripura, *Annona reticulata* from Maharashtra and *Zanthoxylum rhetsa* from Goa has been studied in respective articles for various morphological and biochemical parameters with an objective to identify desirable genotypes. The article on *Trichosanthes dioica* deals with DUS characterization of genotypes for morphological parameters. Studies on parameters influencing germination and seedling vigour in endemic *Garcinia dhanikhariensis* and seasonal performance of vegetative propagation techniques in *Tamarindus indica* have been included in this issue. Effect of vermicompost was studied on an herbal spice- *Eryngium foetidum* in a novel production technique developed for urban and peri-urban cultivation. Significance of weeds found in the interspaces of oil palm plantations has been highlighted in a study from Andhra Pradesh. Standardization of ultrasound assisted extraction process for gymnemagenin in *Gymnema sylvestre* provides interesting information on this medicinally important species. Processing of *Moringa oleifera* leaves using different methods and their effect on its sensory parameters in different packaging materials was also studied and included in the present issue.

Through this issue, an effort has been made to highlight the need and significance of underutilized horticultural species for achieving livelihood, nutritional and ecological security.

Jai Hind!

Prof. Dr. Sisir Kumar Mitra
Dr. Desh Beer Singh
Dr. Pooja Bohra
Dr. Ajit Arun Waman
[Editors, JASA (Special Issue- UHGRCU)]

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Conference Report

Underutilized Horticultural Genetic Resources- Assets for the Present and Future

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Horticulture sector broadly deals with the garden crops *viz*. fruits, vegetables, ornamental plants, spices, plantation crops, medicinal & aromatic plants etc. and for most of these commodities, India is among the major producing nations. However, a number of lesser known and regionally popular species exist in different parts of our country, which have traditionally been used for food, medicines, aesthetics, natural colourants, and other purposes. Most of these species are collected from the natural stands and are rarely cultivated. Commercial production in most crop plants has been hampered by biotic as well as abiotic stresses globally. In the recent past, underutilized horticultural genetic resources (UHGRs) including crop wild relatives (CWRs) have gained importance as potential reservoirs of desirable genes/ traits for addressing the long standing issues of commercial crops. These resources could be of significance as a parent in classical/ advance breeding programmes or as a rootstock for challenging conditions. Besides, few potential species could be brought under cultivation through domestication, which would help in diversifying the cropping system and thereby mitigating the impact of climate change.

In order to sensitize the stakeholders about the importance of rather less-researched genetic resources, ICAR- Central Island Agricultural Research Institute (CIARI), Port Blair in collaboration with Andaman Science Association, Port Blair and Department of Biotechnology, New Delhi organized two days Virtual National Conference on "Underutilized Horticultural Genetic Resources: Conservation and Utilization" during June 3-4, 2022. Dr. E. B. Chakurkar, Director, ICAR-CIARI, Port Blair and Convenor of the event in his opening remarks enlightened the gathering about the need for conducting this event on the occasion of World Environment Day and relevance of native biodiversity in providing nutritional and livelihood security to the masses. The conference was inaugurated by Dr. Chittaranjan Kole, President, International Climate Resilient Crop Genomics Consortium; International Phytomedomics & Nutriomics Consortium and Former Raja Ramanna Fellow. He emphasized upon conserving the precious biodiversity of the Andaman and Nicobar Islands and its sustainable utilization for mitigating the challenges posed by the climate change. He suggested dedicating identified islands as conservation sites for the endemic and rare species of these islands.

Padma Shri Dr. Brahma Singh, Founder, Prof. Brahma Singh Horticultural Foundation, New Delhi and Former Secretary, Life Sciences Research Board, DRDO delivered his Keynote address on importance of UHGRs with special reference to Sea buckthorn. He also sensitized the participants about the potential of local species to cater the global markets. Systematic efforts were envisaged for making the regionally popular species accessible in the domestic as well as international markets through appropriate processing technologies. Dr. Singh elaborated upon how scientific and technological interventions helped in improving the awareness and marketability of sea buckthorn, which was once considered as a weed.

Dr. N.K. Krishna Kumar, Former DDG (HS), ICAR, New Delhi and Former Regional Representative (South and Central Asia), Bioversity International delivered a Keynote Lecture. He highlighted the need of adopting the holistic approach to achieve environmental, ecological and economic security. He elaborated upon the need to develop agrobiodiversity index and identification of keystone species and pollinators in biodiversity rich areas. It was emphasized that focus is required for studying the nutritional requirement of aging population as India would transform from nation with young population at present to one of the old nations in coming 2-3 decades.

Identification of specific crops/ species as a source of specific nutrients/ bioactive molecules would help in targeted production of these commodities in future.

Technical sessions covered four invited talks and 88 presentations on various themes ranging from conservation, domestication, cultivation, improvement, postharvest management and marketing of UHGRs. Need for conservation, documentation and registration of novel germplasm was stressed upon. Especially the regions of high agro-biodiversity, which are under the risk of climate change, need to be focused and systematic plans to be chalked out to preserve the precious germplasm. Dr. Veena Gupta, Principal Scientist, ICAR-National Bureau of Plant Genetic Resource, New Delhi in her invited talk, highlighted the important horticultural plant genetic resources conserved in the national gene bank at its headquarters as well as regional stations.

Promotion of cultivation and value chain development is considered as a key for promoting underutilized species. Dr. P.C. Tripathi, Principal Scientist, ICAR-Indian Institute of Horticultural Research, Bengaluru highlighted various steps taken in this direction and challenges faced in the process, mainly in the perennial fruit bearing species. Dr. P.M. Haldankar, Director of Research, Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli suggested the road map for domestication and sustainable utilization of potential species, citing his personal experiences with *Garcinia indica*. The species was restricted to wild/ semi wild conditions; however, due to systematic efforts, its cultivation and commercial scale processing has now spread across several coastal states of our country. Presentations on diversity of underutilized vegetables, native fruits and spices of the islands and their present state of utilization were made during the event by Dr. Shrawan Singh, Senior Scientist, ICAR-Indian Agricultural Research Institute, New Delhi and Dr. Pooja Bohra, Scientist-SS, ICAR-Central Island Agricultural Research Institute, Port Blair.

During the valedictory session, Dr. A.K. Singh, DDG (HS), ICAR, New Delhi highlighted the need to take up systematic research on perennial lesser known horticultural species, especially in the wake of climate change. He suggested that policy decisions to include potential native species under different government schemes could give impetus for their popularization. Dr. T.V.R.S. Sharma, Former Member, General Body, ICAR, New Delhi stressed upon the vulnerability of island ecosystem to climate change and role of CWRs in meeting the challenges of horticultural sector. Use of local diversity for achieving prosperity especially in the far flung regions was envisaged by Dr. Sharma. Dr. S. Dam Roy, Former Director, ICAR-CIARI, Port Blair highlighted that though the islands as well as other agro-biodiversity hotspots of the country hold precious germplasm, characterization of most of these species are yet to be attempted and the conference could attract the attention of various researchers on such important aspect. Two hundred ninety-one participants from 89 organizations/ institutes of 30 states/Union Territories of the country registered for the event. As most of the underutilized horticultural species are region specific in nature (thus not known to many), an e-book of abstracts (ISBN: 978-81-957481-0-5) with colourful photographs of the discussed species was also published during the event. The book is available online on the website of Authors' Institute and is freely downloadable.

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Invited Article

Promotion of Underutilized Kokum (*Garcinia indica* Choisy.) through Scientific Interventions: an Overview

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Abstract

Kokum (*Garcinia indica* Choisy.), a native species of the Western Ghats in India, is an important tree spice that has largely remained underexploited. It is found in the home gardens and is cultivated at a limited scale as a rainfed crop in the Konkan region, usually mixed with other fruit trees. Fruits are commercially exploited for making traditional products from rind such as Kokum Syrup (*Amrit Kokum*), *kokum agal* (salted syrup) and *amsul* (dried rind). The oil extracted from seeds is edible and remains solid at room temperature. Kokum is useful in the treatment of rashes caused by allergies, burns, scalds and chaffed skin. It is known to relieve sunstroke, dysentery and mucous diarrhea apart from use as an appetizer, liver tonic, cardio-tonic *etc*. It is a source of active ingredients such as hydroxycitric acid (HCA), garcinol (a polyisoprenylated benzophenone), anthocyanins and other compounds with potential antioxidant properties. Apart from traditional value added products, novel products such as rind powder, sherbet mix, *solkadhi* mix, wine, honey, beverage dip bags etc. have recently been prepared from Kokum. In future, emphasis needs to be given on economic exploitation through organized plantations of elite types, development of value chain management and development of novel value added products.

Key words: *Kokum, post harvest management, value addition, phytochemicals*

Introduction

The Western Ghats covers an area of 160,000 km^2 in a stretch of about 1,600 km and is situated parallel to the western coast of the Indian peninsula along the states of Gujarat, Maharashtra, Goa, Karnataka, Kerala and Tamil Nadu. Konkan region of coastal Maharashtra and Goa is a narrow strip of land between Arabian Sea and the Western Ghats. Geography, geomorphology and climate of this region have given rise to high heterogeneity which has contributed to its vast and unique diversity. Various horticultural crops such as mango, cashew nut, coconut, arecanut, kokum, jackfruit, jamun, nutmeg, cinnamon *etc*. are specialty of this region.

Among these, kokum (*Garcinia indica* Choisy) is a unique tree spice belonging to the family Clusiaceae. Some of the species in this family possess medicinal properties, whereas most of the plants are known for their oil glands or secretary canals or cavities, which contain yellow or brightly coloured resins. Kokum is one of the important tree spices that have largely remained underexploited and neglected. It is native to the Western Ghats in India and is mostly found in Konkan region of Maharashtra, Goa, Coastal Karnataka, Kerala, forests of Assam, Khasi and Jaintia hills of Meghalaya, West Bengal and Surat of Gujarat.

Kokum is found in evergreen and semi-evergreen forests, as a home garden tree and is cultivated on a limited scale as a rainfed crop or as a mixed crop in plantations of coconut and arecanut. The defined statistics regarding area under kokum production and productivity is not documented as it is not being planted in an organized pattern as that of commercial fruit crops like mango, cashew, arecanut or coconut. As per a baseline survey (2010), about 1,000 ha area is occupied by kokum in Konkan region with production of 4,500 t fruits. According to survey conducted earlier by the Chief Conservator of Forest, out of the total 46,600 Kokum trees in the state of Maharashtra; 43,000 trees existed in the Ratnagiri and

Sindhudurg districts. It was also reported that in South Konkan, 1,674 t of fruits were used for dried Kokum rind, 757 t for preparation of Kokum syrup and 40 t for Kokum butter.

Uses

Kokum is used in many parts of the country for making several vegetarian and non-vegetarian culinary preparations, including the popular *'solkadhi'* and the sugar syrup '*Amrit kokum'*. *Amrit Kokum* makes an excellent sherbet and is useful in as a cool and refreshing drink. Besides this, *kokum agal* (salted syrup) and *amsul* (dried rind) are traditionally prepared from rind of fruit and oil is extracted from seeds.

Kokum is mostly used in the form of dried rind to give acid flavour to curries and the fresh fruit juice for preparing cooling syrup and curries. It has tremendous potential in curries as a substitute to tamarind. Many therapeutic effects of kokum fruit have been described in traditional medicine based on Ayurveda. These include its usefulness as an infusion in skin ailments such as rashes caused by allergies, treatment of burns, scalds and chaffed skin, to relieve sunstroke, to cure dysentery and mucous diarrhea. It is a good appetizer, liver tonic, cardiotonic and is useful in the treatment of bleeding piles, tumors and heart diseases.

One of the active ingredients of kokum, hydroxycitric acid (HCA), has been patented for use as a hypocholesterolaemic agent. HCA is a potential anti-obesity agent. It suppresses fatty acid synthesis, lipogenesis, food intake and induces weight loss. Garcinol, a polyisoprenylated benzophenone purified from *G. indica* fruit rind, displays antioxidant, anti-cancer and anti-ulcer properties. Apart from HCA and garcinol, kokum contains other compounds such as citric acid, malic acid, polyphenols and ascorbic acid, with potential antioxidant properties.

Recently, *Garcinia* species have received considerable attention worldwide from scientific as well as industrial sectors and several novel structures, bioactivities and potential utilities have been reported. Kokum is being considered as a functional food that provides, in addition to nutritional components, other physiological benefits. The consumption of high value products of kokum has increased tremendously due to the awareness of the potential health benefits associated with the diverse bioactive constituents in the plant.

Improved varieties

Systematic research on collection, characterization and evaluation of kokum was carried out at Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli, Maharashtra. After the evaluation, two varieties of kokum have been developed for promoting commercial cultivation in the region. The important morpho-physiological characters of these varieties are given in Table 1.

Table 1. Key features of improved varieties of kokum

Characters	Konkan Amruta	Konkan Hatis
Yield (7 years, kg)	138	250
No. of fruit per kg	29	11
Length of fruits (cm)	3.74	4.22
Circumference of fruit (cm)	13.15	20.10
Wt. of fruit (g)	34.45	91.50
Wt. of rind (g)	17.55	48.34
No. of seed/fruit	6.40	5.60
Shelf life (days)	15	18
Volume of fruit (ml)	35.50	112.8
Diameter of fruit (cm)	3.95	4.20

Thickness of rind (mm)	4.45	5.58
T.S.S.(°B)	9.08	9.20
Reducing sugar (%)	2.41	2.40
Total sugar(%)	4.52	4.10
Acidity	5.12	5.10
pH	1.81	1.80
Flower bud appearance	1st week of October	2nd week of November
Initiation of flowering	2nd week of November	2nd week of December
Harvesting period	March-April	April-May

Crop cultivation

The seedlings start flowering 7 to 8 years after planting, whereas flowering in grafts is noticed after 3 to 4 years. Generally, kokum plant flowers during December to January. Flowers are borne singly or as fascicular cymes on leaf axils and are tetramerous. The period from flower bud appearance to initiation of flowering is about 30 days. Pollination is through wind. The fruits are harvested after about 120 days of fruit set. Kokum fruits are ready for harvesting from April to May and most of the fruits are harvested in May-June which is the start of rainy season. Nearly 40-70% of the fruits are trapped in rains and hence lost. Presently in Konkan region alone, this loss is estimated to be of Rs. 157 lakh. Not only farmers suffer seriously because of this loss but the processing industry is also adversely affected as large quantity of kokum fruits is required for value addition. Post flowering foliar spray of potassium nitrate and monopotassium phosphate helps to pre-pone harvesting by about 10 to 34 days (Haldankar et al., 2012b).

All kokum fruits on a tree are not ready for harvesting at a time and hence periodical plucking is done. The number of pluckings varies from tree to tree. Generally, 6 – 8 pluckings are required in high yielding plants. Number of pluckings in kokum is a constraint in harvesting. Spraying of ethrel (300 ppm) at the stage of full maturity of kokum fruits helps to facilitate harvesting by reducing the number of pluckings and improving the yield as well as chemical composition of fruits. Fully ripe fruits are plucked by hand. Skilled persons climb on the tree and shake the branches. The ripe fruits which fall down are collected. It leads considerable loss of fruits. Approximately 35-40% fruits are lost, which include immature and broken fruits. In a seedling population, 30-50 kg yield per plant is obtained. In a well managed plantation, 100 kg yield per plant could be obtained (Haldankar et al., 2012a). When kokum is planted as mixed crop in coconut plantation, 15 kg yield per plant is obtained. Annual fruit yield fluctuation is reported in kokum and higher yields are reported every alternate year. Considerable variability in physico-chemical composition of kokum is also noticed. The harvested fruits are exclusively used for processing.

Postharvest handling

The postharvest operations *viz*. grading, packaging, storage and transport play significant role in processing of kokum and shelf life extension (Haldankar et al., 2020). The shelf life of kokum fruits is 5.4 days under ambient temperature storage. It can be extended to 15 days when treated with Waxol (12%) and stored in cool chamber, and up to 28 days when stored at 13 °C ± 1 °C with 86% RH and Waxol (3%) treatment. CFB boxes and paddy straw are good packaging material for kokum.

Value addition

Traditionally, fresh fruits are collected from the forest areas, pooled and marketed. Freshly harvested fruits are reddish green in colour, which turn into full-red to purple colour in a day or two. The fruit has an agreeable flavour and sweetish acid taste. Various value added products prepared from kokum are given below (Thakor et al., 2012).

Dried rind

The normal shelf-life of the fresh fruits is about 5 days. Hence, sun drying is practiced for its preservation. For sun drying, the fresh fruits are cut into halves and the fleshy portion containing the seeds is removed. The rind constitutes about 50-55% of whole fruit. It is repeatedly soaked in the juice of the pulp during the sun drying. About 6-8 days are required for complete drying. The product so dried, constitutes the unsalted Kokum of commerce. A salted variety, wherein common salt is used during soaking and drying of the rind is also marketed. *Lonavala kokum*, *Pakali kokum*, *Khanee* or edible kokum and *Khoba kokum* are some of the trade varieties. The composition of the fresh kokum rind is given in Table 2.

Table 2. Composition of the fresh Kokum rind

Particulars	Value
Moisture (%)	87.50
T.S.S. (°Brix)	16.44
Protein (N × 6.25%)	1.92
Crude fibre (%)	14.28
Total ash (%)	2.57
Tannins (%)	2.85
Pectin (%)	5.71
Starch (%)	1.00
Crude fat (%) (Hexane extract)	10.00
Acid (as hydroxycitric acid)	22.80
Pigment (%)	2.4
Ascorbic acid (%)	0.06
Carbohydrates by difference* (%)	35

Raw Kokum juice extraction and beverage

After destalking and washing of fruits, the seeds are removed from the fruits. Then the rind pieces along with juice from the fruit are passed through hand operated screw type juice extractor. The juice obtained is filtered through four folds of muslin cloth and the clear juice obtained is used to prepare the ready to serve (RTS) beverage, squash and syrup. For RTS, the total soluble solids content and acidity of different juices are observed. Required quantity of citric acid and sugar is added to juice (20%) to raise its °Brix and acidity to 20 °Brix and 0.3%, respectively. Sodium benzoate (140 mg/kg) is added to the product as a preservative. After adding required quantity of sugar, citric acid and water, the product is boiled for few minutes to dissolve the ingredients and preservative is added. Beverage is then filled into the pre-sterilized glass bottles and sealed with crown corks. Then the bottles are pasteurized for 30 minutes in boiling water, removed, cooled, labelled and stored in cool and dry place at ambient temperature.

Squash

The TSS content is noted and required quantity of sugar is added to juice (25%) to raise its TSS to 45 °Brix. Acidity of 1.2% is maintained in this product. After adding the necessary quantity of sugar the product was boiled to dissolve the ingredients. Preservative *i.e.* sodium benzoate is added at the rate of 610 mg/kg of final product. The final product is filled immediately into the pre-sterilized glass bottles followed by sealing of bottles with the crown

corks and pasteurization for 30 minutes in boiling water. The filled bottles are then removed, cooled, labelled and stored at cool and dry place at ambient temperature.

Syrup (Amrut Kokum)

Fresh and sound fruits are selected, washed with water and stalks are removed. The fruits are cut into four pieces, and pulp and seeds are removed. The pieces of rinds are mixed with sugar in 1:2 (rind: sugar) proportion. This mixture is kept in a big stainless steel vessel for 7 days. The mixture is well stirred every day. After 7 days, whole juice from Kokum rind gets extracted due to osmosis and whole quantity of sugar gets dissolved in it. The syrup is then strained through 1 mm stainless steel sieve to separate out the rind portion. The preservative i.e. sodium benzoate at the rate of 610 mg/kg of the finished product is added. The syrup is filled in the pre-sterilized glass bottles. The bottles are then crown corked, labeled and kept in a cool and dry place at ambient temperature conditions.

Ripe Kokum Rind Powder

Fresh fruits are cut into pieces and pulp and seeds are removed. The pieces are then dried in cabinet drier at 50- 55 °C followed by powdering in electrically operated grinder. The powder is then sieved through 1 mm mesh sieve and then packed in polythene bags and kept in a cool and dry place at ambient temperature condition.

Brined Kokum Juice (*Agal*)

The fruits are cut into pieces and inner pulp and seeds are utilized. The pulp along with the seeds is taken into a big stainless steel vessel. The salt at the rate of 160g/ kg pulp is added. The mixture is stirred daily for seven days. After seven days, the whole mixture is strained through stainless steel sieve and filled in pre-sterilized bottles.

Kokum RTS

The RTS of kokum can be prepared by diluting kokum syrup to 1: 5 proportion with UV purified water. Salt and cumin powder is added to it for taste.

Natural kokum juice

Kokum juice extracted from kokum rind can be preserved for longer duration after adding 1,000 ppm sodium benzoate and stored at cool and dry place. This juice can be used for preparation of various processed products.

Kokum Butter

The oil is traditionally extracted by boiling the kernels in water and the oil which collects at the top is skimmed off. Nowadays oil is also extracted by solvent extraction. The yield of oil (fat) is about 25%. The fat is greasy to feel and whitish yellow in colour. Kokum seed contains 23-26% edible oil, known as kokum butter. It remains in solid state at normal mean temperature. The chemical characteristics of the butter are as given in Table 3.

Table 3. Chemical Characteristics of the Fat

Particulars	Value
Melting point	39 - 43°C
Sap value	189
Iodine value	34.7 - 36.7
Unsap matter	1.4%
The component fatty acids	
Myristic acid (%)	0-1.2
Palmitic acid (%)	2.5-5.3
Stearic acid (%)	52.0 -56.4
Oleic acid (%)	39.4-41.5
Linoleic acid (%)	1.7

Kokum fat has been reported to be used in chocolate and confectionery preparation. It is also used in the manufacture of soap, candle and ointments. An ointment made out of Kokum fat, white dammar resin (resin exuded by *Vateria indica* tree) and wax is said to be effective in treating carbuncles.

Table 4. Nutraceutical activity in butter of various *Garcinia* species

Content	Nutraceutical activity
Palmitic acid (%)	• Ionic surfactant (soaps, cosmetics and releasing agents) • Softens skin, heals ulceration, fissures of the lips, hands and soles of feet. • Controls obesity and helps to recover some reproductive abnormalities • Diet enriched, good for diabetes
Stearic acid (%)	• Use in soaps, detergents, shampoo, shaving creams and other cosmetic products • Used in margarine and other spreads • Diet enriched, 14 % total plasma cholesterol decreased
Oleic acid (%)	• Hinders the progression of adrenoleukodystrophy, a fatal disease that affects the brain and adrenal glands
Linoleic acid (%)	• Helping people loose body fat & controlling weight • Possibly preventing colon or breast cancer. • Strong antioxidant, lowering high cholesterol
Arachidic acid (%)	• Anti-inflammatory diet
Vit. E (mg/100g)	• Antioxidant
Phytosterols (0.10 – 1.02)	• Block cholesterol absorption sites in the human intestines • Cardio-tonic
Monounsaturated fats (34.5-39.7 %)	• Improve heart health, help to lose belly fat • Keep cholesterol down
Polyunsaturated fats (1.32-11.38%)	• Lowers LDL cholesterol level, raises HDL cholesterol level • Reduces inflammation

(Utpala and Nandakishore, 2014)

Novel value added products

Kokum Sherbet Mixture

It is an instant product (ready to prepare) in which kokum powder, sugar and spices are added in various concentrations and the mixture is dried in a tray dryer to get the Kokum Sherbet Mixture.

Kokum Solkadhi Mixture

It is an instant product (ready to prepare) in which kokum powder, coconut milk powder, milk powder, salt, sugar and spices are added in various concentration and the mixture is dried in a tray dryer to get the Kokum *solkadhi* mixture.

Kokum Wine

Majority of produce in kokum is used for syrup and juice preparation during summer months apart from some produce which is dried and stored. Remaining part is not harvested and goes as waste. It can be used to produce fermented beverages like wine. The kokum juice is having dark colour and more acidity, hence in order to reduce colour and acidity of wine and to get good amount of quality wine with light alcohol, the wines were prepared from kokum juice by diluting the juice and adjusting the pH levels of must (Pawaskar et al., 2020). Kokum juice has about 4% sugar and can be fermented to produce wine. Kokum wine is prepared in Goa using the traditional method with commercial baker's yeast.

Kokum Honey

Honey is concentrated floral nectar. So far no efforts are reported to establish apiculture unit in Kokum plantations. But, if this is done then 'Kokum honey' can be obtained with excellent medicinal qualities.

Benzophenone derivatives

Garcinol-1 is a polyisoprenylated benzophenone derivative from kokum and other species. The dried rind of kokum contains 2-3% garcinol. Garcinol is structurally similar to a well known antioxidant-Curcumin, which contains both phenolic hydroxyl groups and diketone moiety. Garcinol has been reported to possess antibiotic activities, antiulcer activities, suppressed colonic aberrant crypt foci (AFC) formation, and induction of apoptosis through cytochrome C release and activation of caspases in human leukemia HL-60 cells.

Anthocyanins pigments

Kokum is a rich source of anthocyanins. The red colour in kokum is due to presence of anthocyanins such as Cyanidin 3-glucoside and cyanidine 3-sambuboside. The pigment content in kokum is 1,000-2,400 mg/100g. Anthocyanins are considered as potential replacements to synthetic colours because of their bright attractive hue and water solubility that allows their incorporation into aqueous food systems. They also possess health benefits.

Hydroxycitric acid (HCA)

One of the important uses of kokum is as an anti-obesity agent. The bioactive phyto-chemical responsible for this property is *Para* - hydroxycitric acid and is found in abundance, ranging from 10-30% in the rind of dried fruit of kokum. Attempts were made at author's institute to assess these phytochemical properties of selected kokum types. The highest anthocyanins were observed in two types, which were greater than the earlier reports. Similarly, HCA content was found highest in Variety Kokum Amruta, which was better than previous reports. Further, three types from Diveagar location had superior HCA content.

Development of process of preparation of Kokum beverage dip bag

A novel kokum beverage dip bag product was recently prepared and patented (application ID: 201721009815) by the author's institute. The process includes a) extraction of juice from fresh kokum fruit rind by means of basket press, b) treating the rind residue with sodium chloride (common salt) c) drying the rind residue in cabinet dryer d) grinding the dried residue to obtain a free flowing powder (infusion material) e) filling the residue powder in the double layer filter paper bags and heat sealing The kokum beverage can be prepared by dipping the bags having kokum rind residue infusion material in chilled water for kokum juice infusion and addition of powdered sugar in the reconstituted juice.

Economics

The processing of the kokum fruits is a home scale as well as the commercial (factory) entrepreneurship avenue. The benefit: cost ratio for the processing is given in Table 5. These entrepreneurship options could create more opportunities for employment to family labour (Kshirsagar et al., 2014).

Table 5. Benefit: cost ratio for kokum processing

Particulars	B : C ratio		
	Dried kokum rind	Amrit kokum	Kokum agal
Home scale processing	1.43	2.11	1.40
Commercial (factory)	1.52	1.23	1.83

(Kshirsagar et al., 2014)

The economics of the fresh fruit and value added products of kokum is given in Table 6. It is apparent that the entrepreneurship of value addition of kokum fruits definitely gave higher economic returns to growers.

Table 6. Economics of fresh fruit and value added products of kokum

Particulars	Value
Average yield of kokum fruits/plant	30 kg
Returns from the sale of fruits/plant (@ Rs. 20/- per kg)	Rs. 600/-
Preparation of kokum syrup from 30 kg fruits (15 lit recovery)	Rs. 2,250/-
Sale of RTS by vending machine (90 lit) (@ Rs. 10/- per 200 ml tetra pack)	Rs. 4,500/-

Prospects for exploitation of kokum

Besides the novel medicinal and phytochemicals properties of kokum, it has largely remained commercially underexploited and hence, emphasis needs to be concentrated on economic exploitation through various approaches.

1. Organized plantation of elite types and development of cluster model, contract farming with backward and forward linkages.
2. Development of value chain for reducing postharvest loss.
3. Development of novel value added products.
4. Value addition of immature fruits.
5. Evaluation of diversity of kokum with special reference to phytochemically superior types.

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Genetic Resources and Crop Improvement in Underutilized Vegetables of Andaman and Nicobar Islands

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Abstract

The indigenous vegetables (IVs) constitute important sources of dietary micronutrients and livelihood support for poor and marginal communities in rural and tribal regions. Their role has been well accepted in food-based approaches to fight against micronutrient and vitamin deficiencies which are of main concern for attaining Millennium Development Goals. Although, worldwide conservation efforts resulted in huge germplasm conservation at AVRDC-the World Vegetable Centre, Taiwan and National or Regional Gene Banks, but, received little or negligible attention for breeding of superior genotypes. These vegetables are vital for survival and health of marginal forest dwelling communities and remote rural areas, particularly in underdeveloped and developing countries. In the present paper, the efforts done at ICAR- CIARI, Port Blair for exploring the local diversity of indigenous vegetables of Andaman and Nicobar Islands, India are presented. During 2008-14, around 200 local germplasm of indigenous vegetables were collected and improved varieties were developed which showed remarkable gain in yield, uniformity and quality traits during selection process. Similar efforts to develop promising genotypes in locally adapted indigenous vegetables would help to tap their potential in achieving nutritional and livelihood security of island population.

Key words: *Breeding, Indigenous vegetables, nutrition, tribes, tropical islands*

Introduction

About 3 billion people in the world are malnourished and more than 70% of malnourished children live in Asia alone. Nearly half of the world's micronutrient-deficient population is in India (Stein and Qaim, 2007). It has been estimated that around 6.7 million deaths worldwide were attributed to inadequate intake of fruits and vegetables annually (Lim et al., 2010). The Food and Agriculture Organization (FAO) prescribes for intake of 500 g fruits and vegetables per capita per day. On similar line, the Indian Council of Medical Research (ICMR) has recommended 300 g vegetables and 100 g fruits for Indian population. However, vegetables are perishable foods with variable growing conditions, preferences amongst the consumers and are bulky. In specific ecologies, local or underutilized vegetables are key to food and nutritional security due to their regular access and affordable prices. Developing commercially acceptable cultivars in IVs is essential for tapping their potential in ensuring nutritional and financial security.

Although government schemes in the form of food supply, food fortification and supplementation programs helped significant population to cross the 'line of undernourishment', these programmes need additional activities to substantiate the on-going efforts. Therefore, there is need to have differential and customized approaches including both commercial and local vegetables to ensure regular access to quality vegetables at affordable prices.

Vegetable crops represent a diverse group of culinary plants. A large number of these crops or culinary plant species remain underutilized mainly due to limited area of cultivation, specific consumer base, limited preferences, recent identification or domestication and low commercial prospects in terms of global and national vegetable scenario. These underutilized native vegetable species are described as indigenous vegetables. Their major characteristics are (i) indispensable part of traditional food culture, (ii) lack of standardized agro-techniques for commercial cultivation, (iii) absence of

seed production or marketing systems, (iv) insufficient attempts for genetic improvement and (v) cultivation in wild, home gardens, or small-scale farms. These criteria have been used to describe the terms 'indigenous leafy vegetables' (van Rensburg et al., 2007), 'traditional food crops' (FAO,1997) and 'indigenous traditional vegetables' (Keatinge et al., 2011).

Indigenous vegetables (IVs) are common food for vulnerable communities as these are easily accessible, acceptable and cheapest food source, easy to adopt livelihood option for tribal and rural youth and possess adaptive tolerance to biotic and abiotic stresses. These qualities make them a good choice for climate smart agriculture and organic farming. Development of improved varieties in IVs can significantly contribute in enhancing the nutritional security of island population.

Underutilized vegetables: diversity and local communities

Globally, around 400 plant species are being cultivated as vegetable crops. Of these, 218 vegetable species are distributed across 11 recognized centers of origin. In India, about 50 vegetable species are grown commercially but only a few contribute significantly to the total vegetable production. Besides, 521 wild plant species from 377 genera are used as leafy vegetables by tribes. India has about 550 communities and 227 ethnic groups living in more than 5000 villages and forest areas. This highlights the significance of underutilized vegetables in contributing to the nutritional security of these communities.

Food and nutritional significance of indigenous vegetables

The indigenous vegetables significantly contribute to food and nutritional needs of the tribes and rural communities (Singh et al., 2010) and many of them are nutritionally richer than their exotic counterparts. They are abundant, easily accessible, locally adaptable and acceptable (FAO, 1988). The indigenous vegetables are popular amongst tribes and ethnic communities and occupy major share in vegetable market during rainy or dry seasons (Prioni et al. 2001; Singh and Singh, 2012). Their rich profile in phytochemicals adds the value to their potential in countering the risk associated with non-contagious diseases related to cardiovascular, digestive, respiratory and nervous systems. The regular intake of these vegetables contributes to lowering the incidence of micronutrient (Fe, Ca) and vitamin (A, B, C, K) associated health problems. These are also helpful in global fight against anemia, blindness and other immunity associated diseases.

As per an estimate, the tropical regions will house around 55% of the world's population by 2050 but these are more vulnerable to climate change impacts (https://tropicaldatahub.org). Resource shrinkage due to rise in human population and climatic vagaries in these regions invite attention for measures to ensure food and nutritional security. In this context, the indigenous foods including vegetables could serve better and easy source for dietary microelements (Baruah and Borah, 2009) as they have been playing vital role in fight against micronutrient deficiencies in vulnerable communities due to their easy accessibility and better acceptance in traditional diets (FAO, 1988). Anti-nutritional factors in indigenous vegetables limit the bioavailability of dietary micronutrients and hence, efficient cooking processes are necessary to be devised to minimize the effects of unfavourable dements while retaining the beneficial ascorbic acid, anthocyanins and carotenoids (Udousoro et al., 2013; Singh et al., 2015).

Lack of systematic information on nutritive profiles, inconsistency and inadequacy in supply of nutrient rich vegetables and lack of efficient cooking practices hinder the integration of vegetables in nutritional security schemes. In natural habitats, indiscriminate exploitation lead to situation of 'more efforts less harvest' while poor market and technological support are major constraints in commercial farming of these vegetables. Proper rationing of harvest through community participation is essential to retain the regenerative capacity of the natural habitats and ensuring sustainability of traditional food system. This could be reverted by breeding of nutrient-rich varieties in prioritized indigenous vegetables and development of suitable production technologies (Singh et al., 2015). The IVs are important for food and nutritional security at the

micro level or regional levels in vulnerable communities as shown in Fig. 1.

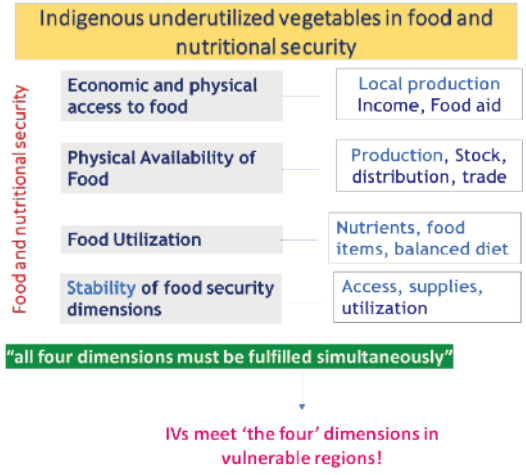

Fig. 1. The IVs in local food and nutritional security

Indigenous vegetables to strengthen regional economy

As in case of Andaman and Nicobar Islands, the Gross State Domestic product (GSDP) was estimated at US$ 810 million with per capita income of US$ 2,132 in 2010 (Basic Statistics, 2010). The contribution of the services sector (49%) was maximum followed by primary sector (17%) and secondary sector (34%). A significant share of income returns back to mainland India as charge of goods and services including vegetable crops. This 'drain of economy' can be reduced by producing vegetables locally to meet the demands of local population and tourists particularly in rainy season when the islands have off-season for commercial vegetable crops. For this, breeding of biotic and abiotic stress tolerant varieties adaptable to heavy rains in open condition and tolerant to partial shade and humid conditions inside the protected structures is a prerequisite. In Andaman Islands, the diversity of indigenous vegetables has potential to substitute the commercial vegetable crops and reduce the demand for import. Few examples include *Hibiscus sabdariffa* for sourness in place of tomato; *Eryngium foetidum* in place of leafy coriander; local tuber crops in place of potato; exotic chilli by local materials for pungency; local legume vegetables to replace import of exotic legumes; multiplier onion for common onion; indigenous pickle items; *Limnophila chinensis* for composite flavours etc.

Indigenous vegetables to mitigate climate change impact

The productivity of vegetables generally remains low in tropical islands (Olasantan, 2007) which could be due to genotypic and environmental factors or their interactions. Bray et al. (2006) reported yield losses of around 50% in vegetable crops primarily due to environmental stresses. In future, the climate associated stress events like high temperature, limited soil moisture and salinity stress will get magnified by climate change impacts (de la Pena and Hughes, 2007). Frequency of extreme events is likely to affect the response of technologies including high yielding genotypes against soil health degradation or changes in disease and pest equilibriums and reproductive biology with modified microclimate. The Andaman and Nicobar Islands are geographically isolated from continental India and form an archipelago of 572 islands fragmented by sea water. The islands have high vulnerability to extreme impact of natural disasters like tsunami and cyclonic storms (Krishnan et al., 2011). Singh and Bainsla (2014) also presented an analysis of breeding for climate resilient traits in vegetable crops with main emphasis on indigenous vegetable crops of the islands. Although, a systematic review was made by de la Peña and Hughes (2007) for improving vegetable productivity in a variable and changing climate with specific attention to tropical continental regions, there is little progress on exploitation of the real potential of these crops to harness their strength in climate change mitigation strategies.

Indigenous vegetables to utilize degraded land and water resources

The Tsunami (26th December 2004) damaged around 8,068.71 ha of precious agricultural land (out of total 50,000 ha agricultural land) of the Andaman and Nicobar Islands and directly affected the livelihood of more than 6,000 farmers. It caused total losses to island agriculture to the turn of INR 321 crores. It caused losses to plantation crops by 28% in Katchal Island, 17% in Car Nicobar

(home of Nicobarese tribes) and 13% in Campbell bay (also commercial plantations of settlers). Small islands like Teressa (744.0 ha), Kamorta (637.0 ha) and Trinket (329.0 ha) Islands were mostly washed out by sea water. These lands were submerged by sea water which created saline soils and lowland areas got flooded during rainy season. Although, the raised bed technique is quite effective but the challenge is to breed varieties which can tolerate high salinity stress. Local germplasm of wild *Ipomea* sp., *Alternanthera* sp., *Portulaca* sp., *Amaranthus* sp. were seen in the Tsunami affected lands indicating the potential to identify varieties suitable for salt stress situations. Under occasional submergence conditions, *Ipomea aquatica* and *Alternanthera* sp. were found to grow well. On the other hand, water is an important limiting factor for vegetable cultivation during later phase of dry season in the islands i.e. March to April months which necessitates breeding of drought tolerant varieties to attain economic yield of quality produce in the islands.

Indigenous vegetables for enriching home gardens

Settlements in tropical islands have home gardens as integral component to meet the day to day requirements of food items. Vegetables, mainly cucurbits, leafy vegetables, chilli, brinjal, okra and cowpea, are the important constituents of these tropical home gardens (Pandey et al., 2007). Perennial tree vegetables such as drumstick, jackfruit and bread fruit are also common on boundaries while low-growing herbs such as *Centella asiatica*, *Eryngium foetidum* and *Bacopa monnieri* are grown in earthen pots. Tsunami also damaged the traditional home gardens of the islands but new home gardens having better crops and varieties were established to meet the daily needs of horticulture-based food items. Intensity of home gardens in rural areas of islands is higher (65%) as compared to peri-urban (40%) areas where manpower is oriented towards commercial farming. The research and development efforts were directed towards modernizing the home gardens with more crops and improved varieties, scientific production technologies, rain shelters, soil mount technique, use of multistorey cropping system, assuring irrigation water for dry phase and providing improved farm implements. Suggested future research efforts would include development of suitable varieties and devising modules for maximum edible product from components of home gardens to meet the maximum share of household needs for food items. Scientific evidence of increase in nutritional status of household members by adoption of home gardens is needed to promote this concept.

Indigenous vegetables for livelihood and entrepreneurship

The IVs are potential options as source of livelihood and entrepreneurship. These crops are more prevalent in areas which are geographically isolated, economically marginal with poor skills of growers to adopt the new and medium to high end economic activities. Though they have ready market in local communities and local markets, their promotion in other regional markets could facilitate the integration of the local farmer communities in economic activities. Also, the women and poor people find it comfortable to deal with economic activities in vegetables since they feel attached with their traditional practices. Other areas which can be seen as potential options are described as (i) production of quality planting material for local and national needs, (ii) growing and marketing of traditional crops having novel traits in 'zero land cultivation' concept for supplementing local income, (iii) tapping the potential of local crops for high end food industry – nutraceuticals and functional foods, (iv) sensitise and market local rare crops having strong traditional value in different forms, (v) local food outlets for tourists and urbanites with reliable and healthy cooking practices, (vii) processing and packaging local unique IVs for national markets, and (viii) integration of vegetables (particularly IVs) in integrated farming systems.

Research Programme on Indigenous vegetables

The research studies on indigenous vegetables are very less across the world including India. Various reasons for it include limited scope/impact of research outcomes, huge diversity of species, different growing requirements, poor acceptance among the non-traditional consumers, region and season specific preference among the growers, breeding beahaviour and lack of improved varieties etc. However, these crops are important in certain

geographical regions particularly in islands, hill regions, tribal regions, desert areas, forest regions etc. These crop plants have evolved in the regional climatic situations over the years and hence, have attained adaptive changes which could be useful for breeding climate resilient crops/ varieties. Therefore, careful planning and implementation of research programme is essential to harness the potential of these neglected vegetable crops.

Since the impact of individual indigenous vegetable crop is limited due to small size of consumer base, simple and rapid breeding methods such as pure line selection and mass selection are to be practiced for developing trait specific varieties. However, need for prioritizing the crops and traits for systematic breeding programme has been given little attention. Efforts in this direction would prove to be effective against climatic and livelihood uncertainties in the geographically isolated islands.

Survey and documentation of IVs and their traditional uses

The target region and dwelling communities should be surveyed using proper surveying techniques for documentation of diverse indigenous vegetables and their consumption pattern. For this, food parameters (preference, food value, perceptions, frequency of use), nutritional perceptions, botanical descriptions (annual, biennial, perennial, edible part, mode of multiplication), habitat and growing situations (season, specific features), cooking parameters, market parameters etc. should be recorded for 2-3 years. In case of perennial IVs, the target plant should be marked with proper GPS location.

Germplasm collection, crop prioritization and conservation

After surveying, the germplasm of target indigenous vegetables should be collected with all the necessary descriptions. The collected germplasm should be grown in germplasm conservation houses located in almost similar geographical conditions for multiplication. The non-propagated germplasm should be collected again for enriching the germplasm and ensuring the representation of different locations in the field gene bank. Further, the edible parts of the target germplasm of different indigenous vegetables should be collected along with the propagating materials for evaluation of food and nutritive values through laboratory analysis. In this process, the major dietary elements, minerals and anti- nutrients should be assessed and they should be given adequate weightage in crop prioritization. In Andaman Islands, scientists of ICAR-CIARI surveyed different inhabited islands, and identified 42 IVs. Thirty species were evaluated for dietary elements and 10 were selected for breeding programme. While prioritizing the crops, food value, market potential and nutritional values were considered adequately.

Abraham et al. (2008) collected germplasm of vegetables and their wild relatives (185 nos.) and tuber crops and their wild relatives (44 nos.) along with other crop germplasm. Singh et al. (2015) also reported existence of diverse germplasm of different vegetables in the islands. The list included 150 species of vegetables, of which perennial vegetables constituted 18% followed by lesser-used-leafy vegetables (15%), local legume vegetables (10%), tuber crops (9%), lesser known cucurbits (7%) and wild related vegetables (5%). Commercial vegetables (18%) and new exotics (11%) were also recorded. Pandey and Diwakar (2008) reported 2,574 floral species including 1,752 dicots, 672 monocots, 8 Gymnosperms and 142 Pteridophytes. Sharma et al. (2010) reported 44 horticultural species including *Colocasia, Momordica, Dioscorea* and *Canavalia* species. Sharma et al. (2018) documented 36 endemic wild relatives including four rare species. They could enlist 308 species from the islands, some of which have potential in pre-breeding for climate change resilience, rare nutrients and biotic and abiotic stresses. They suggested for conservation of this germplasm, systematic screening for biotic and abiotic stresses, and attempting inter-specific hybridization for use in crop improvement programmes.

Germplasm diversity and characterization

Characterization of the unique germplasm in indigenous vegetable crops is particularly important to minimize the biopiracy, trait prioritization and benefit the custodian farmers in 'biodiversity hotspots' (Gautam et al., 2014). Investigations for search of unique traits or genotypes have been made by means of nutritive

profiling of few indigenous vegetables (Singh et al., 2011). Characterization should be done for unique and prospective traits such as climate resilience, disease resistance, development of functional foods etc. In Andamans, extent of diversity was analysed using agro-morphological traits and molecular markers in indigenous vegetables namely *Colocasia*, spp. (Singh et al., 2011), *Capsicum* spp. (Singh, 2013), *Momordica* spp., *Amaranthus* spp., *Alternenthera* sp., *Basella* spp., *Centella asiatica, Ipomea* spp. and *Moringa oleifera* (Singh et al. 2011; 2013; 2018). Although, conservation in field gene garden and in seed form were attempted, there is wide gap in available and conserved species. Farmers and home gardeners are playing crucial role in conservation of agri-horticultural crops but systematic efforts are necessary. Notably, protocols for *in-vitro* conservation of germplasm for tropical regions have been developed which may be further fine-tuned for these underutilized but potential vegetable crop species.

Germplasm improvement methods for yield and related traits

Breeding for nutrient-rich genotypes in indigenous vegetable crops was attempted at ICAR-CIARI. For this, a concept was devised and attempted for improvement of indigenous leafy vegetable crops for nutrient contents which could result into promising genotypes. In self-pollinated crops, pure line selection, hybridization, pedigree method, bulk method, single seed descent (SSD) method and backcross methods are in common use. Pure line selection is the progeny of single self-fertilized homozygous individuals and preferably applied to improve local landrace of indigenous vegetables. From locally popular collection, a large number of plants were selected based on phenotypic basis and their individual progenies were evaluated till homozygosity was attained. It helps in maintenance breeding and attaining the uniformity in appearance and also in nutrient contents. The varieties thus developed and popularized not only increase productivity but also facilitate on farm conservation of local superior genetic resources.

The hybridization method is mainly used to generate variability in segregating progenies from promising parents having better phenotype and high general combining ability. The selected parents are crossed, and selection for desirable characters is made by pedigree and bulk methods. In pedigree method, single plant selection is followed up to $F_{5 \text{ or}} F_6$ generations and in advance generation, families are selected on the basis of their records of phenotypic performance. It has been adopted to improve germplasm and develop variety for nutritional quality. The bulk method is one of the most economical methods of handling segregating population based on natural selection wherein poorly adapted types are eliminated and superior types prevail. Although, this method was employed to develop genetic stocks for tolerance to heavy, drought and waterlogging situation, it did not result in getting superior plants.

The single seed descent method was employed in *Hibiscus sabdariffa*, *Basella alba* and *Capsicum annum* to advance the selected individuals. Backcross method allows transfer of characters controlled by single or oligo-genes but lack of established varieties limits its scope in indigenous vegetable crops. For cross-pollinated vegetable crops, mass selection, line breeding, family breeding and recurrent selection; hybridization and heterosis breeding are suggested to improve the base material. Development of F_1 hybrid is very suitable for enhancing nutraceuticals and edible colours but lack of suitable genetic mechanisms for affordable hybrid seed production is a big challenge. The mass selection is still the practical method applied so far for development of varieties in cross-pollinated indigenous vegetable crops. Based on the extent of available germplasm, floral behaviour and ease breeding programme, the mass selection, pure line selection and clonal selection methods have been generally recommended.

Breeding new varieties in indigenous vegetables

In indigenous leafy vegetable crops, breeding progamme was started in 2008 in the islands, which resulted in development of one variety each of *Eryngium foetidum* (CARI Broad Dhaniya), *Basella alba* (CARI Poi Selection), *B. rubra* (CIARI-Shan), *Amaranthus viridis* (CIARI-Harita) and *A. tricolor* (CIARI-Lal Marsha) for tropical island conditions. The detailed characteristics of

these verities are well documented (Gautam et al., 2016). CARI Broad Dhaniya is the first variety of this crop in India and it was developed through mass selection using local germplasm from Andaman Islands. It produces 30-35% higher yield than the base material. Its leaves are rich in micronutrients and phytochemicals. The variety fits well in 'zero land cultivation' concept. The average yield is around 8-10t/ha/year. CARI Poi Selection is a new and first variety of Indian spinach (green type). It has attractive green and broad leaves, short internodal length and better shelf-life. It is rich in Fe and Ca, ascorbic acid and carotenoids. It is highly suitable to tropical climatic condition of Islands and yields around 18 t/ha (with single harvest) and 54 - 60 t/ha with multi-harvests i.e. 42 - 57 % higher than the local base materials. It was released by the Institute Variety Release Committee of ICAR-CIARI, Port Blair in 2013. CIARI-Shan (*Basella rubra*) was developed using local germplasm through mass selection method. It has dark attractive purple/magenta colour stems and green leaves with coloured veins and short intermodal length. It is rich in anthocyanin (leaf- 280 mg/100g; stem- 410 mg/100g FW) and micronutrients (Fe- 8.4mg/100g; Ca- 202.8mg/100g DW). It is ready to harvest at 35-40 days stage when it attains a height of 25-30 cm. It has yield potential of 48-52 t/ha with multi-harvest and 15-18 t/ha in single harvest. It grows well in partial shade (50%) with organic inputs.

A local germplasm was used in breeding of CIARI-Harita, which is a promising selection of amaranthus (*Amaranthus viridis*) from Andaman and Nicobar Islands. It has attractive green and broad leaves, fast growth habit, high preference among the farmers and consumers and high biomass yield. It is rich in antioxidants like chlorophyll (707.8±7.3 mg/100g) and carotenoids (509.5±2.5 mg/100g). It is well suited to tropical climatic conditions of Andaman and Nicobar Islands and has fast-growing habit. It has green leaf yield potential of 13-15 t/ha in island conditions.

Similarly, another local germplasm of red amaranth (*Amaranthus tricolor* L.) was utilized in development of CIARI Lal Marsha. It is a promising selection for higher yield, attractive leaf colour and better adaptability to tropical warm humid climate of Islands. It has attractive broad and purple/ magenta leaves, fast growth habit and high acceptance by farmers and consumers. It is preferred in home gardens for its attractive colour which also adds ornamental value. It is rich in anthocyanin (288.7±1.8 mg/100g), a strong antioxidant for better health. It has green leaf yield potential of 14-16 t/ha in the tropical islands (CIARI, 2014).

The brinjal variety 'CARI Brinjal-1' has high level of resistant to bacterial wilt. It bears light green oblong shaped fruits. Plants have medium height, semi-spreading type with profuse branching habit. It is especially suitable for growing in rain-fed conditions during dry season in the tropical islands. Four types of wild relatives of brinjal, namely *Solanum torvum* (10 collections), *S. indicum*, *S. surratence* and *S. vairum*, were also collected and characterized. All the 10 accessions of *S. torvum* were observed to be free from all kinds of wilts (bacterial wilt, *Fusarium* wilt and *Verticillium* wilt). Immature berries of *S. torvum* are consumed as spiced dish in the islands. Three accessions of *S. virum* syn. *S. khasianum* from the vicinity of mangroves were also collected, which contain high amounts of secondary metabolites like solasodine with insecticidal and antibacterial properties. *Solanum indicum* is the second most abundant wild relative of brinjal in the islands after *S. torvum*. It is non-edible and acts as carrier of resistance to fruit-and-shoot-borer and wilt pathogen. *Solanum surratence* species contains alkaloids.

In case of chilli, around 60 local genotypes were evaluated for yield and to prevalent biotic stresses (bacterial wilt and leaf curl virus) which resulted in identification of five promising genotypes which were named as CIARI Chilli-1, CIARI Chilli-2, CIARI Chilli-3, CIARI Chilli-4 and CIARI Chilli-5. These genotypes have high yield potential than national and local checks besides better-quality attributes and resistance to leaf curl and bacterial wilt.

Locally collected germplasm of *Momordica cochinchinensis* and *M. subangulata* ssp. *subangulata* were evaluated for quality and horticultural traits (Singh et al., 2013; 2017). Among root and tuber vegetables, sweet potato (*Ipomea batatas*) has good extent of diversity and local germplasm was used for breeding of

two new varieties (C1AR1-SP1 and CIAR1-SP2) through selection method. CIARI- SP-1 is non- twining spreading plant with slow growth rate and short internodal length (Gautam et al., 2016). It has medium sized mature green leaves and purplish-green immature leaves. The individual tuber weight is 81 g and yields 20.87 t/ha. CIARI- SP 2 is another variety, which has twining spreading plant with intermediate growth rate and intermediate internode length. It has medium sized mature green leaves and purplish-green immature leaves. Individual tuber weight is 93 g and yields 22 t/ha.

The elite lines from local germplasm were also developed in indigenous vegetables such as *Momordica subangulata* subsp. *renigera* (CARI Kakrol-1; uniform attractive green fruits with high yield potential (80-90 q/ha) in island condition), *Hibiscus sabdariffa* (CIARI HS-1; attractive green leaves, uniform growth, high yield (130 q/ha), early (35-40 days) and tolerance to foliar diseases); *Centella asiatica* (L.) Urban (CIARI CA-5; broad leaves, rich in micronutrients and yield 30-35 q/ha); *Ipomea aquatica* L. (CIARI NB-4; attractive green leaves and green stem, fast growing habit and yield potential of 80-90 q/ha); *Portulaca oleracea* (CIARI DB-8; rich in polyphenol, Ca, Fe and suitable for problem soils); *Alternanthera* spp. (CIARI MD-1: green leaves, tolerant to heavy rains and waterlogged situation and high yielding and CIARI MD-2: purple green leaves and high yield of 11-14t/ha) (CIARI, 2013; Singh and Bainsla, 2015).

Perennial indigenous vegetables in the islands

The islands have rich diversity of tree vegetable species such as *Moringa oleifera*, *Artocarpus heterophyllus*, *A. incisa*, *Murraya koenigii* and *Tamarindus indica*, which are prevalent in home gardens in the ANI (Abraham et al., 2008; Pandey et al., 2006). *Cycus rumphi* and *Calamus andamanicus* are eaten in the form of stew, soup, pickle, chutney or curry (Singh and Singh, 2012). Perennial vegetables as wild relatives of cultivated species viz. *S. melongena* and *C. annuum* (Abraham et al., 2008) have not been exploited so far. Fruit crops for use as vegetables such as vegetable banana, green papaya, carambola, immature green mango etc. are the dependable sources even during climatic aberrations. Besides, the islands have about 34 mangrove species, which cover 12% of geographical area, few of them being edible (Goutham-Bharathi et al., 2014). Over all, more systematic efforts are required to document and conserve germplasm resources of perennial vegetable species in the islands.

Strengthening 'On-site' production system with indigenous vegetables

Vegetables are perishable food items and their regular supply in adequate quantity remains a big challenge. Hence, measures to enhance production of vegetables 'in locale' through home gardens, kitchen gardens, container gardening, roof gardening etc. are essentially required not only for remote rural areas but also for urbanites. Establishment of new home gardens or enriching the existing traditional home gardens with nutrient rich locally adaptable crops and their superior varieties could contribute much better to the productivity and nutritional security in an environmentally sustainable manner. But, this requires identification of region specific vegetable crops and breeding of varieties for dietary nutrients, prolonged harvest period, adaptation to growing situation and high acceptance among the ultimate beneficiaries. The breeding objectives could include improvement for dietary nutrients and antioxidants, organoleptic scores, tolerance to partial shade, high portion of edible fruits/ parts, low gestation period, resistance to diseases and pests, tolerance to excess rainfall and salinity and response to organic sources etc. So far, research efforts remained targeted towards development of technologies and varieties for commercial scale but some varieties are quite fit for small scale growing in gardens. But, efforts are utmost required to develop plant types ideal for home gardens or other micro-scale production systems.

Way forward

'Indigenous People are the best guardians of the world's forests and biodiversity' as stated by UN Special Rapporteur Victoria Tauli-Corpuz in 2019, should be kept in mind while devising the strategy for development of agriculture in biodiversity hotspots. Andaman and Nicobar Islands have six primitive tribes and

diverse settler communities. However, production-cum-consumer base, low investment in vegetable breeding, climatic constraints in continuation in breeding, narrow germplasm base and poor base of genetic stocks of ready to use genotypes for use in rapid breeding programme and production and marketing preferences are some of the constraints. Seasonal distinctiveness of the islands indicates that a number of indigenous vegetable crops can be grown in islands but it requires proper analysis with reference to crops, growing periods and suitable technological options. Although the indigenous vegetables have better adaptability, these are less popular among the economically better off populace who can afford to use exotic vegetable species due to impact of advertisement and popularization by private players on nutritional and quality grounds, while the local vegetables despite being highly nutritive and more useful are hardly popularized. These crops add to alternative sources for taste, flavor and product mix. Some of the indigenous species are much better sources of important nutraceuticals, flavours, minerals and vitamins. The current research and extension methodology is not enough towards making the islands self-sufficient in vegetable production. It largely helps the farmers to grow scientifically but hardly has any impact on broadening consumer base. The specific awareness programmes like debates, advertisements matching the private sector, comparison with commercial products available in market through more popular media like non-government TV and radio channels, newspapers etc. have to be launched. The vegetables produce harvested from organically certified sites and proper branding in the Islands can fetch more price not only locally but also from mainland states in India. This will create a driving force for island products which are unique, superior and matching in some case with imported items so that stigma of poor man's vegetable species can be eliminated and overruled by its nutritional worth. The breeding effort must be in tandem with the market as well as conservation of natural and biodiversity resources. The efforts must be accelerated for harnessing the power of marker assisted breeding and modern genomic tools this will not only reduce the time cycle but will also result in the development of and genetically tailored vegetable varieties with more precision.

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Characterization of Wild Fagaceous Nut Species for Morphometric Traits from Sub-tropical Forest Area of Kyrdemkulai, Meghalaya

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Abstract

Nuts are rich source of phytonutrients like carbohydrate, fats, protein, vitamins and minerals. Fagaceous nuts are highly diverse and well-distributed across the globe. Diversifying fruit industry and preserving ethnic food habits are necessary for nutritional security and conservation of the species in the face of uncertainties. The basic morphometric traits of wild fagaceous nut found in the forests of Kyrdemkulai circle, Ri-Bhoi district of Meghalaya were characterized in the present study. Few wild fagaceous nuts identified with native Khasi names *viz*. *Soh-ot-saw* (*Castanopsis purpurella* (Miq.) N.P.Balakr.), *Soh-ot-rit* (*Castanopsis indica* (Roxb. ex Lindl.) A.DC), *Soh-ot-langkraw* (*Castanopsis tribuloides* (Sm.) A.DC) and *Soh-ot-dieam* (*Lithocarpus fenestratus* (Roxb.) Rehder) were characterised for morphometric parameters like nut length, diameter, nut weight, hilum length, nut colour, nut shape, burr colour, burr nature, leaf length, leaf width and leaf shape. So far, no attempts have been made to understand the extent of diversity for morphological characters and to select superior types of these species in the region. Hence the descriptions given in this manuscript will serve as a primary information for further selection and detailed characterization studies. The results of the present study revealed that *C. indica* was found to be pomologically superior with respect to nut length and nut weight than the other two studied species of the genus. Locally, the edible wild nuts of these three species are consumed fresh, sun dried and roasted. Nuts of *L. fenestratus* are not preferred for human consumption and solely eaten by wild animals.

Key words: *Characterisation, conservation, Fagaceae, Northeast India, species diversity, utilization*

Introduction

Meghalaya has rich biodiversity with a dense forest area of 17,927 km^2, but the potential of the existing flora is still under-utilized. A total of 151 species (49 families and 86 genera) of wild edible fruits used by the Khasi tribes of Meghalaya have been recorded (Jeeva, 2009). Most common underutilized edible fruit genetic resources in Meghalaya are *Padus napaulensis* (Ser. ex DC.) Schneider, *Elaeagnus latifolia* L., *Myrica esculenta* Buch.-Ham. ex D. Don, *Baccaurea ramiflora* Lour., *Pyrus pashia* Hamilton ex D. Don, *Calamus meghalayensis* (Becc.) A.J. Hend., *Gynocardia odorata* R. Br., *Prunus undulata* Buch.-Ham., *Docynia indica* (Wall.) Decne., *Rhus chinensis* Mill. and *Viburnum foetidum* Wallich (Rymbai et al., 2015; Kharshandi et al., 2015).

Indigenous fruits play a significant role in food and livelihood security of people in the developing nations (Muok et al., 2001; Deshmukh and Shinde, 2010; Mwema et al., 2012; Mabaya et al., 2014) and are reported with richer nutritional value than commercially cultivated fruits (Eromosele et al*.,* 1991; Maikhuri et al., 1994). In the recent times, the younger generations are in denial to even consume the native seasonal fruits due to dilution of traditional knowledge, change in consumption habits, negligence, ignorance and over-dominance by improved commercially-cultivated fruits in the society. Further, increase in urbanization and commercial exploitation of forests and waste lands have threatened the existence of these indigenous species (Makdoh et al., 2014). Hence, diversification in fruit industry is a necessity for addressing health and nutrition insecurity, poverty reduction, unemployment and conservation of the rare species. It is a positive sign that in the recent times, the underutilized species have gained attention by the researchers dealing with various aspects (Devi et al., 2018 a,b,c).

Nuts are important sources of protein, carbohydrate, vitamins, minerals, dietary fibre and other phytonutrients (Ros, 2010). They are genetically very diverse group and distributed throughout the world. In India, fagaceous

group of nuts (those belonging to the family Fagaceae) are found growing both in the orchards and in wild throughout the Himalayas up to Assam and Meghalaya at an altitude of 2000 to 3000 m above MSL (Pandit et al., 2013). The fagaceous nuts have been under-utilised in the country as a whole. In Meghalaya, these under-utilized nuts have tremendous potential and can be popularized for commercialization with proper value addition. Fagaceous nuts can be grown and produced under organic system in areas where other fruit crops cannot be grown (Pandit et al., 2013). Being propagated through seeds, these nut species possesses vast genetic variability and heterogeneity for important traits. Characterization and identification of superior germplasm is necessary to promote these species for commercial horticulture. Hence, the present study was conducted to identify and study the local genetic resources, their economic importance, potential utilities and preliminary pomological traits of different fagaceous nuts found in the Kyrdemkulai forest area of Meghalaya state.

Materials and methods

The study was conducted at sub-tropical forest area of Kyrdemkulai of Umsning Block, Ri-Bhoi District, Meghalaya during 2021-22. The area lies between E 91˚77'30" to E 92˚27'00" Longitude and N 25˚63'00" to N 26˚07'00" Latitude, and at a maximum elevation of 1,242 meters above sea level (Fig 1.). The district receives an annual rainfall of 1242.8 mm with temperature ranging between 9.8°C (min.) and 33°C (max.). Samples were collected through expeditious walks in the selected area with the help of local people. Plant samples were identified by matching the collected samples with authenticated vouchers at Royal Botanical Gardens, Kew gardens (Plants of the World Online, 2022), descriptions given in The Flora of British India (Hooker, 1890) and following specialised literatures and revisionary works and also taking advice from subject specialists.

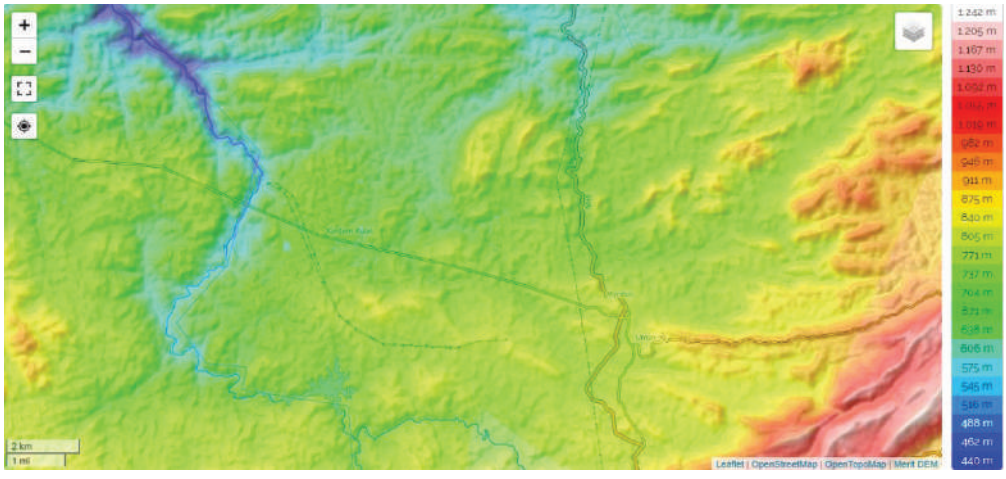

Fig. 1. Topographic map of Umsning, Ri-Bhoi, Meghalaya (https://en-in.topographic-map.com/)

Fruit samples with burr and leaves were collected from 10 trees each. The data was replicated 3 times, consisting of 10 fruits per replication. Information on local names and utilities was received from local inhabitants. The official descriptor list and guidelines of the International Union for the Protection of New Varieties of Plants (UPOV, 1989) were used to characterize the samples. Morphological parameters like nut length (mm), nut diameter (mm), nut weight (g), hilum length (mm), nut shape, nut colour, leaf length (cm), leaf width (cm), leaf symmetry, leaf colour (upper and lower), leaf shape, incision of leaf margin, bur colour and bur nature were recorded. Standard statistical parameters like arithmetic mean, standard deviation (SD) and co-efficient of variation (CV%) were calculated and standard descriptions of the qualitative characters were recorded as per UPOV (1989).

Results and discussion

Diversity and utility

During the study, four fagaceous species were identified from the sub-tropical forest area of Kyrdemkulai, Meghalaya. After comparison and referencing of the characters, the collected species were identified as *Castanopsis purpurella (*Miq.) N. P. Balakr., *Castanopsis tribuloides* (Sm.) A.DC, *Castanopsis indica* (Roxb. ex Lindl.) A.DC. and *Lithocarpus fenestratus* (Roxb.) Rehder (Plants of the World Online, Kew Science, 2022; Hooker, 1890; Singh and Singh, 2016). The vernacular names of the identified species are listed in Table 1. The species identified in this study were also listed among the 24 species of fagaceous nuts reported by Singh and Singh (2016).

Table 1. Wild fagaceous nuts found in the sub-tropical forest of Kyrdemkulai, Meghalaya

Species	Reference	Local name	Nuts Availability	Local utilities	Market
Castanopsis purpurella (Miq.) N. P. Balakr.	http://specimens.kew.org/herbarium/K000832670	*Soh-ot-saw*	November-December	Consumed raw/roasted	Local markets, Umsning, Iewduh, Bara Bazar, Shillong
Castanopsis tribuloides (Sm.) A.DC	http://specimens.kew.org/herbarium/K000832662	*Soh-ot-rit*	November-December		
Castanopsis indica (Roxb. ex Lindl.) A.DC.	http://specimens.kew.org/herbarium/K000832671	*Soh-ot-langkraw*	November-December		
Lithocarpus fenestratus (Roxb.) Rehder	http://n2t.net/ark:/65665/3d33d97ce-0efb-432c-8df3-fb9682081df8	*Soh-ot-dieam*	August-November	Not consumed; eaten by wild animals	

With respect to their consumption pattern, the rural people of the region collected the nuts from the wild forest and consumed raw or roasted, except *L. fenestratus*. Similar consumption pattern was also reported by Dangol et al. (2017). The local harvesters also sold the collected nuts at regional markets like Umsning, Iewduh, Bara Bazar, Shillong (Makdoh et al., 2014; Singh and Singh, 2016). The village residents of the area did not utilise these nut trees for other purposes. However, there are reports of leaves of fagaceous nuts being used for treating stomach disorders, skin infection (Singh and Singh, 2016), bark extracts for anti-cancer and antipyretic activity (Hasan et al., 2022), bark paste for controlling chest pain, curing snake bites (Joshi et al., 2011) *etc*. Other utilities included fodder leaves, firewood and timber (Pokharel et al., 2021; Aye et al., 2012).

Nut length

Among the selected four fagaceous species, the average nut length of *C. indica* was the highest (19.86 mm) followed by *C. purpurella* (15.34 mm) (Table 2). The least average nut length was registered by *C. tribuloides* (12.01 mm). The observations on nut length of the studied species were in line with the studies by Aye et al. (2012) and Pokharel et al. (2022). Meanwhile, Solar et al. (2005) and Poljak et al. (2021, 2022) reported higher mean nut length of European chestnut and sweet chestnut (27 mm).

Nut diameter

L. fensetratus exhibited maximum average nut diameter (15.71 mm), which was closely followed by *C. purpurella* (15.30 mm) (Fig. 2). Like nut length, the lowest values for nut width (11.05 mm) was also observed in *C. tribuloides*. The observed values were in accordance with the report given by Pokharel et al. (2021) in *C. tribuloides*, *C. indica* and *L. fenestratus,* although Aye et al. (2012) reported larger diameter of *C. indica* nuts (30 mm). The nut size of the species is intermediary when referred to *Castanea sativa*, with reported mean diameter range of 12 to 35 mm (Solar et al., 2005; Poljak et al., 2022).

Nut weight

Among the studied species, *C. indica* was recorded with the maximum nut weight (4.89 g). Nuts of *C. tribuloides* and *L. fenestratus* were observed to be comparatively lighter i.e. 3.10 g and 3.08 g, respectively (Table 2, Fig. 2). The values are in accordance with the earlier report (Chou et al., 2011). The nut masses are much lighter than the well-known chestnuts (*C. sativa*) values for which have been reported to be in the range of 5.23 g to 16.37 g (Kim et al., 2005; Solar et al., 2005; Pandit et al., 2013; Poljak et al., 2022).

Hilum length

The present study revealed that nuts of *C. purpurella* had the maximum mean hilum length (14.49 mm), followed by *L. fenestratus* (11.94 mm), *C. tribuloides* (8.87 mm) and *C. indica* (7.90 mm) (Table 2). Studies by Solar et al. (2005) and Poljak et al. (2022) have reported mean hilum length of *C. sativa* to be 22 mm. Higher values for hilum length in common chestnut (*Castanea sativa*) are indicative of its morphological difference from the wild fagaceous nuts.

Nut shape

Nuts of both *C. purpurella* and *C. tribuloides* were broad ovoid-globose, while nuts of *C. indica* were ovoid and those of *L. fenestratus* were globose (Table 3, Fig. 2). Aye et al. (2012) have also reported ovoid shape in *C. indica* and globose in *L. fenestratus.* However, Pokharel et al. (2022) described their observations on the *C. hystrix, C. tribuloides* and *C. indica* as broadly conical, although the referencing of characterisation has not been stated.

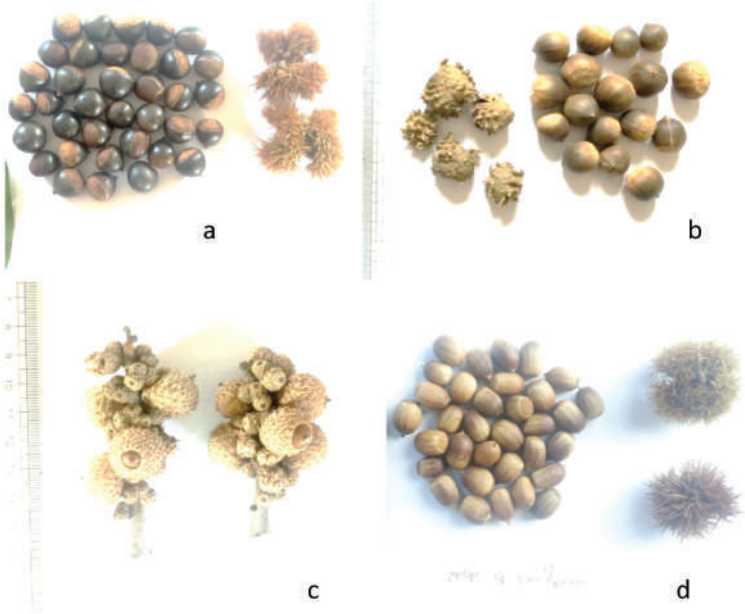

Fig. 2. Fruit and bur of wild fagaceous nuts found in the sub-tropical forest of Kyrdemkulai, Meghalaya: (a) *Castanopsis purpurella* (b) *Castanopsis tribuloides* (c) *Lithocarpus fenestratus* (d) *Castanopsis indica*

Nut colour

The nut colour, as described in UPOV (1989) guidelines *viz*. light brown, brown, dark brown, reddish brown and blackish brown, was recorded in the studied species. While it was blackish brown in *C. purpurella*, nuts of *C. tribuloides, C. indica and L. fenestratus* were brown, light brown-brown and reddish brown with raised stripes, respectively (Table 3, Fig. 2). The result also revealed *C. indica* with the lightest and *C. purpurella* with the deepest hue. In a study by Solar et al. (2005), *C. sativa* was reported to be of intermediate hue, slightly deeper but in closer proximity with that of *C. tribuloides.* Similar observations were also reported by Aye et al. (2012). However, Poljak et al. (2021) showed reddish brown colour in traditional sweet chestnut varieties and reddish brown-dark brown in hybrid chestnuts.

Leaf characteristics

Standard characters like leaf length (cm), leaf width (cm), leaf symmetry, leaf colour (upper and lower), leaf shape and incision of leaf margin were studied as per UPOV (1989). These four species have different foliage shapes and sizes. Among the studied species, *C. indica* was recorded with largest leaf dimension of 21.64 cm length and 9.96 cm width. Leaves of other three species were reported to have mean leaf length and width of 11.20 cm × 4.22 cm, 13.84 cm × 5.72 cm and 16.40 cm × 4.46 cm for *C. purpurella, C. tribuloides and L. fenestratus*, respectively, indicating *C. purpurella* to have shorter and narrower leaves (Table 2, Fig. 3). *C. indica* was observed to have distinctly larger leaf size and sharp incision on the edges (Fig. 3). The leaf sizes are in accordance with the observations presented by Pokharel et al. (2021).

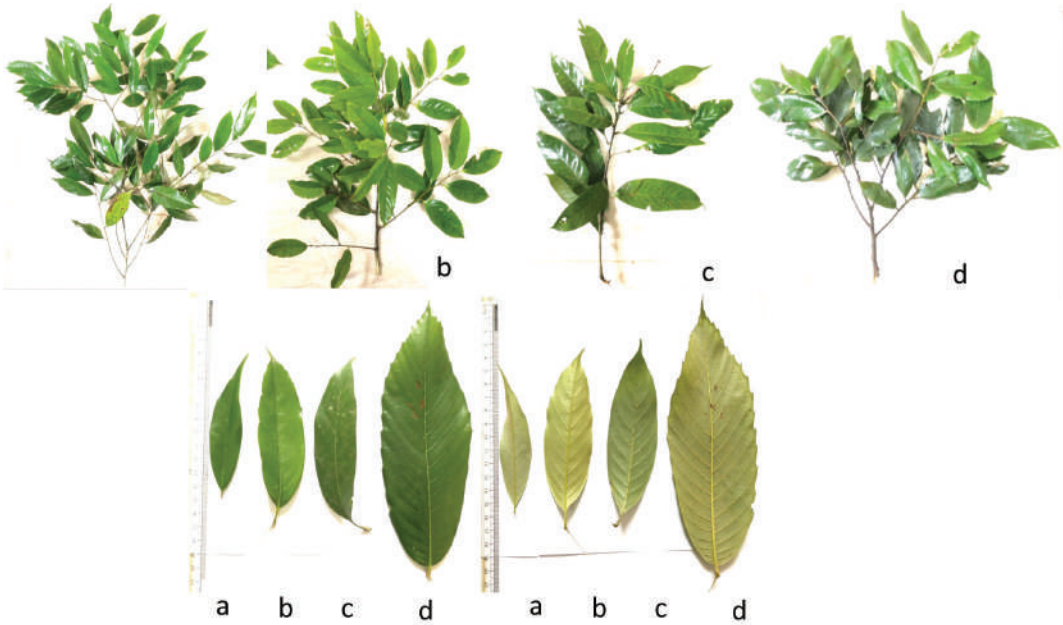

Fig. 3. Branch and leaf of wild fagaceous nuts found in the sub-tropical forest of Kyrdemkulai, Meghalaya (L-R): (a) *Castanopsis purpurella* (b) *Castanopsis tribuloides* (c) *Lithocarpus fenestratus* (d) *Castanopsis indica*

The studied species also varied in the shapes of leaf bases. Leaf base was acute in *C. purpurella*, obtuse in *C. tribuloides and L. fenestratus* and cordate in caser of *C. indica* (Table 3). Further, leaf margins were smooth in case of *C. purpurella* and *L. fenestratus*. Leaves of *C. indica* had mucronate margins, while leaves of *C. tribuloides* were mucronate only from the middle of the leaf to the apical region (Fig. 3). Similar description was also given in the Flora of British India (Hooker, 1890) and Pokharel et al. (2021).

Table 2. Morphometric traits of wild fagaceous nuts found in the sub-tropical forest of Kyrdemkulai, Meghalaya

Traits		Wild fagaceous nuts			
		Castanopsis purpurella	*Castanopsis tribuloides*	*Castanopsis indica*	*Lithocarpus fenestratus*
Nut length (mm)	Mean±SD	15.34±1.16	12.01±2.98	19.86±0.78	12.79±3.56
	Min	14.8	10.33	19.03	11.27
	Max	16.1	13.40	20.40	14.57
	CV (%)	7.56	24.85	3.93	27.82
Nut diameter (mm)	Mean±SD	15.30±1.66	11.05±2.66	14.73±1.57	15.71±0.75
	Min	14.8	10.07	13.07	14.13
	Max	15.77	12.20	15.90	16.40
	CV (%)	10.82	24.06	10.67	4.81
Nut weight (g)	Mean±SD	3.82±0.99	3.10±0.44	4.89±0.70	3.08±0.62
	Min	3.47	2.27	4.03	2.23
	Max	4.13	3.57	5.60	3.80
	CV (%)	25.85	14.06	14.35	20.28
Hilum length (mm)	Mean±SD	14.49±3.65	8.87±0.85	7.90±0.93	11.94±1.52
	Min	13.13	8.03	7.03	11.03
	Max	16.00	9.47	8.83	13.23
	CV (%)	25.20	9.59	11.77	12.71
Leaf length (cm)	Mean±SD	11.20±3.10	13.84±0.90	21.64±3.30	16.40±3.53
	Min	8.17	11.00	18.40	13.60
	Max	11.93	15.23	23.67	18.23
	CV (%)	27.71	6.50	15.27	21.16
Leaf width (cm)	Mean±SD	4.22±0.71	5.72±0.40	9.96±0.78	4.46±1.03
	Min	3.00	4.77	9.03	3.53
	Max	4.87	6.90	11.43	5.67
	CV (%)	16.80	7.07	7.80	23.03

Bur colour and nature

The cupule with the tremendous diversity of scales and spines are the most interested diagnostic characters for individual species (Aye et al., 2012). Observations on mature bur colour were also recorded as per the descriptors (UPOV, 1989). The results revealed bur hues to be reddish brown in *C. purpurella*, brown to dark brown in *C. tribuloides,* brown in *C. indica* and light brown in *L. fenestratus* (Fig. 2). With respect to bur spines, *C. purpurella* and *C. indica* were observed to have sharp

pricky spines. Aye et al. (2012) had also reported sharp spines in various species of the genus *Castanopsis*. *C. tribuloides* had blunt reduced spines in the bur (Table 3). Pokharel et al. (2021) has also described similar features from the species in Nepal. *L. fenestratus* bur had no spine and took an appearance of a scale rather than bur, which did not split easily on maturity unlike the other fagaceous nuts. Cupules of *L. fenestratus* have been recorded to enclose the nut with many curled hook-like scales (Aye et al., 2012).

Table 3. Standard descriptions of the studied fagaceous nut species

Major Details	Name of Descriptor	Wild fagaceous nuts			
		Castanopsis purpurella	***Castanopsis tribuloides***	***Castanopsis indica***	***Lithocarpus fenestratus***
UPOV No. 31	Nut shape	Globose	Globose	Ovoid (broadly conical)	Globose
UPOV No. 35	Nut colour	Blackish brown	Brown	Light brown-brown	Reddish brown
UPOV No. 16	Fully developed leaf: symmetry	Symmetric	Clearly asymmetric	Slightly asymmetric	Symmetric
UPOV No. 19	Fully developed leaf: green colour of upper side	Medium	Medium	Dark	Medium
UPOV No. 20	Fully developed leaf: colour of lower side	Light green	Light green	Light green	Light green
UPOV No. 21	Fully developed leaf: shape of base of blade	Acute	Obtuse	Cordate	Obtuse
UPOV No. 22	Fully developed leaf: incisions of margin	Absent	Mucronate (towards the leaf apex only)	Mucronate	Absent
This study	Bur colour	Reddish brown	Brown-dark brown	Brown	Light brown
This study	Bur nature	Sharp spine	Blunt, reduced spine	Sharp spine	No spine, bur resembles scale

Conclusion

This is the first characterisation study of fagaceous nuts conducted in the state. The present study revealed the variability of the fagaceous nut available in the selected region. The present study suggests superiority in traits of *Castanopsis indica*, especially with respect to nut weight and size. Alternative utilities and traditional knowledge need to be explored for increasing the value of these local nuts. Also, usage of *L. fenestratus* nuts need to be explored since the species is completely underutilized. The data can serve as preliminary information for further studies like selection of the desirable types based on biochemical,

physiological and molecular characterisation and their utilization in a sustainable manner. Such efforts would help to achieve food and nutrition security by making food basket more diverse and to achieve sustainable development based on the use of available genetic wealth, promotion and also conservation of these species.

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Assessment of Genetic Diversity by Using Multivariate Analysis for Morphological Qualitative Traits of Bullock's Heart (*Annona reticulata* L.) Genotypes in the Konkan Plains of Western India

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Abstract

An experiment was carried out to assess diversity of bullock's heart (*Annona reticulata* L.) in Ratnagiri and Sindhudurg districts of Maharashtra. Sampling of home gardens was done using multistage sampling method and samples of 100 elite genotypes were collected from different tehsils of surveyed districts. A total of seventy-eight morphological characters were measured and analyzed in the study. Among them, five morphological characters did not show any variance in the studied genotypes and hence, were removed from the analysis. Eleven morphological qualitative traits were identified and assessed for multivariate analysis. Results demonstrated the presence of substantial variability among the evaluated genotypes for morphological traits. The traits appointed to five principal components of qualitative traits explained 62.15% of total variability. Factor analysis captured more variation within the genotypes as compared to other and five factors from 11 traits explained 150.85% of total variability. Variations among genotypes in fruit shape showed significant positive correlation with fruit symmetry, and fruit symmetry with uniformity in fruit size and colour. Eleven clusters were formed and indicated that the genotypes selected from cluster XI represented highest cluster mean for most of the traits like uniformity in fruit size, fruit symmetry, fruit shape and ripe fruit colour and the same was confirmed by principal component analysis. The genotypes DP-5, VL-22, KL-7, KL-9, SW-12, DP-16, DP-4, VL-7, SW-20 and VL-16 showed potentially good characteristics for international markets. Result suggested that the factors other than geographical separation are responsible for divergence, and genotypes originated from same place may have different genetic architecture or vice-versa.

Key words: *Annonaceae, correlation, diversity, morphological traits, PCA*

Introduction

Bullock's heart (*Annona reticulata* L.) belongs to the family Annonaceae and is a tropical and subtropical fruit tree, widely distributed in Asia, Africa and the America (Nakasone and Paul, 1998). There are about 119 species in the family Annonaceae, of which only six are of commercial importance (Popenoe, 1974). *A. reticulata*, a diploid species with chromosome number 2n=2x=14 and 16 (Darlington and Wylie, 1956), is native of tropical America but its exact native range is unknown and is thought to be in Caribbean from where it has distributed to Mexico and tropical America (Popenoe, 1974). The species varies widely in fruit quality, flavor, habitat, and insect susceptibility (Safford, 1914). In India, it is extensively grown next to custard-apple under diverse conditions from the plains up to 1200 m elevation. It also runs almost wild as an escape, especially in the vicinity of old forts, temples, chapels, villages, etc. throughout the moist as well as drier hot parts of the peninsular, central, western, north-eastern, eastern regions and southern India (Saraswat et al., 2006). It has become intensively naturalized to the extent as to have often appeared being indigenous to India. In some parts of West Bengal, it is planted for utilization of waste land, particularly in heavy soils. In Maharashtra, it is cultivated in Pune, Ahmednagar, Aurangabad, Beed, Dhule and in Nagpur districts, whereas, considerable production is also found in forests and wastelands. The fruits are mainly used for fresh consumption, generally being considered as a 'Fruit of Poor People'. Bullock's heart, which is deciduous in nature, synchronizes the flowering and fruiting during periods of water availability.

In Konkan region of Maharashtra, bullock's heart is commonly grown as a crop of kitchen garden and is found to be regular bearing and does not require any special horticultural practice. The region has potential areas for large-scale bullock's heart cultivation in the future, provided that market outlets are clearly defined (Ghavale et al., 2016). As existing plantation of bullock's heart trees in the Konkan region are of seedling origin and being a cross pollinated crop, large variation in morphological parameters is observed. However, no efforts have been made in selection of superior genotypes of bullock's heart grown in the Konkan region of Maharashtra. By taking into consideration the future importance of this crop under changing agro-climatic conditions, the survey and characterization of bullock's heart genotypes grown under Konkan agro-climatic conditions was made to assess the variability using morphological qualitative traits.

Material and methods

The present investigation was carried out at Department of Horticulture, Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli, Maharashtra, India during 2012 to 2013 to study the diversity of bullock's heart in Ratnagiri and Sindhudurg districts. During survey, samples from 100 genotypes were collected from different tehsils of Ratnagiri and Sindhudurg using multistage sampling method for morphological characterization (Table 1). In order to increase the precision of sampling, a large number of clusters were used according to the suggestions given by Thattil and Samita (2007). Scion sticks of all the studied trees were given to the nursery of Horticulture department for further grafting and conservation. Since a descriptor list for *A. reticulata* is not available, the descriptor list of *A. cherimola* compiled by the IPGRI, 2008 was used in this study. A total of seventy-eight morphological characters were measured and used to analyze this study. Among these, 5 morphological characters did not show any variance in 100 genotypes and accordingly removed from the analysis (Table 2). From each tree ten fully expanded and healthy leaves, ten flowers from four directions and two well-developed fruits were randomly selected for measurements of characters. Eleven morphological qualitative traits were identified and assessed for multivariate analysis (Table 2). Munsell Color System chart published by the *Azalea Society of America* (Anon., 1999) was used to record the parameters such as trunk colour, colour of young branches, leaf colour, flower colour, exocarp color, pulp colour and seed colour.

Table 1. List of experimental material used for multivariate analysis in bullock's heart

Genotypes	Number of collections	Place of collection
SW-1, SW-2, SW-3, SW-4, SW-5, SW-6, SW-7, SW-8, SW-9, SW-10, SW-11, SW-12, SW-13, SW-14, SW-15, SW-16, SW-17, SW-18, SW-19, SW-20, SW-21, SW-22, SW-23, SW-24	24	Sawantwadi, Sindhudurg
VL-1, VL-2, VL-3, VL-4, VL-5, VL-6, VL-7, VL-8, VL-9, VL-10, VL-11, VL-12, VL-13, VL-14, VL-15, VL-16, VL-17, VL-18, VL-19, VL-20, VL-21, VL-22, VL-23, VL-24, VL-25	25	Vengurla, Sindhudurg
KL-1, KL-2, KL-3, KL-4, KL-5, KL-6, KL-7, KL-8, KL-9, KL-10	10	Kudal, Sindhudurg
MN-1, MN-2	2	Malwan, Sindhudurg
KN-1, KN-2	2	Kankawali, Sindhudurg
DV-1, DV-2, DV-3, DV-4	4	Devgad, Sindhudurg
VB-1, VB-2, VB-3, VB-4, VB-5	5	Vaibhavwadi, Sindhudurg
DP-1, DP-2, DP-3, DP-4, DP-5, DP-6, DP-7, DP-8, DP-9, DP-10, DP-11, DP-12, DP-13, DP-14, DP-15, DP-16, DP-17, DP-18	18	Dapoli, Ratnagiri
KD-1, KD-2	2	Khed, Ratnagiri
CN-1, CN-2, CN-3, CN-4, CN-5, CN-6, CN-7	7	Chiplun, Ratnagiri
GH-1	1	Guhagar, Ratnagiri

Table 2. Variation of qualitative characters measured and used in the analysis

Qualitative parameters	Observed Variation
*Trunk colour	1. Light grey (43%), 2. Dark grey (40%), 3. Pale grey (17%).
Tree crown shape	1. Ellipsoid (11%), 2. Spheroid (9%), 3. Oblong (42%), 4. Irregular (38%).
Tree growth habit	1. Erect (64%), 2. Spreading (8%), 3. Semi-spreading (28%) and 4. Drooping (0%).
*Colour of young branches	1. Light green (37%), 2. Dark green (24%), 3. Moderate green (18%), 4. Pale green (21%).
*Defoliation at the end of fructification phase	0. Absent (0%), 1. Partial (82%). 3. Complete (18%).
Leaf blade shape	1. Ovate (15%), 2. Elliptic (19%), 3. Obovate (0%), 4. Lanceolate (66%).
*Shape of leaf base	1. Acute (87%), 2. Rounded (13%), 3. Obtuse (0%) and 4. Cordate (0%).
*Shape of leaf apex	1. Acute (85%), 2. Rounded (0%) and 3. Acuminate (15%).
*Colour of mature leaves	1. Light green (23 %), 2. Brilliant green/Green (23 %), 3. Greyish green (0%) and 4. Dark green (54 %).
*Leaf margin	1. Entire (100%) or 2. Undulated (0%).
*Leaf blade venation	3. Submerged (0%), 5. Intermediate (100%) or 7. Raised (0%).
Petal outer colour	1. Yellowish green (55.0 %), 2. Light yellowish green (30.0 %), 3. Deep yellowish green (15.0 %) and 99. Other (0%).
*Colour of the internal petal base	1. Pink (63%), 2. Light reddish purple (11%), 3. Dark red (26%) and 4. Deep reddish purple (0%).
Location of fructification	1. Base of the crown (10%), 2. Middle of the crown (71 %) and 3. Top of the crown (19%).
Fruit shape	1. Round (10%) 2. Oblate (6%) 3. Cordate/Heart (60%) 4. Broadly cordate (17%) 5. Oval (7%).
Fruit symmetry	0. Asymmetric (40 %) or 1. Symmetric (60 %).
Uniformity in fruit size	0. No (48%) or 1. Yes (52%).
Fruit exocarp type	1. *Laevis* (smooth) (65%), 2. *Impressa* (slight depressions) (33%), 3. *Umbonata* (small protrusions) (2%), 4. *Tuberculata (*medium protrusions) (0%) and 5. *Mamillata* (large protrusions) (0%).
Ripe fruit colour	1. Reddish yellow (32%), 2. Reddish brown (52%), 3. Reddish green (13%) and 4. Pink (3%).
*Pulp colour	1. White (0%) and 2. Creamy white (100%).
Pulp texture	1. Watery (46%), 2. Creamy (30%), 3. Granular (24%), 4. Hard (0%) and 5. Hard areas in the pulp (%).
*Seed coat colour	1. Grey (0%), 2. Brownish black/Dark brown (28%), 3. Black (72%) and 99. Other (0%).

(* indicated characters with no variation)

Statistical analysis

The data collected on individual characters were tabulated and subjected to statistical analysis and two seasons' data for fruits characters was pooled to arrive at a proper conclusion (Panse and Sukhatme, 1985).

Multivariate analysis

Non-parametric data were converted to scales as proposed in descriptors for *A. cherimola* (IPGRI, 2008). Multivariate analysis *viz*., Principal Component Analysis (PCA), Factor analysis (FA), Principal Co-ordinate Analysis (PCO), Canonical Vector Analysis (CVA), Biplot, Score plot, Scree plot, 2D scatter plot, correlation coefficients, covariance matrix, D^2-analysis and Hierarchical cluster Analysis (HCA), as developed by Mahalanobis (1936), were performed using the mean data for each character following the widely used Windostat version 9.1 and JMP@10.0.2 statistical computer software packages program. For multivariate analysis, total 65 characters were used out of 78 characters and remaining 13 characters were also removed from the analysis due to low coefficient of variation. Furthermore, among 65 characters, 11 morphological qualitative traits were analyzed separately. The collected data were summarized and subjected to diversity analysis.

Results and discussion

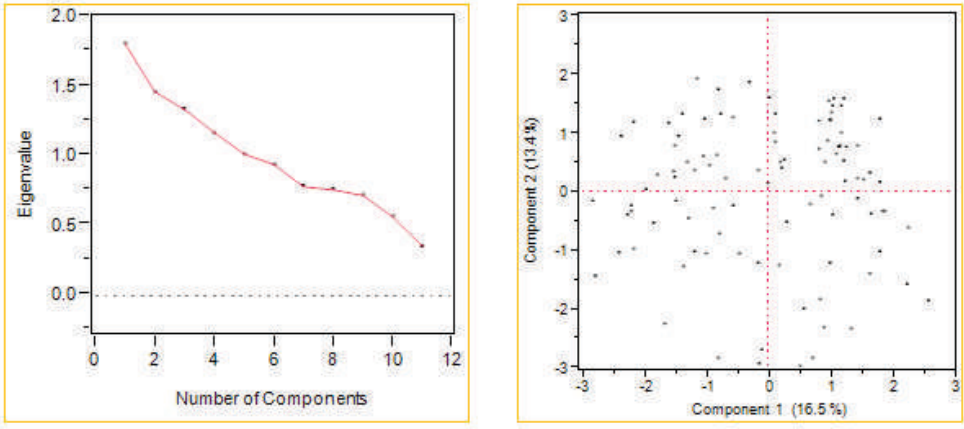
Principal Component Analysis

Results revealed that the 1st principal component (PC) largely accounted for variation among genotypes which contributed to 16.52% of the variation followed by 2nd PC (13.38%). PCA identified 5 PCs with Eigen values >1 explaining 62.15% of total variation. Most of the variability of analyzed genotypes has been explained by first 3 PCs. Results suggested that the 1st PC represented mainly uniformity in fruit size, fruit symmetry, tree growth habit, fruit shape, exocarp types and location of fructification. 2nd PC represented mainly exocarp type, fruit symmetry, leaf blade shape, fruit shape, tree crown shape, location of fructification, tree growth habit and fruit colour, while 3rd PC represented location of fructification, fruit shape, tree crown shape, petal outer colour, leaf blade shape, exocarp type, fruit symmetry and uniformity in size (Table 3). The scatter diagram distributed genotypes into 11 groups and showed that the 10 genotypes occupied distinct position, out of which 8 genotypes were far from the origin while 2 were near to the origin of scatter plot (Fig. 1). A 2D representation of the relative position of the genotypes in the biplot graph was found adequate and indicated the structure of population (Fig. 2). Biplot analysis revealed significant positive associations among uniformity in fruit size, fruit symmetry, ripe fruit colour, fruit shape (Fig. 2). 2D plot demonstrated that genotypes of divergent clusters scattered far apart, while genotypes of similar clusters were placed close to one another (Fig. 3). The 2D PCA plot was consistent with the grouping of genotypes obtained using cluster analysis. Results of PCA-I revealed that the traits responsible for genetic divergence in major axis of differentiation were uniform fruit size, symmetry, tree growth habit and fruit colour (Fig. 4). In PCA-II, the traits having a major role in determining genetic divergence in the 2nd major axis of differentiation were fruit exocarp type, fruit symmetry, leaf blade shape, petal outer colour, fruit shape and tree crown shape. Positive values in both vectors across two axes indicating the important component of genetic divergence among the studied characters were tree crown shape, location of fructification, fruit shape, fruit symmetry and ripe fruit colour, while negative values of both vectors for pulp texture indicated lowest contribution towards divergence. This was also confirmed by relative character contribution percentage towards genetic diversity and rank distribution, i.e. uniformity in fruit size (20%), fruit symmetry (18%), ripe fruit colour (16%), fruit shape (13%) and exocarp type (11%). Aforementioned 5 major traits together accounted for 78% contribution towards divergence (Fig. 4). These results are in accordance with the observations reported by Padmini *et al.* (2013), where the 5 PCA from 15 characters explained 69% of total variability of *A. muricata* germplasm. The findings analogous to this observation have also been reported by Rahman and Al Munsur (2009) in lime, Sudha et al. (2013) in papaya, Majumder et al. (2013) in mango and Rajasekhar et al. (2013) in sapota.

Table 3. Eigen/Latent vectors (V), loading matrix (L), eigen values, Bartlett test and percentage of total population of variance explained by 7 principal components of 11 morphological qualitative traits of 100 bullock's heart genotypes

Sl. No.	Morphological qualitative characters	PC I		PC II		PC III		PC IV		PC V		PC VI		PC VII	
		Vector	Loading	Vector	Loading	Vector	Loading	Vector	Loading	Vector	Loading	Vector	Loading	Vector	Loading
1	Tree Crown shape	0.036	0.048	**0.233**	**0.283**	**0.419**	**0.486**	**0.421**	**0.457**	**-0.417**	**-0.422**	-0.002	-0.002	0.080	0.071
2	Tree growth habit	**-0.447**	**-0.603**	0.069	0.083	-0.030	-0.035	0.125	0.136	**0.355**	**0.359**	**-0.406**	**-0.396**	0.114	0.102
3	Leaf blade shape	**-0.242**	**-0.327**	**0.406**	**0.493**	0.138	0.159	-0.187	**-0.203**	0.162	0.164	**0.590**	**0.575**	0.083	0.074
4	Petal outer colour	0.082	0.111	**-0.425**	**-0.516**	**0.326**	**0.378**	0.168	0.182	0.084	0.085	**0.355**	**0.346**	**0.644**	**0.572**
5	Location of fructification	0.011	0.015	0.121	0.147	**-0.509**	**-0.590**	**0.338**	**0.367**	**0.398**	**0.403**	**0.383**	**0.373**	-0.026	-0.023
6	Fruit shape	0.182	**0.245**	**0.234**	**0.283**	**0.430**	**0.498**	-0.197	**-0.214**	**0.527**	**0.534**	**-0.341**	**-0.332**	0.144	0.128
7	Fruit symmetry	**0.496**	**0.669**	**0.414**	**0.502**	0.042	0.049	0.113	0.122	**0.238**	**0.241**	0.036	0.036	0.073	0.065
8	Uniformity in fruit size	**0.602**	**0.812**	-0.043	-0.052	0.009	0.010	0.054	0.059	-0.029	-0.029	0.036	0.036	**-0.316**	**-0.281**
9	Fruit exocarp type	0.135	0.182	**-0.584**	**-0.709**	0.092	0.106	-0.139	-0.151	**0.353**	**0.357**	0.085	0.083	-0.195	-0.173
10	Ripe fruit colour	**0.269**	**0.363**	0.033	0.040	**-0.494**	**-0.573**	-0.141	-0.153	-0.145	-0.146	**-0.246**	**-0.240**	**0.626**	**0.556**
11	Pulp texture	-0.025	-0.034	-0.103	-0.125	-0.010	-0.011	**0.732**	**0.795**	0.163	0.165	-0.168	-0.164	-0.046	-0.041
Eigen values		1.82		1.47		1.34		1.18		1.02		0.95		0.79	
Total population variance explained (%)		16.52		13.38		12.22		10.72		9.31		8.64		7.17	
Cumulative percentage		16.52		29.90		42.12		52.84		62.15		70.78		77.95	
Chi Square		94.04		69.18		54.38		40.92		30.80		24.03		17.53	
DF		55.03		47.15		39.31		32.05		25.27		19.06		13.56	
Prob>ChiSq		0.001*		0.02*		0.06		0.14		0.21		0.20		0.20	

Highlighted values of each column represented selected characters of each principal component



Scree plot (a) **Scatter plot (b)**

Fig. 1. Principal component analysis scree plot (a) and scatter plot (b) depicting the genetic diversity based on PC scores of morphological qualitative data of 100 bullock's heart genotypes

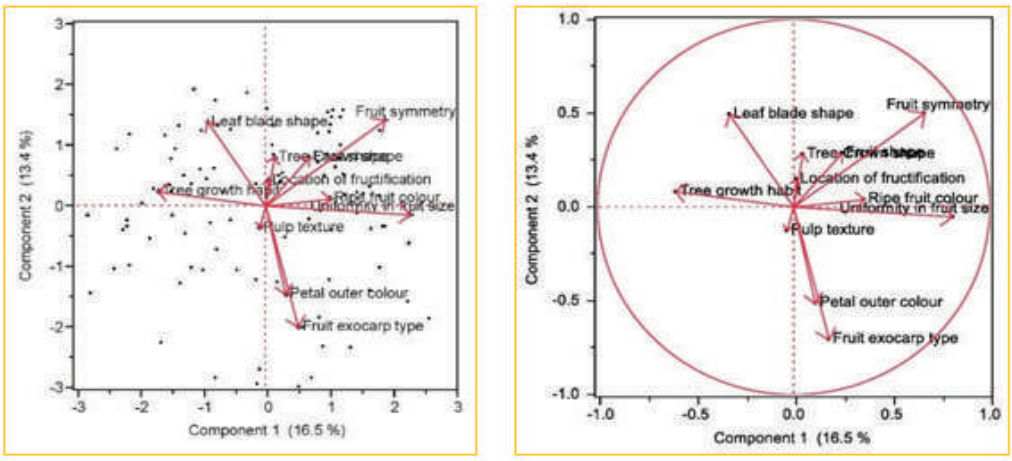


Fig. 2. Principal component analysis (PCA) Biplot (a) and loading plot (b) of PC1 and PC2 factor loadings for genotypes-by-traits and correlation analysis among various morphological qualitative traits using cumulative data of 100 bullock's heart genotypes

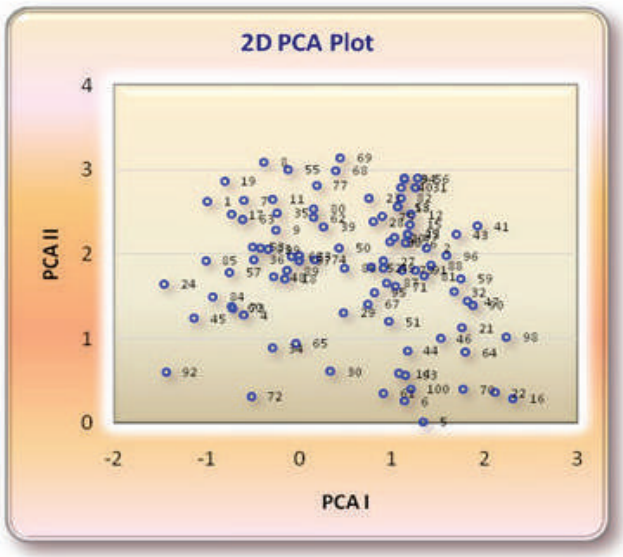


Fig. 3. Scattered diagram: Two dimensional ordination showing the relative position of 100 bullock's heart genotypes based on PCA scores (PC 1 and PC 2) of morphological qualitative traits

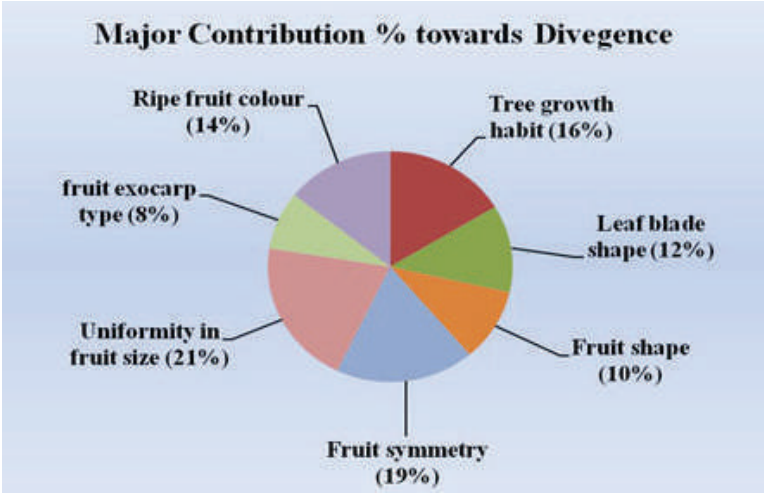


Fig. 4. Graphical representation of relative proportionate contribution of studied major traits (in parentheses value) out of 11 morphological qualitative traits towards genetic divergence of 100 bullock's heart genotypes

Correlation studies

Tree crown shape registered significant positive correlation with fruit symmetry and positively associated with pulp texture, fruit shape and petal outer colour (Table 4). Tree growth habit positively associated with pulp texture, leaf blade shape and location of fructification. Leaf blade shape positively associated with fruit shape, fructification, fruit symmetry. Petal outer colour showed significant positive correlation with fruit exocarp type and positively associated with pulp texture and uniformity in fruit size. Location of fructification recorded significant positive correlation with fruit symmetry, pulp texture and ripe fruit colour. Fruit shape had significant positive correlation with the fruit symmetry and positively associated with fruit exocarp type and uniformity in fruit size. Fruit symmetry had significant positive correlation with uniform fruit size and fruit colour. Uniformity in fruit size showed significant positive correlation with fruit exocarp type and ripe fruit colour and positively associated with pulp texture (Table 4). These results are in line with the observations reported by Verma et al. (2012) in pomegranate, Majumder et al. (2013) in mango and Rajasekhar et al. (2013) in sapota.

Table 4. Correlations coefficients among 11 morphological qualitative traits of 100 bullock's heart genotypes

Characters → ↓	Tree Crown shape	Tree growth habit	Leaf blade shape	Petal outer colour	Location of fructification	Fruit shape	Fruit symmetry	Uniformity in fruit size	Fruit exocarp type	Ripe fruit colour	Pulp texture
Tree Crown shape	**1.000**										
Tree growth habit	-0.053	**1.000**									
Leaf blade shape	0.025	0.048	**1.000**								
Petal outer colour	0.040	-0.067	-0.082	**1.000**							
Location of fructification	-0.092	0.048	0.039	-0.087	**1.000**						
Fruit shape	0.062	-0.002NS	0.072	-0.014	**-0.147***	**1.000**					
Fruit symmetry	**0.103***	**-0.170***	0.014	-0.028	**0.138***	**0.279***	**1.000**				
Uniformity in fruit size	0.008NS	**-0.344***	**-0.216***	0.049	-0.026	0.050	**0.441***	**1.000**			
Fruit exocarp type	**-0.153***	**-0.101***	**-0.181***	**0.229***	-0.012	0.063	**-0.164***	**0.144***	**1.000**		
Ripe fruit colour	**-0.129***	**-0.146**	**-0.144***	-0.095	**0.106***	-0.047	**0.132***	**0.128***	-0.031	**1.000**	
Pulp texture	0.098	0.071	-0.096	0.071	**0.116***	-0.038	-0.020	0.011	0.003NS	-0.048	**1.000**

Cell Contents= Simple correlation; NS=Non Significant; Bold value and * indicates significant at p=5 % level (significant at p<0.05).

Factor Analysis: Varimax Rotation

Factor analysis identified 6 factors retained by positive Eigen value criterion, which explained 154.82% of the total genotypes variation. Most of the variability of the analyzed genotypes was explained by first 3 factors (Table 5). First factor with Eigen value of 1.10 accounted for 62.70% of the variation and was primarily related to uniformity in fruit size, fruit symmetry, tree growth habit and ripe fruit colour, while uniformity in fruit size, fruit symmetry, and ripe fruit colour showed highest positive correlation. The second factor that accounted for 37.35% of the total variance was mainly loaded by fruit exocarp type, fruit symmetry, leaf blade shape and petal outer colour, while fruit symmetry and leaf blade shape revealed highest positive correlation. The third factor accounted for 25.96 % of the total variation and was mainly associated with location of fructification, ripe fruit colour, fruit shape and tree crown shape, while fruit shape and tree crown shape showed highest positive correlation. The communality values ranged from 0.49 to 0.13. Even so, the factor analysis identified traits which contributed most to the variation of the analyzed genotypes and could serve as a useful tool for facilitating the selection of desirable characteristics in bullock's heart breeding. The findings are in accordance with those reported by Manigandan and Vijayakumar (2014).

Table 5. Factor loading (unrotated and rotated factor), Eigen values, cumulative variance, percentage of total (standardized) population of variance explained by 6 factor model and communalities of the 11 morphological qualitative traits of 100 bullock's heart genotypes from factor analysis

Sr. No.	Morphological qualitative characters	Factor I		Factor II		Factor III		Factor IV		Factor V		Factor VI		Communalities
		UFL	RFL	UFL	RFL	UFL	RFL	UFL	RFL	UFL	RFL	UFL	RFL	
1	Tree Crown shape	0.048	0.034	0.171	-0.044	0.242	0.064	**0.221**	**0.395**	-0.165	-0.050	-0.009	0.026	0.17
2	Tree growth habit	**-0.409**	**-0.461**	0.118	-0.086	-0.009	0.015	0.084	0.011	0.175	0.098	-0.106	0.008	0.23
3	Leaf blade shape	-0.193	-0.167	**0.325**	-0.189	0.096	0.089	-0.105	0.054	0.030	-0.026	**0.166**	**0.342**	0.19
4	Petal outer colour	0.053	0.034	**-0.295**	**0.376**	0.208	-0.008	0.123	0.099	0.031	-0.038	0.079	-0.041	0.16
5	Location of fructification	0.022	0.022	0.098	-0.116	**-0.353**	-0.070	0.154	**-0.111**	0.169	**0.407**	0.100	0.011	0.20
6	Fruit shape	0.191	0.049	0.173	0.019	**0.324**	**0.455**	-0.118	0.055	**0.199**	-0.116	-0.077	0.062	0.23
7	Fruit symmetry	**0.595**	**0.483**	**0.350**	-0.195	0.019	**0.414**	0.056	0.114	0.107	0.189	0.011	0.017	0.49
8	Uniformity in fruit size	**0.663**	**0.620**	-0.117	0.120	-0.010	0.161	0.032	0.011	-0.043	0.048	0.006	-0.170	0.46
9	Fruit exocarp type	0.092	0.056	**-0.492**	**0.482**	0.091	0.002	-0.059	**-0.200**	0.170	-0.052	0.039	-0.120	0.29
10	Ripe fruit colour	**0.230**	**0.256**	-0.013	-0.163	**-0.326**	-0.064	-0.114	**-0.235**	-0.033	0.085	-0.075	-0.144	0.18
11	Pulp texture	-0.021	-0.067	-0.052	0.098	-0.023	-0.045	**0.349**	0.196	0.058	**0.234**	-0.037	-0.144	0.13
Eigen values		1.10		0.66		0.46		0.26		0.17		0.07		-
Total population variance explained (%)		62.70		37.35		25.96		14.91		9.93		3.97		-
Cumulative percentage		62.70		100.05		126.01		140.92		150.85		154.82		-

UFL- Unrotated Factor Loading; RFL- Rotated Factor Loading and 6 factors will be retained by the positive Eigen value criterion.

Non-hierarchical clustering

Non-hierarchical clustering was done by using correlation coefficients and covariance matrix where100 bullock's heart genotypes were grouped into 11 clusters. By application of this non-hierarchical clustering pattern of the genotypes, the PCA was confirmed. The clustering patterns obtained through different techniques coincided with the grouping patterns done by PCA suggesting that the results obtained through PCA were established by non-hierarchical clustering.

Hierarchical Cluster Analysis (HCA)

Cluster contributions based on dendrogram

The genotypes based on qualitative traits were grouped into 11 distinct clusters with each cluster containing genotypes that were morphologically similar

(Table 6 and Fig. 5). The number of genotypes in the different groups ranged from 3-21. The distribution pattern indicated that the majority of the genotypes in cluster-II, cluster-I and cluster-VIII, whereas the minimum genotypes were in cluster-XI. The clusters II, I, VIII and X together accounted for 59% of total genotypes.

Table 6. Cluster composition of all genotypes regarding morphological qualitative traits based on dendrogram by Ward's minimum distance method with their source of collection

Clusters	Genotypes and distinct characters
I	Cluster-I is consisted of fourteen genotypes, SW-1, SW-19, CN-5, SW-8, KL-6, SW-7, DP-5, SW-9, SW-18, KL-4, KN-2, SW-11, DP-12 and DP-13 from Ratnagiri and Sindhudurg districts which had highest mean values for most of the studied traits such as tree growth habit (-1.04), followed by uniformity in fruit size (-0.99) and fruit symmetry (-0.88) and almost lowest for fruit shape (0.18).
II	Cluster-II comprising of twenty one genotypes, SW-3, SW-12, SW-15, VL-1, VL-18, DP-4, KD-1, VB-4, SW-13, KL-9, VL-16, VL-7, KL-7, DP-10, DP-3, VL-11, VB-2, DP-2, DP-17, VL-19 and VB-1 from Sawantwadi, Vengurla, Vaibhavwadi and Kudal tahsils of Sindhudurg districts and Dapoli and Khed tahsils of Ratnagiri district which had the extreme mean values for location of fructification (0.81).
III	Cluster-III comprising of four genotypes, VL-5, VL-15, VL-20 and DP-11 from Ratnagiri and Sindhudurg districts which had the extreme mean values for tree crown shape (1.35) and fruit shape (1.33).
IV	Cluster-IV comprising of eight genotypes, SW-23, VL-4, KN-1, VL-25, CN-2, VL-14, KL-10 and GH-1 from Ratnagiri and Sindhudurg districs which had highest mean values for leaf blade shape (1.73) and location of fructification (1.45) other than tree crown shape (1.35) and fruit shape (1.33).
V	Cluster-V comprising of five genotypes, SW-2, SW-20, DP-8, KL-3 and DV-4 from Ratnagiri and Sindhudurg districts which had the extreme mean values for fruit shape (2.48) other than tree crown shape (2.50).
VI	Cluster-VI comprising of seven genotypes, SW-17, VL-21, KL-8, VL-24, DP-14, VB-5 and KD-2 from Ratnagiri and Sindhudurg districts which had the extreme mean values for location of fructification (2.09) other than tree crown shape (3.07), leaf blade shape (3.17) and fruit shape (3.05).
VII	Cluster-VII comprising of seven genotypes, SW-4, VL-12, SW-24, VL-9, CN-7, DV-3 and VL-10 from Ratnagiri and Sindhudurg districts which had the extreme mean values for ripe fruit colour (2.32) other than leaf blade shape (3.89), tree crown shape (3.64) and fruit shape (3.62) and which were also greater than mean value of all eleven clusters.
VIII	Cluster-VIII comprising of fourteen genotypes, SW-5, SW-22, VL-2, VL-13, SW-14, VL-6, KL-5, DP-6, SW-21, VL-8, CN-3, DV-1, VB-3 and KL-2from Ratnagiri and Sindhudurg districts which had possessed first position in case of leaf blade shape (4.61) followed by tree crown shape (4.22) and fruit shape (4.20).
IX	Cluster-IX comprising of three genotypes,SW-10, MN-2 and VL-22, had almost lowest for fruit symmetry and uniformity in fruit size, no remarkable feature was noticed in this cluster for these two traits, while highest mean values for leaf blade shape (5.33), tree crown shape (4.79) and fruit shape (4.77).
X	Cluster-X comprising of ten genotypes, VL-17, DP-18, DP-16, CN-4, CN-6, DP-9, VL-23, DP-15, SW-6 and SW-16 from Ratnagiri and Sindhudurg districts which had the extreme mean values for leaf blade shape (6.05), tree crown shape (5.36), fruit shape (5.35), tree growth habit (3.78) and pulp texture (3.77).
XI	Cluster-XI comprising of seven genotypes, MN-1, DP-1, VL-3, DP-7, CN-1, KL-1 and DV-2 from Ratnagiri and Sindhudurg districts which had the extreme mean values for leaf blade shape (6.76) followed by tree crown shape (5.94) and fruit shape (5.92) and also the remarkable feature was noticed in this cluster for location of fructification, fruit symmetry and uniformity in fruit size.

[Hierarchical clustering]
Method=Ward
No. of clusters=11

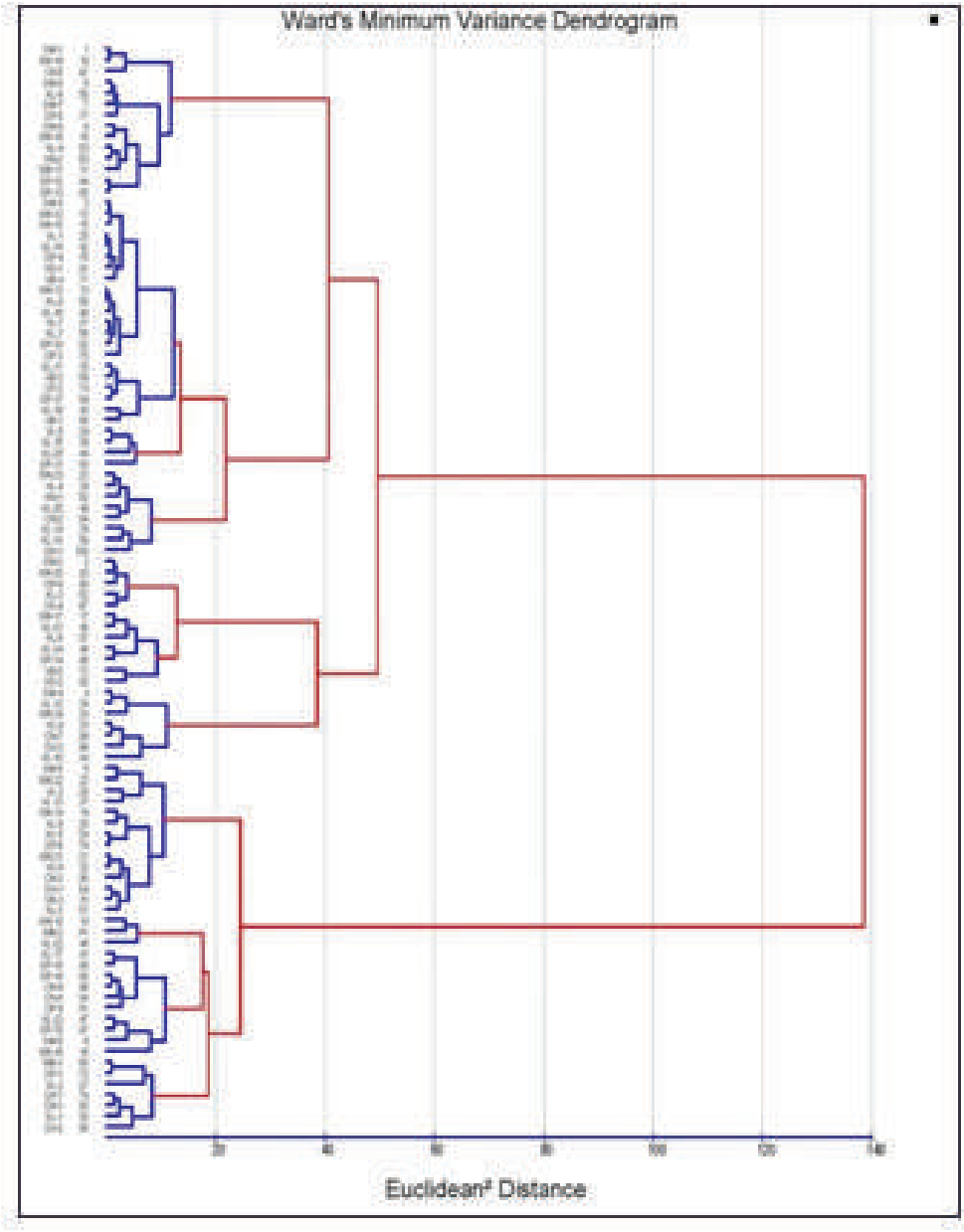


Fig. 5. Consensus tree diagram (Dendrogram) representing relationships of 100 elite bullock's heart genotypes produced by Ward's minimum distance cluster analysis based on 11 morphological qualitative traits (Scale: Euclidean^2 distance)

Cluster means with distinct characters

Differences among the genotypes for leaf blade shape, tree crown shape, fruit shape and location of fructification were more pronounced as compared to the other traits. Some of these characters, though not very significant in our study, could be effectively exploited in future crop improvement. In particular, the comparison between non-hierarchical clustering and HCA revealed the uniformity in fruit size to be the most important character its contribution was maximum (21%) in the genetic divergence (Fig. 4). Cluster-XI and cluster-X had the highest cluster mean values for most of the traits (Table 7). Based on the cluster means, the important clusters were cluster-I for uniformity in fruit size and fruit symmetry, and cluster-XI and cluster-X for fruit shape and pulp texture.

Table 7. Cluster means of 100 bullock's heart genotypes for 11 clusters with respect to eleven studied morphological qualitative traits based on dendrogram by Ward's minimum distance method

Morphological qualitative characters	<-------Clusters mean------>											Mean
	I (14)*	II (21)	III (4)	IV (8)	V (5)	VI (7)	VII (7)	VIII (14)	IX (3)	X (10)	XI (7)	
Tree Crown shape	0.20	**0.78**	**1.35**	**1.92**	**2.50**	**3.07**	**3.64**	**4.22**	**4.79**	**5.36**	**5.94**	**3.07**
Tree growth habit	**-1.04**	-0.50	0.03	0.57	1.10	1.64	2.18	2.71	3.25	3.78	4.32	**1.64**
Leaf blade shape	-0.42	0.29	1.01	1.73	**2.45**	**3.17**	**3.89**	**4.61**	**5.33**	**6.05**	**6.76**	**3.17**
Petal outer colour	-0.62	-0.17	0.27	0.71	1.16	1.60	2.04	2.49	2.93	3.37	3.82	**1.60**
Location of fructification	0.49	**0.81**	1.13	1.45	1.77	2.09	2.41	2.73	3.05	3.37	3.69	**2.09**
Fruit shape	0.18	**0.75**	**1.33**	**1.90**	**2.48**	**3.05**	**3.62**	**4.20**	**4.77**	**5.35**	**5.92**	**3.05**
Fruit symmetry	-0.88	-0.58	-0.29	0.01	0.30	0.60	0.90	1.19	1.49	1.78	2.08	**0.60**
Uniformity in fruit size	**-0.99**	-0.69	-0.38	-0.08	0.22	0.52	0.82	1.12	1.42	1.73	2.03	**0.52**
Fruit exocarp type	-0.21	0.11	0.42	0.74	1.05	1.37	1.69	2.00	2.32	2.63	2.95	**1.37**
Ripe fruit colour	-0.37	0.08	0.52	0.97	1.42	1.87	2.32	2.77	3.22	3.66	4.11	**1.87**
Pulp texture	-0.65	-0.17	0.32	0.81	1.29	1.78	2.27	2.75	3.24	3.73	4.21	**1.78**

Highest values with bold figure in column indicating important characters of the cluster and * indicating number of genotypes in the cluster

Principal Co-ordinate Analysis

Intra-cluster distances ranged from 4.38 to 9.33 (Fig. 6). Highest intra-cluster distance was observed in cluster-IX indicating that the genotypes belonging to this cluster were far diverged from cluster-VII. Minimum intra-cluster distance was observed in cluster-II which included maximum number of genotypes. Cluster-II was far diverged with the rest of the clusters indicating that these genotypes could be crossed with other genotypes in order to incorporate the desired characters like fruit shape, fruit symmetry, uniformity in fruit size, fruit exocarp type, ripe fruit colour and pulp texture into the cultivated types.

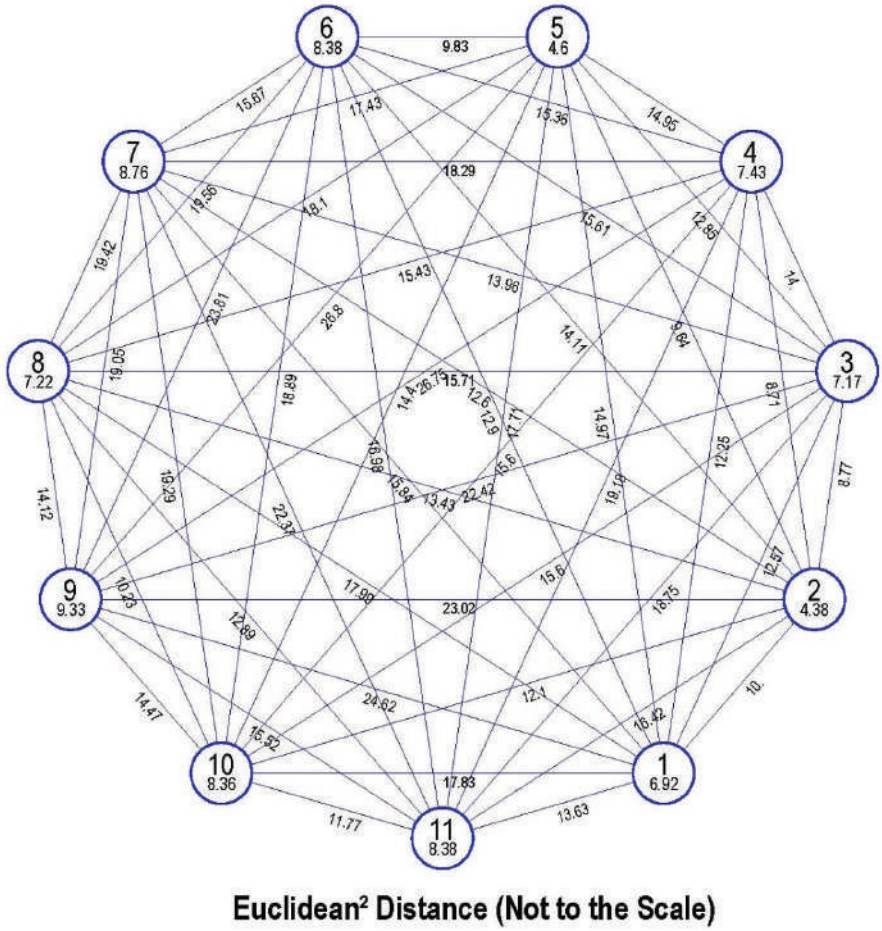


Fig. 6. Cluster diagram showing the average intra and inter cluster distance ($D = \sqrt{D^2}$) based on Euclidean² distance for 11 morphological qualitative traits in 11 clusters of 100 bullock's heart genotypes (The values along the lines indicate inter cluster distances and the values within the circle indicate intra cluster distances)

Canonical Vector Analysis

Results revealed that inter-cluster distances ranged from 8.71 to 26.80 (Fig. 6). Maximum inter-cluster distance was observed between cluster-V (SW-2, SW-20, DP-8, KL-3, DV-4) and cluster-IX (SW-10, MN-2, VL-22) indicating wide range of genetic diversity between these two clusters, followed by cluster-IV and IX, cluster-I and IX, cluster-VI and IX and cluster-II and IX. Lowest inter-cluster distance was between cluster- II and cluster- IV meaning more genetic similarity. The putative parents for a systematic crossing programme should belong to diverse clusters characterized by a large inter-cluster distance. The hybridization among the genotypes drawn from widely divergent clusters with high yield potential is likely to manifest maximum heterotic combinations as well as new recombination with desired traits. From the observations, it was apparent that there was a considerable degree of divergence at inter-genetic stock (between genotypes), inter-cluster (between clusters), and intra-cluster (within cluster) levels of diversification in *A. reticulata.* These results are in accordance with the observations reported by Rajasekhar et al. (2013) in sapota and Sharma et al. (2013) in apple.

Conclusion

Results of morphological diversity analysis demonstrated the presence of substantial variability among the evaluated bullock's heart genotypes for the studied traits and it was enough to distinguish between them. All desired characteristics were not found in one unique genotype, although some genotypes DP-5, VL-22, KL-7, KL-9, SW-12, DP-16, DP-4, VL-7, SW-20 and VL-16 (Fig. 7) had showed potentially good characteristics for international markets. Hierarchical and non-hierarchical algorithms, based on the multivariate statistical techniques, are common methods used by breeders to identify diverse genotypes for developing varieties that suit the target environment. Application of these three methods was useful for classification, documentation and characterization of bullock's heart genotypes. Results suggested that the genotypes originated from same place may have different genetic architecture or vice-versa. It is therefore recommended that the genetic conservation and improvement of bullock's heart based on the selected materials should be ecouraged.

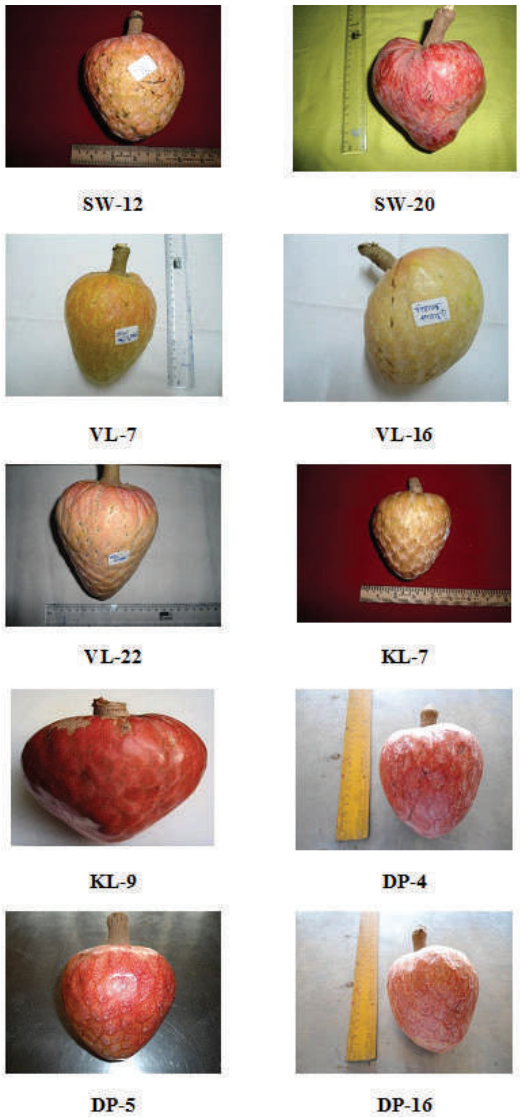


Fig. 7. Variability in fruit characteristics in selected genotypes of bullock's heart

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Studies on *in situ* Characterization and Evaluation of Non-descript Jackfruit Genotypes of Tripura, India

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Abstract

Tripura state in the Northeastern India is the reservoir of diverse jackfruit genotypes possessing traits of importance and hence, identification of trait specific superior jackfruit genotypes in the region is desirable. An investigation was carried out to characterize,evaluate and identify superior jackfruit genotypes from Tripura. Surveys were undertaken for 3 consecutive seasons in the West Tripura district of Tripura and 25 genotypes were collected and evaluated for morphological and biochemical parameters using IPGRI descriptors. Wide variability was observed among the studied accessions for morphological characters *viz.* crown shape, shape of leaf blade, fruit, flakes and seed characters. Values for traits *viz*. fruit weight, number of flakes/kg fruit and total soluble solids(TSS) ranged from 2.0-8.6 kg, 6- 46 and 12.5- 28.2 °Brix, respectively. Higher fruit weight was recorded in genotypes TJS 1 (8.6 kg), TJS 18 (6.82 kg) and TJS 9 (6.35kg), while higher TSS was observed in genotypes TJS 20 (28.2 °Brix) and TJS 21 (24 °Brix). These genotypes were found to be suitable for table and processing purpose. The results of the present study may be used for future breeding and crop improvement purpose to develop superior jackfruit genotype suitable for commercial cultivation.

Key words: *Genotypes, morphology, Northeastern India, physicochemical characters*

Introduction

Jackfruit (*Artocarpus heterophyllus* Lam.) is an important nutritious fruit crop belonging to the Moraceae family. It is native to parts of South and Southeast Asia and is believed to be originated in the rainforests of the Western Ghats of India (Rowe-Dutton, 1985). It is cultivated throughout the low lands in South and Southeast Asia, parts of Central and Eastern Africa and Brazil. In India, it has wide distribution in the states of Assam, Tripura, West Bengal, Bihar, Uttar Pradesh, Kerala, Tamil Nadu, Karnataka andfoothills of the Himalayas. Of these, Tripura, Assam and West Bengal produce major share of jackfruit in India.

Tripura is a reservoir of diverse jackfruit genotypes including those exhibiting year-round fruiting. Area under jackfruit cultivation in Tripura is 5,491 ha with annual production of 1,33,251 lakh t (Anon., 2021). Tripura has the highest productivity (24.27 t/ha) of jackfruit in India followed by Odisha, Assam and West Bengal. There is huge local demand of jackfruit in the state. The best quality jackfruits are available from May to July and are transported to neighboring states and countries. According to some report, every year large quantity of jackfruits is legally and illegally exported to Bangladesh from Tripura (Sarkar, 2017).

Jackfruit cultivation is preferred by the farmers of the state due to its remunerative prices, hardy nature, low input requirement, easy cultivation and suitability for rainfed condition. Favorable agro-climate coupled with availability of cultivable (hilly and undulated) land for the crop offers immense potentiality for its cultivation.The Government aimed to set up a jackfruit mission to promote its production, processing, value addition and marketing which could help to determine its export potential to foreign countries (Anon., 2022). Thus, there is an immediate need to identify trait specific superior jackfruit genotypes in the region. Despite the wider genetic diversity available, no commercial variety/ superior genotypes have been developed for the region. Thus, an attempt was made to identify unique germplasm from West Tripura through systematic morphological and physico-chemical characterization.

Materials and methods

The survey was carried out in Lefunga block of West Tripura district of Tripura during 2019-2021. The study area has tropical climate with average rainfall of 2,200 mm, extending over a period of 8 months in a year. Average temperature falls to 10°C in winter and the summer temperature is 35°C. The humidity ranges from 60 to 98%. The soils of the hillocks and undulated (*tilla*) land are deep, well drained and acidic in nature (pH: 4.5 to 6.0).

Survey was undertaken for three consecutive seasons in the West Tripura district of Tripura and 25 identified genotypes were evaluated for morphological and biochemical characters using IPGRI descriptors (IPGRI, 2000). For the identified 25 genotypes, GPS coordinates and data on the various morphological parameters of trees, leaves, fruits, seeds and quality characters were recorded. The qualitative characters were measured by observation and frequency distribution analysis was performed. The variations among the different types of trees were determined by calculating the coefficient of variation. The quantitative characters were analyzed using statistical tools such as mean, standard deviation, and coefficient of variations (Panse and Sukhatme, 1967).

Table 1. GPS coordinates of the identified accessions of jackfruit

Sl. No.	Genotypes	Location	Altitude (m)	Latitude (N)	Longitude (E)
1	TJS1	West Tripura	108.02	23.963955	91.315794
2	TJS2	West Tripura	102.31	23.903882	91.316115
3	TJS3	West Tripura	101.07	23.902873	91.31607
4	TJS4	West Tripura	102.35	23.90274	91.315921
5	TJS5	West Tripura	86.68	23.904756	91.316406
6	TJS6	West Tripura	93.39	23.904952	91.316346
7	TJS7	West Tripura	87.13	23.903638	91.31499
8	TJS8	West Tripura	104.98	23.903401	91.315016
9	TJS9	West Tripura	98.13	23.902915	91.314681
10	TJS10	West Tripura	101.43	23.902463	91.314703
11	TJS11	West Tripura	103.28	23.902574	91.314611
12	TJS12	West Tripura	85.43	23.902419	91.314587
13	TJS13	West Tripura	93.27	23.902487	91.314637
14	TJS14	West Tripura	86.54	23.902442	91.31457
15	TJS15	West Tripura	82.89	23.902449	91.314611
16	TJS16	West Tripura	81.28	23.902466	91.314639
17	TJS17	West Tripura	86.23	23.902433	91.314514
18	TJS18	West Tripura	81.7	23.90244	91.314489
19	TJS19	West Tripura	77.78	23.902368	91.314539
20	TJS20	West Tripura	98.63	23.905545	91.314259
21	TJS21	West Tripura	100.61	23.902649	91.314855
22	TJS22	West Tripura	89.75	23.90228	91.314673
23	TJS23	West Tripura	83.35	23.902529	91.314455
24	TJS24	West Tripura	51.21	23.905283	91.31532
25	TJS25	West Tripura	89.88	23.904438	91.316482

Results and discussion

During the survey it was observed that being underutilized fruit, jackfruit was mainly grown in the backyards or homestead gardens without any management practice, mixed with other forest/ fruit trees or randomly at roadside. However, at two locations (Cocotilla and Belbari, West Tripura), a systematic seedling progenies plantation of 35 - 40 yearsold with around 80-100 trees was recorded. The crop is found to be suitable in different land situation *viz*. *lunga* (lowland) and *tilla* (upland) lands in Tripura**.** The peak season starts from March – April and ends in June – July. Das and Saha (2020) reported the broad classification of jackfruit *viz*. (1) Normal bearing, (2) Early bearing, (3) Late bearing, (4) Twice bearing and (5) All season types (*Baramasi*) based on the season at Tripura**.**

Jackfruit, being cross-pollinated and mostly seed propagated, exhibits great variation in economic traits, which is considered as a prerequisite for any crop improvement programme. Wide variability was observed among the selected jackfruit genotypes for morphological characters *viz*.growth habit, tree characters, leaf characters, inflorescence characters, fruiting behaviour and yield attributing characters. The frequency distribution of the qualitative characters is presented in Table 2. Irregular (52%), Elliptical growth habit (16%), cluster bearing (64%), spiny surface (84%), soft texture flakes (68%), reddish yellow rind colour (56%) were found more frequent among the accessions studied. Mitra and Mani (2000) also observed wide variations among seedling progeny of jackfruit with regard to growth habit, canopy structure, leaf size, fruit bearing, fruit shape, fruit size and fruit quality. Dey and Baruah (2019) also reported wide variability among 24 jackfruit accessions of Assam for various tree, leaf, fruit and seed morphological characters. Similarly, Reddy et al. (2004) observed enormous variability in the qualitative and quantitative traits of jackfruit in South Karnataka.

Table 2. Frequency distribution of jackfruit genotypes of the polymorphic qualitative characters

Characters	Frequency	Frequency (%)	Characters	Frequency	Frequency (%)
Tree Vigor			**Fruit shape**		
Low	11	**44**	Ellipsoid	6	24
Medium	13	**52**	Spheroid	7	28
High	5	**20**	Clavate	7	28
Trunk surface			Oblong	5	20
Very rough	4	**16**	**Fruit surface**		
Rough	10	**40**	Spiny	21	84
Smooth	11	**44**	Smooth	4	16
Crown Shape			**Flake texture**		
Elliptical	4	**16**	Coarse	5	20
Irregular	13	**52**	**Soft**	**17**	68
Broadly Pyramidal	1	**4**	Firm	3	12
Pyramidal	1	**4**	**Pulp flavor**		
Semi-circular	1	**4**	Strong	8	32
Tree growth habit			Weak	1	4
Semi erect	2	**8**	Intermediate	16	64
Spreading	4	**16**	**Fruit rind colour**		
Erect	1	**4**	Green	2	8
Elliptical	18	**72**	Yellow	5	20
Fruit clustering habit			Light yellow	1	4
Cluster	16	**64**	Greenish yellow	3	12
Solitary	9	**36**	Reddish yellow	14	56

Great variability existed with regard to desirable characters *viz*. bearing habits (cluster/ solitary), number of fruits per tree, average fruit weight (2.05 – 9.70 kg), number of flakes per kg fruit (7 – 46), total soluble solids (TSS) content of flakes (12.5 to 28.2 °Brix), shelf life at room temperature (2 -10days). Wide variation in yield, fruit quality and seed characters have also been reported by earlier researchers (Azad and Haq, 1998; Singh and Srivastava, 2000).Such considerable range of variation in morpho-agronomic characters observed in jackfruit may be due to cross pollination and seedling origin. Wangchu *et al.* (2013) also reported great variation in the morpho-physiochemical characters among 44 superior jackfruit genotypes from West Bengal.

Table 3. Estimates of performance for range in growth and yield characters

Characters	Range		Stdev	Mean	CV
	Max	Min			
Trunk circumference (m)	2.25	0.68	0.36	1.3252	26.90
No. of fruits/tree	53	6	12.77	30.16	42.34
Stalk length (cm)	33.2	7.2	6.79	14.7228	46.14
Stalk diameter (cm)	2.7	1.5	0.31	2.1064	14.68
Fruit length (cm)	41.4	19.3	6.67	31.274	21.32
Fruit diameter (cm)	28.30	13.25	4.52	19.408	23.30
Fruit weight (kg)	9.70	2.05	2.12	4.9344	42.99
No. of flakes/kg fruit	46	7	9.81	29	33.81
Weight of flakes/kg fruit (g)	739.6	418.0	93.13	642.016	14.51
Weight of fresh flakes with seed (g)	700.23	264.61	134.13	461.9348	29.04
Weight of fresh flakes without seed (g)	512.11	147.00	119.64	318.8156	37.53
Flake length (cm)	9.2	3.5	1.52	5.496	27.59
Flake width (cm)	5.85	2.30	0.94	3.482	27.09
Rind thickness (mm)	13.8	10.1	1.05	11.46	9.14
TSS (°Brix)	28.2	12.5	3.76	20.836	18.06
Rachis length (cm)	34.0	13.1	6.16	24.656	24.97
Rachis diameter (cm)	20.3	5.3	4.01	9	44.57
Seed length (cm)	4.25	1.75	0.68	2.994	22.57
Seed width (cm)	3.4	0.9	0.60	1.894	31.73
100-seed weight (g)	800.0	131.2	167.99	592.5772	28.35
No. of seeds/kg of fruit	46	17	8.35	29.4	28.40
Shelf life (days)	10	2	2.49	5.68	43.90

The tree morphological characters mainly observed in the genotypes collected are medium tree vigor, smooth trunk surface, irregular crown shape, erect growth habit, sparse branching density, verticillate branching and cluster fruit bearing habit. The genotypes *viz.*TJS1, TJS2, TJS3, TJS4, TJS8, TJS12, TJS13, TJS15, TJS17, TJS18, TJS19, TJS20, TJS21, TJS23, TJS24 and TJS25 showed cluster fruit bearing habit, which is a desirable character for future jackfruit improvement (Table 4).

Table 4. Tree morphological characters of the different Jackfruit accessions as per the IPGRI descriptor

Accessions/ Genotypes	Tree Vigor	Trunk surface	Crown Shape	Tree growth habit	Branching density	Branching Pattern	Fruit clustering habit
TJS1	Medium	Rough	Irregular	Semi-erect	Medium	Irregular	Cluster
TJS2	High	Rough	Semi-circular	Spreading	Verticillate	Horizontal	Cluster
TJS3	Low	Very Rough	Erect	Erect	Sparse	Erect	Cluster
TJS4	Low	Smooth	Broadly Pyramidal	Spreading	Medium	Horizontal	Cluster
TJS5	Low	Smooth	Irregular	Erect	Sparse	Erect	Solitary
TJS6	Low	Smooth	Sparse	Elliptical	Sparse	Erect	Solitary
TJS7	Low	Smooth	Irregular	Spreading	Sparse	Horizontal	Solitary
TJS8	Medium	Very Rough	Irregular	Spreading	Medium	Horizontal	Cluster
TJS9	Medium	Smooth	Paramidal	Erect	Medium	Verticillate	Solitary
TJS10	Low	Rough	Elliptical	Erect	Sparse	Erect	Solitary
TJS11	Low	Smooth	Elliptical	Erect	Sparse	Erect	Solitary
TJS12	Medium	Rough	Spherical	Erect	Medium	Verticillate	Cluster
TJS13	Medium	Smooth	Spherical	Erect	Medium	Verticillate	Cluster
TJS14	Medium	Smooth	Irregular	Erect	Sparse	Verticillate	Solitary
TJS15	Low	Very Rough	Irregular	Erect	Sparse	Verticillate	Cluster
TJS16	Medium	Rough	Irregular	Erect	Sparse	Erect	Solitary
TJS17	Low	Rough	Irregular	Erect	Sparse	Erect	Cluster
TJS18	Low	Smooth	Elliptical	Erect	Sparse	Opposite	Cluster
TJS19	Low	Smooth	Irregular	Erect	Sparse	Erect	Cluster
TJS20	Medium	Smooth	Elliptical	Erect	Medium	Verticillate	Cluster
TJS21	Medium	Rough	Irregular	Erect	Sparse	Verticillate	Cluster
TJS22	Medium	Very Rough	Irregular	Erect	Sparse	Verticillate	Solitary
TJS23	Medium	Rough	Irregular	Semi-erect	Medium	Irregular	Cluster
TJS24	Medium	Rough	Irregular	Erect	Medium	Erect	Cluster
TJS25	Medium	Rough	Irregular	Erect	Medium	Erect	Cluster

Large variation was also recorded among the genotypes (Table 5) for characters *viz*. fruit shape (ellipsoid, spheroid, clavate and oblong), stalk attachment to fruit (flat and intermediate), shape of spines (sparse and sharp pointed), fruit rind colour (yellow, reddish yellow, light yellow, greenish yellow and green), latex exudation (high, medium & low) and fruit quality (excellent, moderate & good). Fruit shape (obovate, oblong with curved tip, irregular, cordate, twisted and rectangular), flakes texture (coarse, soft & firm), pulp flavor (weak, intermediate & strong), flakes thickness (thick, medium & thin), fibre content (low, medium & high), pulp taste (insipid, slight sweet, moderate sweet, very sweet & sweet), pulp colour (light yellow, yellow & deep yellow) and seed shape (irregular, ramiform, ellipsoid, oblong & spheroid) were recorded (Table 6).

Table 5. Fruit quality characters of the jackfruit accessions as per the descriptor

Accessions/ Genotypes	Fruit shape	Stalk attachment to fruit	Shape of spine	Fruit rind colour	Latex exudation	Fruit quality
TJS1	Ellipsoid	Flattened	Intermediate	Yellow	High	Good
TJS2	Ellipsoid	Flattened	Intermediate	Reddish Yellow	High	Excellent
TJS3	Spheroid	Flattened	Flat	Green	Low	Moderate
TJS4	Ellipsoid	Flattened	Intermediate	Reddish Yellow	Medium	Good
TJS5	Spheroid	Flattened	Intermediate	Reddish Yellow	Intermediate	Good
TJS6	Clavate	Flattened	Intermediate	Greenish Yellow	Medium	Good
TJS7	Spheroid	Flattened	Sharp Pointed	Reddish Yellow	Medium	Moderate
TJS8	Spheroid	Flattened	Sharp Pointed	Yellow	Medium	Good
TJS9	Ellipsoid	Flattened	Intermediate	Greenish Yellow	High	Good
TJS10	Clavate	Flattened	Sparse	Reddish Yellow	Medium	Good
TJS11	Clavate	Flattened	Intermediate	Reddish Yellow	Medium	Excellent
TJS12	Spheroid	Flattened	Intermediate	Reddish Yellow	High	Moderate
TJS13	Oblong	Flattened	Sharp Pointed	Reddish Yellow	High	Excellent
TJS14	Clavate	Flattened	Intermediate	Reddish Yellow	Medium	Good
TJS15	Ellipsoid	Flattened	Sharp Pointed	Yellow	Medium	Good
TJS16	Clavate	Flattened	Sharp Pointed	Reddish Yellow	Medium	Good
TJS17	Clavate	Flattened	Sharp Pointed	Reddish Yellow	Low	Good
TJS18	Spheroid	Flattened	Sharp Pointed	Reddish Yellow	Low	Good
TJS19	Clavate	Flattened	Sharp Pointed	Yellow	Medium	Good
TJS20	Oblong	Flattened	Intermediate	Reddish Yellow	Medium	Good
TJS21	Oblong	Flattened	Sharp Pointed	Yellow	High	Good
TJS22	Ellipsoid	Flattened	Sharp Pointed	Reddish Yellow	Medium	Good
TJS23	Oblong	Flattened	Sharp Pointed	Green	Medium	Excellent
TJS24	Spheroid	Flattened	Flat	Yellow	Medium	Good
TJS25	Oblong	Flattened	Flat	Light Yellow	Medium	Excellent

Wide variations were also observed for various fruits physical parameters (Table 7). Genotypes *viz*.TJS1, TJS8, TJS13, TJS20 and TJS21 recorded higher number of fruits per tree *i.e*. 53, 47, 46, 46 and 41, respectively. The genotypes *viz*. TJS1, TJS11, TJS14, TJS19 and TJS 21 recorded longer fruit length of 38.9 cm, 35.6 cm, 36.5 cm, 38.6 cm and 41.4 cm, respectively as compared to genotype TJS 3 withthe lowest fruit length of 19.3 cm. Higher fruit diameter of 25.35 cm, 25.1 cm, 28.3 cm, 26.3 cm were recorded in genotypes TJS1, TJS13, TJS 21, TJS 23, respectively while it was the lowest in genotype TJS 24 (13.7cm). Heavier fruits were observed in genotypes TJS 21 (9.7 kg), TJS 1 (8.6 kg) and TJS 22 (7.85 kg), while fruit weight was lower in genotypes TJS 3 (2.05 kg), TJS 24 (2.5 kg) and TJS 9 (2.9 kg). Higher weight of flakes/kg fruit of 725.5 g, 739.6 g, 734.4 g and 719.6 g was recorded in genotypes TJS 1, TJS 20, TJS 21 and TJS 23, respectively as compare to minimum number of flakes/ kg fruit of 418 g observed in genotype TJS 7. Genotype TJS12 recorded the maximum number of flakes/kg fruit (46).

Table 6. Morphological and sensory characters of the flakes for the jackfruit accessions as per the descriptor

Accessions/ Genotypes	Flake Shape	Flake Texture	Pulp Flavor	Flake Thickness	Fibre Content	Pulp Taste	Pulp Colour	Seed Shape
TJS1	Obovate	Coarse	Strong	Thick	Low	Insipid	Yellow	Irregular
TJS2	Irregular	Soft	Strong	Medium	Medium	Sweet	Deep Yellow	Irregular
TJS3	Cordate	Soft	Weak	Medium	Medium	Slight Sweet	Light Yellow	Reniform
TJS4	Oblong With Curved Tip	Soft	Intermediate	Medium	Low	Sweet	Yellow	Irregular
TJS5	Spheroid	Firm	Intermediate	Medium	Medium	Sweet	Yellow	Irregular
TJS6	Irregular	Soft	Strong	Medium	Medium	Sweet	Light Yellow	Ellipsoid
TJS7	Cordate	Coarse	Intermediate	Medium	Low	Slight Sweet	Light Yellow	Ellipsoid
TJS8	Irregular	Soft	Intermediate	Medium	High	Sweet	Light Yellow	Ellipsoid
TJS9	Twisted	Firm	Intermediate	Medium	Medium	Sweet	Yellow	Ellipsoid
TJS10	Rectangular	Soft	Intermediate	Thin	Low	Moderate Sweet	Light Yellow	Ellipsoid
TJS11	Twisted	Soft	Strong	Medium	High	Sweet	Deep Yellow	Oblong
TJS12	Cordate	Soft	Intermediate	Thin	High	Sweet	Yellow	Spheroid
TJS13	Rectangular	Soft	Strong	Thick	High	Sweet	Light Yellow	Ellipsoid
TJS14	Oblong	Coarse	Intermediate	Thick	Low	Sweet	Yellow	Oblong
TJS15	Cordate	Soft	Intermediate	Medium	Low	Sweet	Light Yellow	Irregular
TJS16	Rectangular	Coarse	Intermediate	Thick	Low	Sweet	Yellow	Ellipsoid
TJS17	Irregular	Firm	Intermediate	Thick	Low	Sweet	Yellow	Spheroid
TJS18	Rectangular	Coarse	Intermediate	Thick	Low	Sweet	Light Yellow	Irregular
TJS19	Spheroid	Soft	Intermediate	Thin	Medium	Sweet	Yellow	Spheroid
TJS20	Rectangular	Soft	Intermediate	Medium	High	Sweet	Yellow	Irregular
TJS21	Oblong	Soft	Strong	Medium	Medium	Very Sweet	Light Yellow	Reniform
TJS22	Twisted	Soft	Strong	Medium	Medium	Sweet	Deep Yellow	Irregular
TJS23	Spheroid	Soft	Strong	Thin	Medium	Sweet	Yellow	Irregular
TJS24	Rectangular	Soft	Intermediate	Thin	Medium	Slight Sweet	Light Yellow	Ellipsoid
TJS25	Spheroid	Soft	Intermediate	Medium	Medium	Sweet	Light Yellow	Irregular

Table 7. Physico-chemical characters of the flakes for the jackfruit accessions as per the descriptor

Genotypes	No. of fruits/ tree	Fruit length (cm)	Fruit diameter (cm)	Fruit weight (kg)	No. of flakes/kg fruit	Weight of flakes/kg fruit (g)	Weight of fresh flakes without seed (g)	Flake length (cm)	Flake width (cm)	TSS (°Brix)	Seed length (cm)	Seed width (cm)	100-seed weight (g)	No. of seeds/kg of fruit	Shelf life at room temperature (days)
TJS1	53	38.9	25.4	8.6	31	725.5	353.2	7.5	3.8	19.8	4	2.1	692	31	3
TJS2	37	27.4	16.3	4.3	23	614.0	392.1	9.2	5.9	23.6	4.3	3.4	516	23	5
TJS3	26	19.3	13.3	2.1	18	555.0	469.0	6.4	4.3	12.5	2.6	1.9	679	18	8
TJS4	6	27.0	16.7	5.2	26	675.0	368.0	5.1	4.2	19.8	2.3	1.3	703	26	2
TJS5	17	23.3	16.9	3.4	22	570.9	313.2	5.2	3.0	20.4	2.3	1.2	539	22	5
TJS6	25	25.2	14.0	3.2	7	595.6	512.1	6.0	4.8	22.4	1.8	0.9	398	17	6
TJS7	31	26.3	18.3	3.9	29	418.0	161.0	5.9	3.7	17.4	3.0	1.6	689	29	8
TJS8	47	29.9	22.1	4.3	36	478.3	183.2	3.5	2.6	19.2	2.4	1.2	400	36	8
TJS9	29	34.9	19.5	6.4	28	679.6	322.1	6.3	4.0	22.6	3.2	1.7	613	28	10
TJS10	25	31.8	17.2	3.4	28	508.6	256.9	4.5	3.5	18.6	3.0	1.8	569	28	4
TJS11	33	35.6	22.9	4.2	32	694.4	251.0	4.5	3.3	23.0	3.3	2.2	800	32	5
TJS12	19	19.9	15.8	2.9	46	680.8	159.9	4.9	2.8	20.6	3.8	2.6	632	46	10
TJS13	46	35.2	25.1	5.5	34	707.2	350.3	5.2	2.3	26.2	2.7	1.5	620	34	8
TJS14	19	36.5	16.6	5.6	21	682.5	510.0	8.5	3.5	22.8	3.0	1.8	460	21	10
TJS15	33	30.4	22.6	4.5	26	689.5	370.2	5.5	2.4	18.0	3.7	2.0	712	26	3
TJS16	20	37.2	18.5	6.0	26	715.0	455.0	5.4	3.2	21.8	2.5	2.0	468	26	5
TJS17	16	33.6	20.8	3.0	41	600.7	173.9	4.1	2.8	19.6	3.0	2.1	609	41	4
TJS18	27	33.9	22.1	6.8	36	646.5	209.9	4.9	2.9	20.8	3.1	2.1	717	36	5
TJS19	21	38.6	18.5	4.2	30	639.2	301.2	5.2	3.6	19.3	3.9	2.1	631	30	5
TJS20	46	28.5	16.7	5.1	43	739.6	232.1	4.3	2.9	22.0	2.3	2.2	566	43	7
TJS21	40	41.4	28.3	9.7	27	734.4	409.9	6.1	4.2	24.2	3.2	2.0	671	27	5
TJS22	42	36.0	22.2	7.9	23	696.9	465.1	4.8	3.6	20.1	3.0	2.2	675	23	4
TJS23	28	34.8	26.3	5.2	32	719.6	311.3	5.2	4.3	28.2	2.8	1.6	131	32	4
TJS24	31	22.0	13.7	2.5	38	638.4	147.0	4.1	2.8	16.2	3.0	2.1	735	38	5
TJS25	37	34.4	15.6	5.6	22	645.3	292.7	5.1	2.8	21.8	2.9	2.0	588	22	3
S.Em	2.29	1.23	0.83	0.38	1.70	16.65	22.56	0.27	0.16	0.64	0.12	0.10	28.29	1.52	0.46
CV	37.90	19.69	21.42	38.38	29.26	12.97	35.38	24.12	23.58	15.32	20.17	26.71	23.87	25.83	40.89

With regard to the fruit quality characters, considerable variations were observed. Maximum TSS was recorded in the genotypes TJS 23 (28.2 ºBrix) followed by TJS 13 (26.2 ºBrix), TJS 21 (24.2 ºBrix) and TJS 2 (23.6 ºBrix). Maximum flake dimension (9.2 cm length, 5.85 cm width) and seed size (4.25 cm length, 3.4 cm width) was observed in TJS 2. Genotype TJS 11 recorded the highest 100 seed weight (800 g) followed by genotype TJS 24 (735.4 g). Lower number of seeds per kg fruit was recorded in genotypes *viz*. TJS 6 (17), followed by TJS 3 (18) and TJS 14 (21), which is a beneficial trait for future breeding programmes.

Crop improvement in perennials is a complex process and use of available genetic resources is crucial for any crop improvement programme. Variation in economic traits is a prerequisite for any crop improvement programme. Thus, there is a need to identify the areas holding rich genetic diversity of jackfruit that can be used for improving the productivity and quality. From this study, the following genotypes from Tripura are identified for their specific traits.

1.Types suitable for use as a table fruit (Excellent fruit quality and high TSS content):TJS 2, TJS 5, TJS 6, TJS 12, TJS 11, TJS 13, TJS 23 and TJS 25.

2.Types suitable for vegetable/cooking purpose (small-medium size fruits with less fibre content, green or greenish yellow rind and tender flakes): TJS 3, TJS4, TJS 6, TJS9, TJS7, TJS14, TJS15, TJS18 and TJS 23.

3. Types suitable for processing purpose (Medium large size fruits, high TSS content and soft flesh): TJS 11, TJS 13, TJS 14, TJS 18, TJS21, TJS22, TJS 23 and TJS 25.

The information generated during the study could be used as a baseline for fruit breeders in selecting genotypes with superior fruit qualities for jackfruit improvement programmes in the future.

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Ultrasound Assisted Extraction: A Quick and Efficient Method for Extraction of Gymnemagenin from *Gymnema sylvestre* (Retz.) R.Br. ex Sm.

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Abstract

Ultrasound assisted extraction is one of the promising green techniques for extraction of bioactive molecules from medicinal plants, however limited reports are available regarding most of the Indian medicinal plants. Present experiment was carried out to explore the possibility of using ultrasound-assisted extraction as a tool to extract gymnemagenin from an important anti-diabetic medicinal plant, *Gymnema sylvestre*. Mature leaves were shade dried, powdered and extracted using ultrasound processor at 30% amplitude. Extraction was carried out using three levels of hydro-alcoholic solvent (25, 50 and 75%) for five durations (20, 30, 40, 50 and 60 min). Treatments were compared with 24 h cold percolation as control.The extracts were hydrolyzed prior to RP- HPLC analysis in order to convert the gymnemic acids into gymnemagenin. The HPLC analysis of the samples revealed significant variations with respect to gymnemagenin recovery amongst the treatments and ultrasound assisted extraction was found to be faster and more efficient technique for extraction.

Key words: *HPLC, madhunashini, medicinal plant, solvent*

Introduction

Gymnema sylvestre (Retz.) R.Br. ex Sm. is an important medicinal plant of the Apocynaceae family. The species is popularly known as *madhunashini* or *gurmar* owing to its anti-diabetic properties (Dhanani et al., 2015). A number of bioactive macromolecules have been isolated and reported from the leaves of this species (Dateo and Long, 1973; Khramov et al., 2008; Yoshikawa et al., 1991). Gymnemagenin is a common aglycone of gymnemic acids produced after hydrolysis, which is known to inhibit the glucose absorption in the body. This makes the species valuable in the treatment of diabetes in the ayurvedic medicines and gymnemagenin is regarded as a marker compound (Puratchimani and Jha, 2004).

There are a variety of methods used for bioactive molecules extraction from medicinal plants in order to maximize recovery. Solvent extraction is one of the most popular methods in which the solvent enters the solid matrix (generally powder), dissolves the extractable material (solute) and diffuses out the solutes out of the matrix (Zhang et al., 2018). The extracted solutes are then collected and quantified using appropriate methods. In order to recover bioactive compounds from medicinal plants, different solvent extraction methods have been used, including soxhlet extraction, cold percolation, microwave assisted extraction, ultrasound assisted extraction *etc*. Effectiveness of the extraction process is known to vary greatly with a number of factors including the solvent type and amount, method, time, particle size, genotype used, growing conditions, season of cultivation/ collection *etc*. (Li et al., 2008; Zhang et al., 2018).

Ultrasound assisted extraction (UAE) is one of the promising methods, which has been found to be successful in a number of plant species (Barba et al., 2016). The present study was undertaken with the objective to investigate the effect of solvent concentration and extraction time on gymnemagenin recovery from the leaves of *G. sylvestre*.

Materials and methods

Leaf samples were collected from the experimental block of *Gymnema sylvestre* maintained at the ICAR-Directorate of Medicinal and Aromatic Plants, Boriavi, Anand, Gujarat. Mature leaves were shade dried and powdered in a blender. Accurately weighed (5 g) powder samples were extracted with 100 ml of methanol at different solvent compositions and times (Table 1). Briefly, the extraction was performed using three levels of hydro-alcoholic solvent (25, 50, and 75%) for five different time durations (20, 30, 40, 50, and 60 min.) using Sonic Vibra Cell ultrasound processor (VCX 500 Watt) at 30% amplitude. The cold percolation method was also used in the study, using the same amount of powder with three different solvent compositions for a period of 24 h. After extraction, it was filtered under vacuum and concentrated to dryness using vacuum rotary evaporator at 60 °C. Extracts were stored in glass bottles at 4 ºC until further analysis.

Table 1. Effect of time of extraction and methanol concentration on extract yield and gymnemagenin content in *Gymnema sylvestre*

Treatment	Time (min)	Methanol (%)	Extract yield (%)	Gymnemagenin content in extract (%)	Gymnemagenin content in dry powder (%)
T_1	20	75	24.64 ± 1.12 cd	1.460 ± 0.011 cde	0.365 ± 0.003 ef
T_2	20	50	35.59 ± 2.09 a	1.650 ± 0.029 abc	0.588 ± 0.010 a
T_3	20	25	31.67 ± 2.30 ab	0.995 ± 0.016 g	0.314 ± 0.005 fg
T_4	30	75	21.25 ± 2.27 d	1.765 ± 0.033 a	0.375 ± 0.007 de
T_5	30	50	27.48 ± 2.85 bc	1.540 ± 0.010 bcd	0.423 ± 0.003 cd
T_6	30	25	34.80 ± 1.26 a	1.385 ± 0.005 de	0.482 ± 0.002 b
T_7	40	75	24.29 ± 0.67 cd	0.710 ± 0.000 hij	0.172 ± 0.000 j
T_8	40	50	34.73 ± 0.98 a	0.310 ± 0.007 k	0.105 ± 0.002 k
T_9	40	25	34.12 ± 1.53 a	0.795 ± 0.001 h	0.275 ± 0.000 gh
T_{10}	50	75	27.65 ± 1.53 bc	1.635 ± 0.252 abc	0.455 ± 0.070 bc
T_{11}	50	50	32.07 ± 0.60 ab	1.410 ± 0.001 de	0.453 ± 0.000 bc
T_{12}	50	25	33.83 ± 2.36 a	1.315 ± 0.022 ef	0.445 ± 0.008 bc
T_{13}	60	75	22.18 ± 2.65 d	1.700 ± 0.008 ab	0.375 ± 0.002 de
T_{14}	60	50	35.43 ± 2.15 a	0.590 ± 0.011 ij	0.215 ± 0.004 ij
T_{15}	60	25	30.81 ± 1.76 ab	0.530 ± 0.088 j	0.164 ± 0.027 j
T_{16}	24 h	75	31.71 ± 0.16 ab	0.765 ± 0.036 hi	0.245 ± 0.011 hi
T_{17}	24 h	50	34.60 ± 0.62 a	1.175 ± 0.013 fg	0.405 ± 0.005 cde
T_{18}	24 h	25	34.97 ± 0.45 a	1.165 ± 0.007 fg	0.405 ± 0.002 cde

Values presented are the mean ± standard error of mean. Mean values followed by same lower case letter in a column did not differ significantly at 5% level of significance using least significant difference.

The extracts were hydrolyzed prior to HPLC analysis in order to convert the gymnemic acids into gymnemagenin following the method described earlier (Manika et al., 2013). Briefly, in a flask, 50 mg of extract was suspended in 5 ml of methanol, and 1 ml of KOH was added. The flask was boiled on a water bath for 60 min. After cooling the flask, 0.9 ml of concentrated HCl was added. The mixture was once again refluxed on a water bath for 60 min. and then cooled. The pH of the sample was adjusted between 7.5-8.5 using saturated KOH solution. A final volume was then made up to 20 ml using 50% methanol in water. Samples were centrifuged and the supernatants were passed through syringe filter (0.22 µm). Samples were stored at 4 °C until HPLC analysis.

The samples were analyzed using previously developed HPLC protocol (Dhanani et al., 2015). Yield of gymnemagenin were calculated using the equation obtained from the standard curve developed using standard gymnemagenin at different concentrations. Data was subjected to analysis of variance and mean separation was carried out using Web Agri Stat Package (WASP, v. 2.0., ICAR-CCARI, Ela, Goa, India).

Results and discussion

The extraction of bioactive compounds from plant materials is the crucial first step in the analysis of medicinal plants since the plant matrix is complex in nature. Additionally, the extraction conditions determine the recovery of bioactive compounds in the extracts, which govern the efficacy of the extraction. The present study examined the use of ultrasound assisted solvent extraction to reduce the time needed for cold percolation extraction, *i.e.* 24 h. For this, three solvents compositions i.e. methanol (25, 50 and 75%) in water were used for extraction with five extraction durations (20, 30, 40, 50 and 60 min.). Analysis of the data revealed significant differences among the solvent compositions as well as extraction time (Table 1).

Cold percolation is the commonly employed method for extraction in this species (Dhanani et al., 2015). In the present study, in cold percolation treatments, yields of extract remained statistically similar (31.71% to 34.97%), irrespective of the solvent used for the extraction (Table 1). It means, with the prolonged extraction duration of 24 h, the solvent concentration had minimal role in conventional extraction process. The yields obtained from nine out of 15 UAE were comparable to those obtained from cold percolation treatments. As a result, UAE was efficient with short duration treatments. A 75% methanol solvent concentration in the UAE treatment resulted in significantly lower extract yields than 50 or 25% solvent concentrations in the same extraction period, which indicates that solvent concentrations played a significant role in the UAE extraction process. These results are in accordance with earlier researcher (Chemat et al., 2017), who observed solvent concentration dependent variations in extraction efficiency.

The extracts obtained using different treatments were subjected to HPLC analysis to determine the gymnemagenin contents. Results suggested significant variations among the treatments studied. Gymnemagenin content in the extract varied between 0.310% (T_8 involving 50% methanol for 40 min) and 1.765% (T_4 involving 75% methanol for 30 min). The treatment T_4 remained statistically similar with T_2, T_{10} and T_{13}. It means that, though the extract yield was low in treatments involving 75% methanol, the extraction recoveries were not necessarily affected.

Recovery of bioactive compounds was measured in terms of yield from the leaf powder to take into account the extract yield as well as gymnemagenin content. Results suggested that the highest yield of gymnemagenin (0.588%) was obtained in powdered samples extracted using UAE with 50% methanol and 20 min. duration (T_2), while the recovery was the lowest (0.105%), when UAE was carried out for 40 min. using 50% methanol. Gymnemagenin content in conventional cold percolation treatments remained statistically inferior when compared with T_2. Thus, use of UAE with 50% methanol for 20 min. was found to be optimum for extraction of gymnemagenin from leaf powder of madhunashini. The results are in accordance with earlier report by Jovanović et al. (2017) in *Thymus serpyllum*, who obtained higher polyphenols recovery using UAE with 50% hydroalcoholic solvent.

The process of UAE employs energy generated through ultrasonic waves for extraction of the active

ingredients. Cavitation effect hastens the extraction and diffusion of solutes from solid matrix thereby improving the extraction process (Barba et al., 2016). Further, requirement of low amounts of solvents and quicker extraction have been regarded as the prime characteristics of this method (Chemat et al., 2017). Thus the method has advantage over traditional cold percolation method as also witnessed in the present investigation.

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Studies on Indian Prickly Ash (*Zanthoxylum rhetsa* (Roxb.) DC) Collections from Goa, India

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Abstract

Seven collections of *Zanthoxylum rhetsa* (Roxb.) DC from different locations of Goa were studied for tree and fruit morphological traits, essential oil content and oleoresin content in fruit skin. Variations were noticed in parameters *viz*. tree height (15 to 24 m), number of main branches (3-5), number of prickles/10 cm^2 on main stem (5.25 to 7.25), number of prickles at terminal branch (22.2 to 25.0) and number of leaflets per terminal branch (228.6 to 273.4). Essential oil content in dried fruit skin ranged between 6.0% (Tisal-07) and 8.4% (Tisal-06). Oleoresin content was determined using acetone as a solvent, which revealed as high as 12.22% oleoresins in Tisal-04, while 8.86% was obtained in Tisal-01.

Key words: *Essential oil, morphological parameters, oleoresin, pericarp*

Introduction

Indian Prickly Ash or Tisal belonging to the family Rutaceae is found growing in wild form in the forests of Goa, the Western Ghats and the North East Himalaya. The tree has vast traditional significance and has potential for commercial utilization. The tree wood is termite resistant (Boer et al., 1998) and could be used for preparation of furniture and handicrafts. Forest dwellers use the dried branches as firewood. Branches have thick sharp prickles and hence, are used for fencing to keep away the wild cattle. The shoots are consumed as vegetables by *Adi* tribe of East Siang District of Arunachal Pradesh (Payum et al., 2013). Fresh and dried fruits have been traditionally used in culinary items in many parts of India. In Goa, dried fruit pericarp is used in culinary items *viz*. fish curry, *solkadhi*, biryani, pumpkin dish *etc*. (Karanjalker et al., 2021). Fruit powder is reported to be effective against rancidity in peanut (Antony et al., 2019). Locals of Goa use the raw fruits for preservation of raw mango (Karanjalker and Karanjalker, 2021).

Tisal fruits have multiple pharmaceutical values and have been traditionally used against manifold human ailments. For example, leaves are being used for de-worming by Naga tribes of North East India (Yadav and Tangpu, 2009) and prickles for breast pain by *Kanikkar* tribes of Tamil Nadu (Medhi et al., 2013). It is used against toothache, dizziness and bloating in Thailand (Duangyod et al., 2020). It also helps in increasing lactation in nursing mothers (Lalitharani et al., 2010). It is reported to be have anti-cancerous (Theeramunkong and Utsintong, 2018), antibacterial (Pooja et al., 2012), anti-diabetic, anti-spasmodic, anti-inflammatory, anti-nociceptive and anti-diarrheal properties (Pai et al., 2009; Duangyod et al., 2020).

Botanical description of the species has been given by the earlier researchers. Plant attained height of 25–30 m (Shankaracharya, 1994; Brophy et al., 2000), hard- conical shaped spines (Lalitharani et al., 2013), compound-imparipinnate leaves, terminal cymose (20 cm length) flowers and simple follicle fruits. However, information on characterisation of individual trees of tisal for variability in morphological traits and essential oil content is scarce and hence, the present study was undertaken.

Materials and Methods

The plants were identified from different locations of Goa as per the information provided by the locals where the marketing and consumption of fruits are observed. The collections were done from Hankane (Tisal-01),

Sangolda (Tisal-02), Sakorda (Tisal-03), Madkaim (Tisal-04), Velguem (Tisal-05), Dongrim (Tisal-06) and Cudnem (Tisal-07). The plants were of different unknown age and no cultivation practices were followed. The morphological parameters *viz*. plant height, number of main branches, number of leaves per terminal end, number of fruits per cluster, number of prickles at 1.3 m height (breast height), number of prickles at terminal branch, fresh fruit diameter (mm), seed length (mm), seed width (mm), seed thickness (mm) and 100 seed weight (g) were recorded. All values were subjected to statistical analysis using Web Agri Statistical Package (WASP v. 2.0) software (ICAR-CCARI, Ela, Goa, India).

Fresh matured fruits were harvested and sun dried for five days. Pericarp was separated from seed and packed in air tight bags till further analysis. Oleoresin and essential oil content in the pericarp were determined using Soxhlet apparatus and Clevenger's apparatus, respectively. The content was expressed as percentage.

Results and discussion

Tree morphological characters

The plant height amongst the collections varied from 15 m in Tisal-03 to 24 m in Tisal-06 (Table 1), which closely resembled with the height range (25–30 m) reported by Shankaracharya (1994) and Brophy et al. (2000). In all the collections, trunk was observed to be upright, corky and straight. The number of main branches varied from 3-5. Trunk and branches of *Z. rhetsa* are characterized with prickles all over the surface (Lalitharani et al., 2013). The conical prickles on branches were sparse and thick on the main trunk, while those were thin and pointed at the terminal end of the stem. Number of prickles per 10 cm^2 area at 1.3 m varied from 5.3 ± 0.70 (Tisal-03) to 7.3 ± 0.70 (Tisal-05), while at terminal end, it varied from 22.2 ± 0.70 (Tisal-03) to 25.0 ± 3.53 (Tisal-05). Leaves were compound- imparipinnate and were mostly present at the terminal ends of the branches. Number of leaves per terminal branch significantly varied from 14.0 (Tisal-01) to 27.5 (Tisal-05).

Table 1. Tree morphological parameters of Tisal collections from Goa, India

Collection code	Tree height (m)	No. of main branches	No. of prickles at breast height per 10cm^2 area	No. of prickles at Terminal branch	No. of leaves per terminal end	No. of fruits per cluster
Tisal-01	17	4	6.0 ± 0.70	23.0 ± 1.41	14.0c	237.2 ± 45.97
Tisal-02	19	3	5.8 ± 0.70	23.5 ± 2.82	25.0a	273.4 ± 25.97
Tisal-03	15	5	5.3 ± 0.70	22.2 ± 0.70	24.3a	228.6 ± 47.97
Tisal-04	19	4	6.0 ± 2.12	23.8 ± 0.70	14.5bc	249.6 ± 22.02
Tisal-05	22	4	7.3 ± 0.70	25.0 ± 3.53	27.5ab	256.5 ± 17.51
Tisal-06	24	3	6.5 ± 1.41	23.3 ± 3.50	12.1abc	261.1 ± 40.24
Tisal-07	23	5	7.0 ± 1.70	24.5 ± 0.70	19.7c	241.6 ± 34.08

*Values followed by similar alphabet in a column do not differ significantly at 5% level ofsignificance using least significant difference

Very few studies on systematic characterisation of tree diversity in *Z. rhetsa* are available in the literatures. Plant is deciduous or evergreen in nature (Yadav and Tangpu, 2009, Hartley, 2013). Flowers are borne terminally in cymose (20 cm long) and both male and female flowers are evident on the panicle. Male flowers have 3 mm long stamens and are of disc or conical shape, lobed or grooved along with rudimentary gynoecium, whereas female flowers are of disc or columnar shaped, 1.5 mm long with single/double carpelled gynoecium, excentric style and flattened stigma (Hartley, 2013).

Fruit and Seed morphological parameters

Fruits of tisal are borne in cluster and number of fruits per cluster varied between 228.6 (Tisal-03) and 273.4 (Tisal-02). However, the difference among the collections were not significant. Diameter of fresh fruits varied from 5.48 mm (Tisal 01) to 6.55 mm (Tisal 03) in present study (Table 2). Seeds were globose, hard with smooth and black surface. Morphological characterization of seven collections from various parts of Goa suggested distinct variations for seed morphology (Table 2). Significant differences were noticed for seed length, which varied between 5.535 mm (Tisal-02) and 6.525 mm (Tisal-06), while seed width varied from 4.835 mm (Tisal-05) to 6.376 mm (Tisal-01). Thickest seeds of 5.385 mm were noticed in collection Tisal-01, while it was the lowest in Tisal-07. Weight of 100 seeds varied between 8.403 g (Tisal-03) and 14.773 g (Tisal-07) among the collections studied.

Table 2. Fruit and seed morphological parameters of Tisal collections from Goa, India

Collection code	Diameter fresh (mm)	Seed length (mm)	Seed width (mm)	Seed thickness (mm)	100 seed weight (g)
Tisal-01	5.48 d	6.111 b	6.376 a	5.385 a	11.883 c
Tisal-02	5.81 cd	5.535 c	5.825 c	5.207 ab	9.630 e
Tisal-03	6.55 a	5.656 c	5.725 c	5.119 bc	8.403 f
Tisal-04	6.36 ab	6.250 b	5.678 c	5.154 b	13.410 b
Tisal-05	6.23 ab	6.145 b	4.835 d	4.955 cd	10.340 d
Tisal-06	6.47 a	6.525 a	6.066 b	5.130 bc	13.896 b
Tisal-07	6.06 bc	6.495 a	6.085 b	4.845 d	14.773 a

*Values followed by similar alphabet in a column do not differ significantly at 5% level ofsignificance using least significant difference

Essential oil and oleoresin content

Fruits are highly aromatic that smell like lemon skin (Duangyod et al., 2020) and hence, essential oil and oleoresin contents were determined in different collections. Essential oil content in dried fruit skin ranged between 6.0% (Tisal-07) and 8.4% (Tisal-06). Oleoresin content was determined using acetone as solvent, which revealed as high as 12.22% oleoresins in Tisal-04, while 8.86% was obtained in Tisal-01. The recovered oleoresin varied in terms of colour as well.

As synthesis and accumulation of essential oils in a species is governed by several internal and external factors, variations in this trait could be expected. Theeramunkong and Utsintong (2018) observed volatile oil yield of 8.1 to 13.6% (v/w) from fresh fruit skin, while it was 13.17 to 15.33% (v/w) in skin from dried fruits collected from Thailand (Northern Nan, Southern Nan, Phayao and Chiang Rai). Volatile oil recovery of 2.3% (v/w) has been reported by Duangyod et al. (2020) in dry fruits collected from Thailand, while mere 1.94% essential oil was recovered in a study by Rana and Blazquez (2010), who obtained the dry seed coats from Imphal, Northeast India. Visual colour of oleoresin was 'Golden yellow to light green' in four collections (Tisal-01, Tisal-02, Tisal-03, Tisal-05), while it was 'dark green to blackish' in remaining three collections (Tisal-04, Tisal-06 and Tisal-07). Such variations indicate presence of diversity, which needs to be systematically exploited. Identification of genotypes with higher content of essential oil and oleoresins is a pre-requisite for promotion of small scale extraction units.

Vegetative and reproductive parts of plants of tisal possess pleasant flavour. As the aroma is akin to lemon, fruit skin oil could be used as a substitute for the citrus oil. Essential oils extracted from various parts of plants and fruits could be used in manifold commercial products like perfumes, preservatives, cosmetic natural sunscreen, anti-ageing cosmetic, antiseptics *etc*. (Shantanu et al., 2011; Reddy and Beena, 2011; Antony et al., 2019; Santhanam et al., 2013).

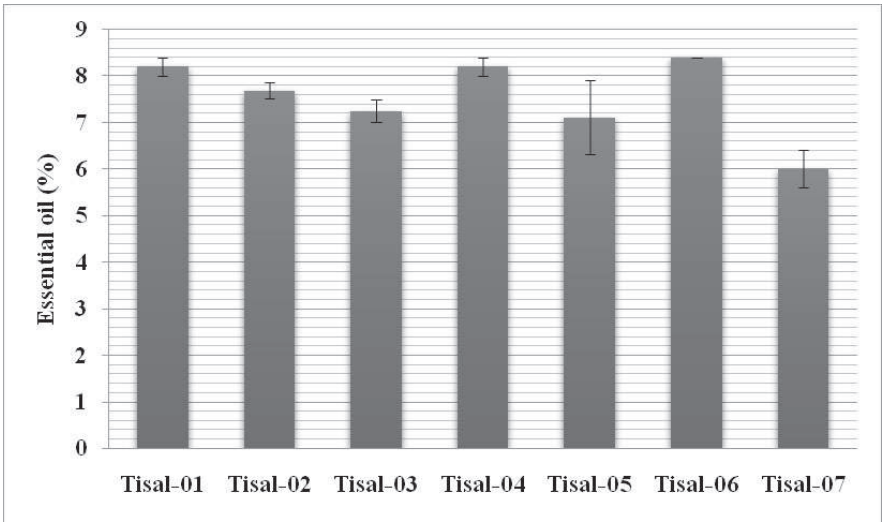

Fig. 1. Variability in essential oil content among tisal collections from Goa, India

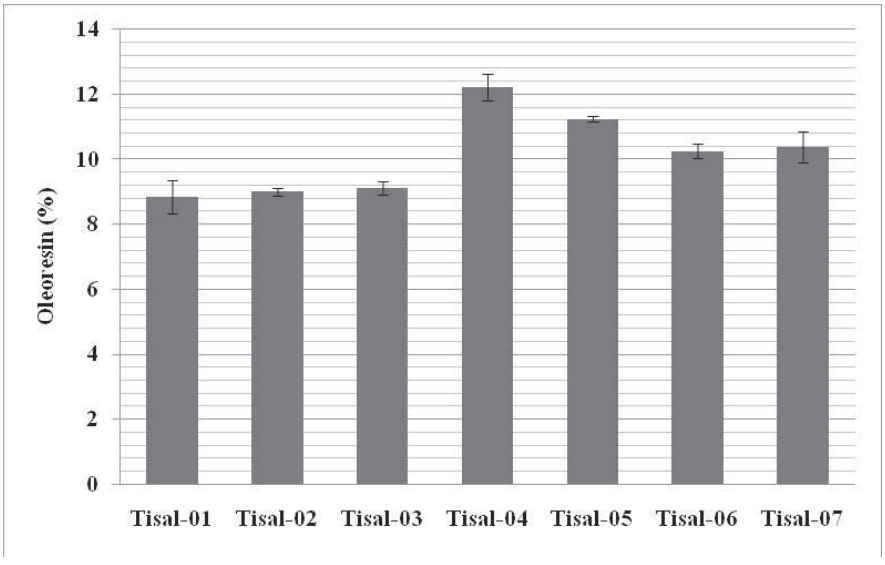

Fig. 2. Variability in oleoresin content among tisal collections from Goa, India

Conclusion

In the present study, seven collections of an underutilized but traditionally valued spice 'tisal' from different locations of Goa were studied. Variations were observed for tree, fruits and seed morphological parameters. Further, collections showed variability for volatile oil content and oleoresin content, which indicated scope for identification of superior germplasm for further utilization in aroma based industries.

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Standardization of Vegetative Propagation Methods for Tamarind (*Tamarindus indica* L.) under Kerala Conditions

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Abstract

Tamarind is an underutilized crop known for its climate resilience. The state of Kerala is known for its rich diversity of local and wild tamarind trees. However, there is no standardized vegetative propagation method that is practiced locally in the state. Hence, to produce vegetative propagules of tamarind plants for conservation of local elite trees and to meet demands of homestead gardeners and farmers, five different vegetative propagation methods were evaluated in four different seasons (March, June, September and December) of a year. Amongst the different treatment combinations studied, veneer grafting showed highest success (70%) during March, which was on par with softwood grafting (65%) and approach grafting (60%) methods. Mean of all the grafts prepared in March showed highest shoot length (6.91 cm). Correlation study showed a significant association of mean maximum temperature with graft success rate (r=0.97). Hence, veneer grafting or softwood grafting methods could be recommended for tamarind propagation during March in order to get maximum saleable plants. This would be helpful in conservation of elite local genotypes as well.

Key words: *Humid climate; seasons; softwood grafting; veneer grafting*

Introduction

Tamarind is a multipurpose species, which is known for its drought tolerance and has become popular in most of the tropical and subtropical countries due to its variety of industrial and non-industrial uses (El-Siddig et al., 2006). In India, it is mainly distributed in warm and drier tropics and subtropics with an area of 48,000 ha. In Kerala, tamarind is being distributed on an area of 10,610 ha with an average annual production of 36,470 Mt (Indiastat, 2022). The major districts of Kerala in terms of tamarind production are Palakkad, Thiruvananthapuram, Malappuram, Kozhikode and Thrissur. Landraces such as Valanpuli, Thenpuli and Madhurapuli are popular amongst the people of Kerala. In most households of the country, tamarind is being widely used as a souring agent in indigenous cuisines. Even though Kerala is one of the largest tamarind producing states of India, majority of tamarind grafts for planting are currently being procured directly from nurseries of adjacent Tamil Nadu state or through local nurserymen who procure grafts from Tamil Nadu based on prior orders.

Since long, tamarind has been one of the major components of homestead gardens. But, due to the fragmentations of land and population pressure, homestead gardens are facing severe threat and at the verge of losing many tree components including tamarind. The area under tamarind in Kerala has seen a drastic reduction of 25% and 42% in 2017-18 compared to 2008-09 and 1997-98, respectively (Indiastat, 2022). Being perennial, tamarind seedling trees take about 8-12 years for bearing fruits whereas grafted/ budded plants are reported to yield in 3-4 years after planting (Farooqi et al*.,* 2005).

The climate pattern of Kerala has drastically changed over the past few decades and frequency of droughts has increased considerably (Abhilash et al., 2019). So, integration of climate-resilient crops like tamarind could ensure sustainability and farm income to the farmers. Hence, to reintroduce tamarind in existing cropping systems like homestead gardens and to provide quality planting material in bulk for commercial cultivation and germplasm conservation, efficient vegetative propagation method and season needs to be standardised. There is hardly any scientific literature available on tamarind

propagation in Kerala and hot humid climatic regions, and hence the present study was undertaken to identify the best propagation method in hot and humid climatic region of Kerala.

Materials and Methods

The present study was conducted at the Department of Plantation crops and spices, College of Horticulture, Kerala Agricultural University (KAU), Thrissur during the year 2017 to 2019. The study area is located at 10°32'58.3"N 76°17'02.4"E with an elevation of 30 m. The area receives an annual rainfall of about 2,500 mm from the rains of both South West and North-East monsoons. The experiment was designed using Factorial CRD with five propagation treatments (veneer grafting, wedge grafting, approach grafting, patch budding, air layering) and four seasons **[**March (summer), June (monsoon), September (post-monsoon), December (winter)]. Each treatment was conducted with four replications with 40 plants per treatment per season.

For grafting and budding, one-year-old local tamarind seedlings of uniform growth and pencil thickness were used as rootstocks. Scion sticks of 10-12 cm with 6-8 buds were used for softwood grafting and veneer grafting; whereas, single bud taken from scion stick was used for patch budding. Scion shoots were collected from a selected elite tree present in the main campus of KAU. Scion shoots were defoliated just before grafting in all seasons except during summer when naturally defoliated shoots were available on the trees. Air layering was done by girdling the pencil thick shoots at 20 cm from the tip. Girdled ends were treated with indole-3 butyric acid (IBA) 1,000 ppm, wrapped with wet moss and covered with polythene cover. Approach grafting was performed on the same elite tree. Observations were recorded for the time taken by the grafts to sprout, success percentage at 30 days after grafting (DAG), shoot length at 60 DAG, number of branches per graft at 90 DAG and final success percentage at 90 DAG. Statistical analysis and correlation studies were carried out using IBM- SPSS STATISTICS 22 and WASP 2.0 software.

Results and Discussion

Time taken for sprouting is an important parameter with respect to success of grafting and its establishment. Significant difference was observed in the time taken for sprouting among grafting and budding methods (Table 1). Time taken for sprouting was lesser for softwood grafting (4.5 days) followed by veneer grafting (9.3 days). Patch budded plants failed to sprout in all the seasons and hence, they were excluded from further statistical analysis. Time taken for sprouting among four seasons was comparatively more during September. Softwood grafts took least days (4.5 days) to sprout during March, whereas veneer grafts took least days to sprout during June. The temperature of 32 °C has been reported to favour callusing in apple grafts resulting in successful graft union; however, temperature above 32°C causes injury and beyond 40°C tissue death could occur (Shippy, 1930). Higher temperature prevailing during the March might be the reason for early sprouting and faster establishment of vascular connection across the graft union due to quick cell multiplication and growth (Agasimani et al., 2019).

Table 1. Performance of different propagation methods in tamarind

	*Time taken for sprouting (days)	*Success (%) at 30 days	*Shoot length at 60 days	Success (%) at 90 days	*No. of branches at 90 days
SEASON					
S_1-March	6.15	73.75	6.91	51.25	4.72
S_2-June	6.63	0.00	0.00	1.88	0.00
S_3-Sept	8.19	0.00	0.00	10.00	0.00
S_4-Dec	6.65	7.50	4.46	16.88	1.06
METHOD					
P_1-Air layering	-	-	-	16.88	-
P_2-Approach grafting	-	-	-	25.63	-
P_3-Softwood grafting	4.50	17.50	1.82	16.25	1.248
P_4-Veneer grafting	9.30	23.13	3.87	21.25	1.643
INTERACTION					
S_1P_1	-		-	10.00	-
S_1P_2	-		-	60.00	-
S_1P_3	3.33	70.00	7.26	65.00	4.99
S_1P_4	8.98	77.50	6.55	70.00	4.45
S_2P_1	-		-	0.00	-
S_2P_2	-		-	7.50	-
S_2P_3	5.38	0.00	0.00	0.00	0.00
S_2P_4	7.88	0.00	0.00	0.00	0.00
S_3P_1	-		-	30.00	-
S_3P_2	-		-	10.00	-
S_3P_3	4.88	0.00	0.00	0.00	0.00
S_3P_4	11.50	0.00	0.00	0.00	0.00
S_4P_1	-		-	27.50	-
S_4P_2	-		-	25.00	-
S_4P_3	4.43	0.00	0.00	0.00	0.00
S_4P_4	8.86	15.00	8.93	15.00	2.13
CD					
Season (S)	**NS**	**7.26**	**2.50**	**5.50**	**0.69**
Propagation method (P)	**1.55**	**5.14**	**1.77**	**5.50**	**NS**
Factor (S × P)	**NS**	**NS**	**3.54**	**10.99**	**0.97**

*Observations and statistical analysis were carried only for detached method of grafting (softwood grafting and veneer grafting).

NS – non significant, S – season, P – propagation method

The tamarind softwood grafts took 24 to 27 days to sprout during March, April and May months and complete mortality was observed in grafts prepared during December, January and February. Time taken for bud sprouting is known to get affected as the temperature starts rising and humidity starts dropping

(Singh and Srivastava, 1962). Two independent studies from Chhattisgarh and Tamil Nadu reported that bud breaking in tamarind softwood grafted plants took 9 to 17 DAG (Anil et. al., 2022; Mayavel et. al., 2022). In the present study, it was observed that time taken to sprout was lesser with increasing temperature, which might be due to higher relative humidity at this region. Softwood grafting in tamarind between the period of leaf fall and new growth was found to be best in Rahuri condition, which was quite opposite to normal propagation season of major tropical crops like mango and sapota (Lalaji, 2001). Hence, the present study is in agreement with the earlier reports (Singh and Srivastava, 1962; Lalaji, 2001; Mayavel et. al., 2022).

Patch budding completely failed during the experiment as there was no sprouting even after 3 months of budding, probably due to nonunion of cambial cells of scions and rootstock. Prominent observation in this study was that most of the veneer and softwood grafts sprouted within 3-5 days as compared to other regions where it generally took 20 to 35 days to sprout (Singh and Singh, 2007). In the present study, early sprouting of scion might have been accelerated due to combined effect of high temperature and high humidity inside the mist house which led to the sprouting of scion at the expense of reserved food in its tissues without the proper cambial union. This led to exhaustion of scion making it difficult for further growth after the union and subsequent drying up of sprouts before reaching even a minimum length of 2 cm. Unlike scion stick used in grafting, bud patch lacks good food reserves, which might have resulted in failure of patch budding.

Success percentage after 30 days grafting is an indicator for early estimation of grafting efficiency. There was a significant difference among the seasons and propagation methods for establishment success percentage (Table 1). Irrespective of the propagation methods followed, the highest success rate (73.75%) was recorded in March. Sprouting was observed initially in the grafts prepared during June and September but complete mortality was noticed within 30 days of grafting. Veneer grafting was recorded with the highest success rate (23.13%).

Length of the shoot at 60 days after grafting is another parameter for assessing the vigour of the grafted plant and ensuring proper contact between the scion and the stock. The differences in shoot length amongst different propagation methods were highly significant (Table 1). Maximum shoot length was recorded by veneer grafting (3.87 cm) whereas softwood grafting recorded lowest (1.82 cm). Grafts prepared in March season recorded highest shoot length (6.91 cm) which was on par with shoot length of December grafts (4.46 cm). There was no success in veneer grafting (produced during June and September) and softwood grafting (produced during June, September and December).

Shoot length is significantly and negatively correlated with number of branches present in the graft (Table 2). The influence of interaction effect of season and propagation method on shoot length was highly significant. Maximum shoot length was recorded for veneer grafting carried out during December (8.93 cm). This might be due to lesser branches per graft as a result of less sprouting of buds during winter. This was on par with shoot lengths of softwood grafts produced during March (7.26 cm) and veneer grafts prepared during March (6.55 cm).

Table 2. Correlation between length of the shoot and number of branches

	Length of shoot	Number of branches
Length of shoot	1	
Number of branches	-0.61*	1

* significant at 0.05 level.

Number of branches per graft at 90 days after grafting expresses the proper union of graft, health of scion and plant vigour. The differences in number of branches were not significantly influenced by method of grafting (Table 1). Observations were recorded only for detached method of grafting. Number of branches remained statistically

similar between veneer grafting (1.64) and softwood grafting (1.25). Season of grafting had significant influence on number of branches. Overall, March season grafts recorded highest number of branches (4.72) which might be due to higher temperature and more active bud sprout in March. Contrastingly, least number of branches was observed in December grafts (1.06) which might be due to unavailability of good quality scion wood as the tree normally remains in fruit development stage. Less bud sprouting during this season might be due to lower temperature.

Influence of interaction of season of grafting and propagation method was highly significant with respect to number of branches. Softwood grafts produced during March recorded maximum number of branches (4.99) which was statistically on par with those produced by veneer grafts prepared during the same season (4.45). Lowest number of branches was recorded by veneer grafts prepared during December (2.13). Lower temperature and scion in the active growth stage are responsible for reduction in number of branches in December whereas in March, all the buds on scions are dormant and high temperature coupled with high humidity enhanced sprouting of more buds to form branches.

Successful establishment after three months of grafting/layering represents the saleable plants those are suitable for transplanting after another three months. The differences in rate of success among the seasons were highly significant. Overall highest success was obtained in propagules produced during March month (51.25%), irrespective of propagation methods.

Among the different treatment combinations, veneer grafting recorded highest success (70.00%) during March, which was on par with softwood grafting (65.00%) and approach grafting (60.00%) prepared during the same period. The union was not successful in softwood grafts prepared during June, September and December even after normal sprouting of scion. Similarly, in case of veneer grafts produced during June and September, the success was nil. Air layers produced during June recorded zero establishment percentage even though there was successful rooting observed. In Air layering, layers produced during September recorded maximum survival (30.00%) followed by December (27.50%) and March (10.00%). Season dependent success in air layering has been previously reported in tropical species such as *Spondias pinnata* and *Cinnamomum verum* (Tomar, 2016; Waman and Bohra, 2018). Among the approach grafts, grafts prepared during March recorded highest survival (60.00%) followed by grafting during December (25.00%) and September (10.00%).

In a study conducted at Andhra Pradesh on grafting of tamarind, 68% and 49% success were observed in softwood grafts and veneer grafts, respectively during April (Purushotham and Narasimharao, 1990). In a similar study from Chhattisgarh, tamarind softwood grafts recorded highest success of 63% to 80% during March month (Anil et al*.,* 2022). Higher success percentage during March, April and May months might be due to the favourable environment with optimum temperature and relative humidity. This might have enhanced the union of the scion and stock cambial layers coupled with precocious callus formation and development of good vascular network between stock and scion (Hartmann et al*.,* 2010; Agasmani et al*.,* 2019; Praveenakumar et al*.,* 2019; Umadevi et al*.,* 2021) as also observed in the present study.

The studies in jackfruit conducted under Tarai conditions of Uttarakhand reported wedge grafting as the best propagation method to be performed from December to April (best in March) (Rai et al*.,* 2021). Among temperate trees (e.g. apple, pear), when dormant scions were used for grafting on active rootstocks, the graft success percentage was found to be higher (Leakey, 2014). Similar result was obtained in this study during March, when naturally defoliated scions were used for grafting and significantly higher success percentage was obtained.

The present study revealed that patch budding was not a reliable technique for tamarind propagation in this region as also noticed in Godhra region of Gujarat (Singh and Singh, 2007). Probable causes for failure of union could be non-optimal temperature during cell division and formation of union, loss of cell turgidity due to desiccation of scion, physical movement in the junction

of scion and rootstock and microbial infection (Hartmann et al., 2010). In the present study, grafts prepared during June, September and December failed to survive even after 100.00% sprouting within 15 days in all the seasons.

Understanding of season and weather is essential for ensuring success of propagation. Graft callus formation and healing are highly influenced by environmental factors such as temperature, relative humidity and grafting season (Hartmann et al., 2010). Correlation of weather parameters with the final success percentage revealed interesting results. The maximum success in veneer grafting, softwood grafting and approach grafting were obtained, when the monthly mean maximum temperature ranged between 33 and 37 °C (Fig. 1). Success was nil in the months, when mean maximum temperature dropped below 32.9 °C. Correlation studies revealed that maximum temperature was the most influencing parameter (r=0.97*) for overall success of propagation, irrespective of propagation methods studied (Table 3).

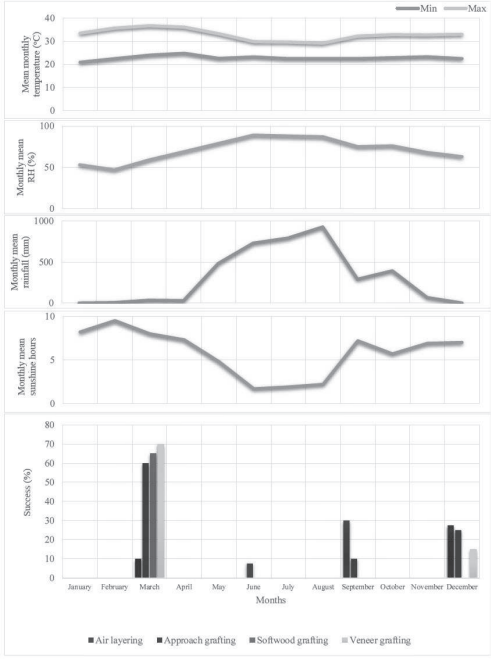

Fig. 1. Influence of weather parameters on graft success

Table 3. Correlation between weather parameters and final success percentage at 90 days after grafting.

	Min. Temperature (°C)	Max Temperature (°C)	RH (%)	Rainfall (mm)	Sunshine (hours)	Final graft success
Min. Temperature (°C)	1					
Max Temperature (°C)	0.70	1				
RH (%)	-0.19	-0.81	1			
Rainfall (mm)	-0.23	-0.84	1.00**	1		
Sunshine (hours)	0.26	0.86	-0.90	-0.93	1	
Final graft success	0.84	**0.97***	-0.68	-0.72	0.73	1

* significant at 0.05 level.

** significant at 0.01 level.

The softwood and veneer grafts showed success in the months having average relative humidity of 60-65% (Fig. 1) with zero precipitation, whereas approach grafting recorded maximum success of 25 to 60% during zero precipitation month and it was as low as 7.5 to 10% during months with 290 to 790 mm rainfall.

Higher rainfall of above 500 mm per month with heavy downpour in short period caused complete leaf fall of the air layers and approach grafted plants along with complete leaf fall of mother trees. Trees recovered after heavy rains by putting forth new flush, but the air layered and approach grafted shoots along with rootstocks failed to recover after heavy rains receded, which led to higher mortality of grafts and layers prepared during the present study. Higher success in the grafts appears to be slightly associated with longer sunshine hours, generally in the range of 7-10 h.

In a similar study under Dharwad region of Karnataka, the lowest success (12.03%) was observed in December whereas highest success (48.16%) during January followed by February (38.79%) in softwood grafting (Kumar et al., 2003). They reported that relative humidity of 60-65% coupled with maximum and minimum temperature of 34.0 °C and 16.7 °C, respectively after December month favoured higher grafting success in January and February. These factors might have accelerated cell activity and quick union of cambium across juxtaposed regions. The lower rate of graft success in December might be due to drop in temperature. In Rahuri region of Maharashtra, grafts prepared during December to March failed to sprout probably due to low minimum temperature (6.3 to 11.0 °C) and low relative humidity (around 30%) during the period (Lalaji, 2001), which reduced cell division rate and callus formation across the union. They also obtained very less success during September which was reported to be due to unavailability of good bud woods as the tree was in pod development stage.

Conclusion

From the present study, it is evident that ideal season for propagation of tamarind is March under Kerala conditions, which coincides with the period between leaf fall and new flushing. Significant differences were noticed in different seasons and the highest survival was obtained in March with veneer grafting followed by softwood grafting. Based on the results, air layering, approach grafting and patch budding methods were not recommended for tamarind propagation in the region. The maximum temperature and relative humidity were identified as key parameters that influence the success percentage of grafts. The results would help in taking up mass multiplication activities of elite genotypes of tamarind in the region.

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Effect of Vermicompost on Growth and Quality of Culantro (*Eryngium foetidum* L.) Grown Under Novel Pro-tray Cultivation System

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Abstract

Culantro (*Eryngium foetidum* L.) is an underutilized spice used for flavouring in the Andaman Islands and North Eastern states of India. The produce fetches premium prices in the urban parts of these regions. To promote cultivation of this high value herb in urban areas, novel methods of pro-tray cultivation (Dweep ProDhaniya) has been developed. This system is suitable for cultivation in urban spaces including shaded terraces. To study the effect of vermicompost supplementation on cultivation in this system, an experiment was conducted with five ratio of soil: vermicompost (1:0, 0:1, 1:1, 1:2 and 2:1, v/v). Each treatment was replicated six times and pro-trays were maintained in shade net house (50% shade). Results revealed that soil or vermicompost alone did not support growth of culantro, while combination of substrates showed positive results. Leaf parameters such as leaf length (10.23 to 13.51 cm), leaf width (1.67 to 2.01 cm) and number of leaves per plant (6.33 to 11.13) were significantly influenced by the treatments and highest mean plant weight was observed in soil: vermicompost (1:1) combination. Quality of the produce in terms of chlorophyll content, total carotenoids content and ascorbic acid content also showed significant variations. These results will be helpful in enhancing the efficiency of the system for production of culantro in urban areas.

Key words: *Apiaceae, herb, substrate, urban horticulture*

Introduction

Culantro (*Eryngium foetidum* L., Apiaceae) is a tropical aromatic culinary rosette herb that was introduced by the Chinese into Southeast Asia (Malaysia, Indonesia, Thailand, Vietnam, Singapore, Myanmar, Sri Lanka, India and Bangladesh) as a substitute to the cilantro (*Coriandrum sativum*) in the late 1800s to the beginning of 1900s (Singh et al., 2014; Ramcharan, 1999; Chowdhury et al., 2007). The herb possesses strong aroma, better shelf life and is hardy in nature (Waman and Singh, 2019). It has been commonly employed for garnishing, marinating, flavouring and seasoning purposes in cuisines and food products (Ramcharan, 1999; Singh et al., 2014). It has been an integral part of the traditional Manipuri cuisines such as *morak-metpa*, *Oottii*, *Chagem-pomba* and *Hawaijar* (Singh and Sundriyal, 2003).

The herb is known to be rich source of various bioactive ingredients as saponins, flavonoids and essential oils have been reported from the aerial parts of the plant, while underground parts contain terpenes, saponins, monoterpene glycosides, phenolic compounds, aldehydes, esters, acetylenes and oligosaccharides. Eryngial (E 2 dodecenal) has been identified as the major constituent of *Eryngium* essential oil and it is commonly used in the flavour and fragrance industry (Shavandi, 2012). The herb is also valued in the traditional medicines for treating fever, burns, hypertension, stomach ache and epilepsy (Natraj et al., 2020). Further, anthelmintic, anti-inflammatory, analgesic, anti-convulsing, anti-carcinogenic and anti-diabetic properties have been reported in it (Saenz et al., 1997; Simon and Singh, 1986; Honeychurch, 1980).

To promote cultivation of this herb in urban areas, novel pro-tray cultivation system has been developed recently (Waman and Bohra, 2022). The growth, yield as well as quality of the produce are known to vary with the substrate. Vermicompost, a product of non-thermophilic biodegradation of organic matter, is a material of choice for use as a soil conditioner due to its high porosity,

aeration, drainage, water holding capacity, humic acids and microbial activity (Arancon et al., 2006). It is easily available and is a nutrient-rich manure which could be used by the urban dwellers for growing the herbs. Hence, the present study was undertaken to investigate the influence of using vermicompost as a constituent of substrate for cultivation of culantro in pro-tray system.

Materials and methods

The study was conducted at the Division of Horticulture and Forestry, ICAR-Central Island Agricultural Research Institute, Port Blair, Andaman and Nicobar Islands. Seedlings of *Eryngium foetidum* var. CARI-Broad Dhaniya-1 were planted in pro-trays (53 cm × 28 cm × 4.5 cm) having 50 cavities maintained in the shadenet house (50% agro-shade net). The experiment was laid out in completely randomized design with six replications per treatment (120 plants per treatment). Irrigation was provided with micro-sprinkler system during the course of development of plant.

Initial pH of the substrate was determined in 1:2.5 water-substrate suspensions by digital pH meter (Hanna). Treatments consisted of T_1- soil: vermicompost (1:0, v/v), T_2- soil: vermicompost (0:1, v/v), T_3- soil: vermicompost (1:1, v/v), T_4- soil: vermicompost (2:1, v/v) and T_5- soil: vermicompost (1:2, v/v). Urea (0.1%) application was carried out through foliar sprays at weekly interval. All necessary cultural practices and plant protection measures were followed uniformly for all the treatments during the entire period of study.

Morphological observations on leaf length (cm), leaf width (cm) and number of leaves were recorded on 20 plants per treatment. The crop was harvested by uprooting after 4 months of transplanting and used for estimating yield parameters. Moisture content, photosynthetic pigments content using DMSO as a solvent, ascorbic acid content and total carotenoids (β- carotene equivalent) content were estimated following standard procedures (Shivashankara et al., 2017; Waman et al., 2022). The experimental data was subjected to statistical analysis by using Web Agri Stat Package WASP 2.0 (ICAR-CCARI, Ela, India).

Results and discussion

Promotion of cultivation of high value horticultural crops in urban areas is crucial for meeting the ever increasing demand of these commodities. Culantro is a popular herb that fetches premium prices in the island markets. To promote its cultivation, novel pro-tray based systems of cultivation have been developed in recent past by authors' institute. In the present study, effect of vermicompost was studied on performance of this herb, which revealed significant differences among the treatments studied.

Morphological growth parameters

Perusal of data suggested significant influence of vermicompost-soil substrate interaction on morphological traits. Leaves are the economic part of culantro. In some places of cultivation, individual mature leaves are plucked and used while in other parts whole herbs are uprooted and sold with roots. In Andaman Islands, the produce reaching in the market is of the latter type (Waman and Singh, 2019). Number of leaves produced on a seedling and their morphological parameters showed variations when observed after 30, 60, 90 and 120 days of planting (Fig. 1). In general, mean number of leaves increased from 30 to 90 days of planting, while there was a decline in this number after 120 days in all the treatments studied. As the plant reaches maturity, lower leaves start senescing and thus, such reduction in number of leaves per plant at harvest is justifiable.

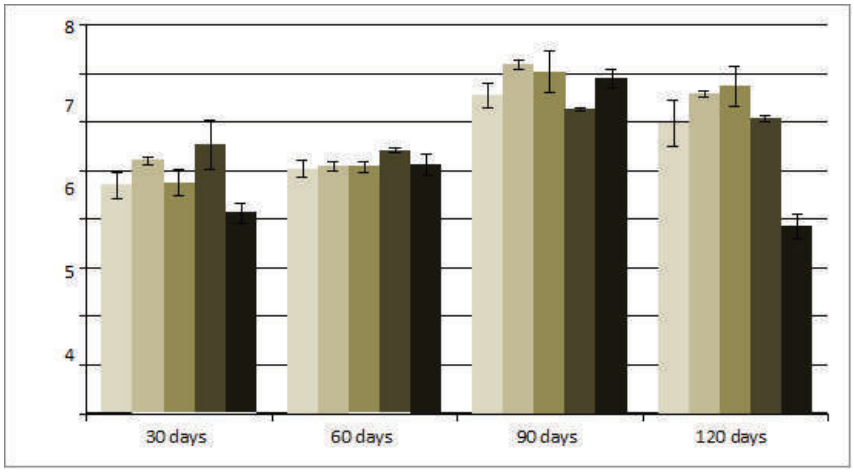

Fig. 1. Mean number of leaves per plant as influenced by vermicompost supplementation over a period of time [Soil: VC (v/v):: T_1 (1:0), T_2(0:1), T_3(1:1), T_4(2:1), T_5(1:2)]

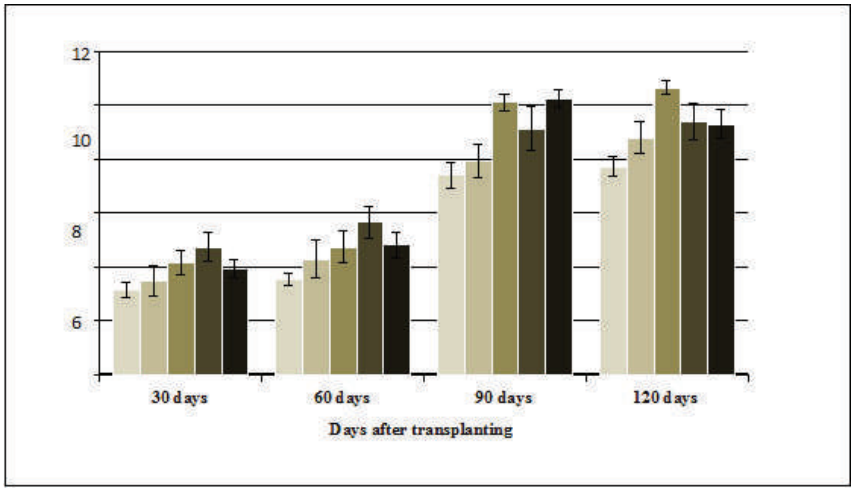

Fig. 2. Mean leaf length (cm) as influenced by vermicompost supplementation over a period of time [Soil: VC (v/v):: T_1 (1:0), T_2(0:1), T_3(1:1), T_4(2:1), T_5(1:2)]

Mean length of the recently matured leaf increased progressively with the age of the plant (Fig. 2). Significant differences were noticed among the treatments throughout the study period and use of soil alone was found to be the poorest of all. This clearly suggested that the herb responded to application of organic nutrient. However, identification of optimum levels of constituents is required as pure VC showed decline in the leaf length at later stage of crop growth. Irrespective of the treatment used, mean width of leaf increased with crop age up to 120 days. Use of soil alone showed lowest values for this parameter throughout the experimental period (Fig. 3). These findings are in accordance with earlier report (Rayhaneh and Shahrzad, 2017), who observed that success obtained with use of vermicompost is influenced by proportion of vermicompost as well as plant developmental stage in thyme.

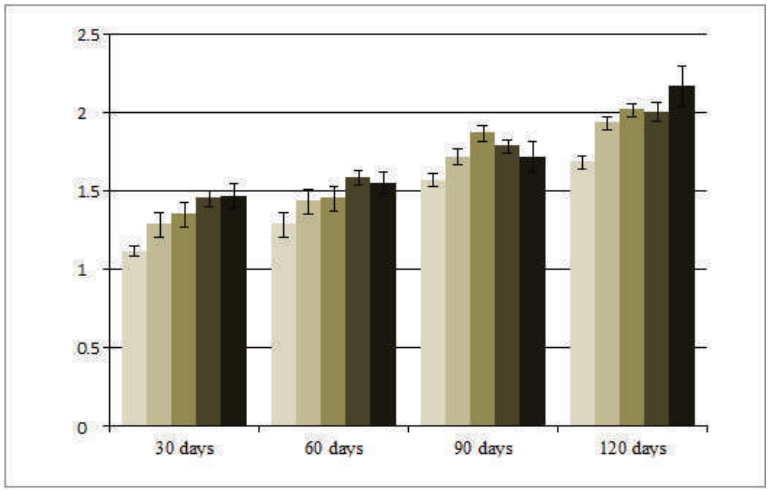

Fig. 3. Mean leaf width (cm) as influenced by vermicompost supplementation over a period of time [Soil: VC (v/v):: T_1 (1:0), T_2(0:1), T_3(1:1), T_4(2:1), T_5(1:2)]

Morphological parameters recorded at the time of harvesting the herb revealed significant differences among the treatments (Table 1). In general, use of equal proportion of soil and vermicompost as substrate significantly improved the plant growth, when compared with other treatments (Fig. 4). Mean length of leaves varied between 10.2 cm (T_1) and 13.5 cm (T_3). Widest leaves (2.0 cm) were observed in vermicompost alone; however, this treatment remained statistically similar with T_3. Leaf width is an important parameter influencing the interception and conversion of solar energy (Sarkar et al., 1995). Soil derived seedlings weighed 2.8 g at harvest, while the highest weight of individual plants was recorded in T_3 (5.7 g). Number of leaves per plant was the highest in T_4 (7.4), which was closely followed by T_1 (7.1) and T_3 (7.0). Importance of identification of optimum level of vermicompost in the substrate for better growth has been emphasized by Rayhaneh and Shahrzad (2017).

Table 1. Morphological parameters at harvest as influenced by vermicompost supplementation

Treatment (Soil: VC, v/v)	Leaf length (cm)	Leaf width (cm)	Weight of seedling (g)	No. of leaves/ plant
T_1 (1:0)	10.2 ± 0.49c	1.6 ± 0.04c	2.8 ± 0.18a	7.1 ± 0.42
T_2 (0:1)	11.7 ± 0.43b	1.6 ± 0.05c	3.0 ± 0.17b	6.8 ± 0.27
T_3 (1:1)	13.5 ± 0.30a	1.8 ± 0.04ab	5.7 ± 0.30c	7.0 ± 0.23
T_4 (2:1)	11.8 ± 0.30b	1.8 ± 0.03bc	3.9 ± 0.27d	7.4 ± 0.27
T_5 (1:2)	12.1 ± 0.40b	2.0 ± 0.05a	4.8 ± 0.32d	6.9 ± 0.22

Values are presented as mean ± standard error of mean. Values followed by similar alphabets in a column do not differ significantly following least significant different at 5% level of significance.

Vermicompost has been reported to be rich in beneficial microflora such as nitrogen fixers, P-solubilizer and cellulose decomposers, apart from being rich source of enzymes, vitamins and plant hormones (Perucci 1990). These micro-flora and phytohormones along with humic acids might have contributed for superior leaf morphological parameters in the vermicompost supplementation treatments in the present study. These compounds are known to promote the plant growth due to proliferation of root hairs, release of mineral nutrients, and assisting in oxidative phosphorylation, cellular respiration, photosynthesis and other enzymatic activities. Improvement in plant morphological parameters due to vermicompost has already been reported in marigold (Ali et al., 2014).

Fig. 4. General view of harvested plants from different treatments (top to bottom, T_1 to T_5)

Biochemical parameters

Significant changes were noticed for biochemical parameters due to different treatments assigned. Apart from the basic function of photosynthesis in a plant system, chlorophyll pigments impart 'greenness' to the produce, which is used as a parameter to judge the quality of fresh herbs. During present study, chlorophyll a, chlorophyll b, total chlorophylls and ratio of chlorophyll a: chlorophyll b were influenced by the treatments studied (Table 2). Substrates involving higher proportions of soil *viz*. T_1 and T_4 showed poorest accumulation of chlorophyll a (1.5 ± 0.01 mg/g and 1.5 ± 0.00 mg/g, respectively), chlorophyll b (0.5 ± 0.02 mg/g and 0.5 ± 0.02 mg/g, respectively) and total chlorophylls (2.0 ± 0.03 mg/g and 2.0 ± 0.02 mg/g, respectively). On the other hand, these parameters were significantly boosted in treatments involving equal or higher proportions of VC. In general, use of soil: VC in equal proportions helped in maximum accumulation of chlorophylls in the leaves. Ratio of chlorophyll a: chlorophyll b varied between 2.82 (T_3) and 3.24 (T_5).

Table 2. Photosynthetic pigments variation due to vermicompost supplementation

Treatment (Soil: VC, v/v)	Chlorophyll content (mg/g)			
	Chl. a	Chl. b	Total chl.	Chl. a: Chl. b
T_1 (1:0)	1.5 ± 0.01	0.5 ± 0.02	2.0 ± 0.03	2.91
T_2 (0:1)	2.5 ± 0.00	0.8 ± 0.00	3.3 ± 0.00	2.92
T_3 (1:1)	2.5 ± 0.01	0.9 ± 0.01	3.4 ± 0.02	2.82
T_4 (2:1)	1.5 ± 0.00	0.5 ± 0.02	2.0 ± 0.02	3.06
T_5 (1:2)	2.4 ± 0.00	0.8 ± 0.14	3.2 ± 0.14	3.24

Vermicompost is known to enrich the substrate with essential nutrients, many of which are directly or indirectly involved in the process of photosynthesis (Sinha et al., 2011) and hence, higher accumulation of chlorophyll pigments could be observed in VC supplemented treatments (Tadayyon et al., 2018). Positive influence of vermicompost on increasing photosynthetic pigments has been demonstrated in thyme and marigold (Ali et al., 2014; Rayhaneh and Shahrzad, 2017).

Moisture content of the produce is generally high in case of leafy greens/ herbs as earlier reports suggest presence of 60.23 to 88.33% moisture in culantro and 82.4% in coriander var. Arka Isha (Varalakshmi et al., 2012; Lepcha et al., 2018). In the present study, moisture content of the freshly harvested leaves did have high moisture content of 85.508% to 86.524%; however, the values did not differ significantly among the substrates studied (Fig. 5).

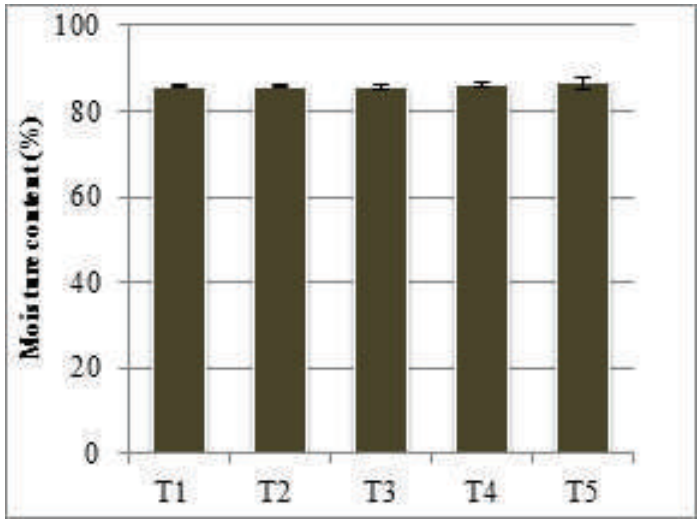

Fig. 5. Moisture content in freshly harvested leaves as influenced by different substrates [Soil: VC (v/v):: T_1 (1:0), T_2(0:1), T_3(1:1), T_4(2:1), T_5(1:2)]

Ascorbic acid content is one of the important nutritional parameter in the herbs (Varalakshmi et al., 2012; Lepcha et al., 2018). In the present study, the ascorbic acid content in the fresh leaves was the lowest (23.97 mg/ 100g) in plants grown in soil alone, which was followed by vermicompost alone (29.97 mg/ 100g). The highest content of ascorbic acid (Fig. 6) was reported from treatment T_5 (65.93 mg/ 100g). As the substrates had different levels of VC, the release of various growth promoting substances would have been different and thus the variations could be justified. Earlier reports have suggested 18.33 to 32.33 mg/100g ascorbic acid in culantro grown in Sikkim (Lepcha et al., 2018), while the levels of ascorbic acid observed in the present study was higher.

Carotenoids are non-enzymatic antioxidants that are known to protect the chlorophylls from oxidative stress. Carotenoid content (β carotene equivalent) showed drastic differences among the treatments studied. Plants grown with soil alone as a substrate showed the lowest content of carotenoids (15.4 mg/ 100g) among the treatments studied (Fig. 6). Use of VC at all the proportions had beneficial influence on the accumulation of carotenoids in the herb and soil: VC (1:2) had the highest content (74.7 mg/100g) of carotenoids in the present study. Similar findings have been reported by earlier researchers who observed improved carotenoids in vermicompost supplied plants than soil alone (Ali et al., 2014; Rayhaneh and Shahrzad, 2017).

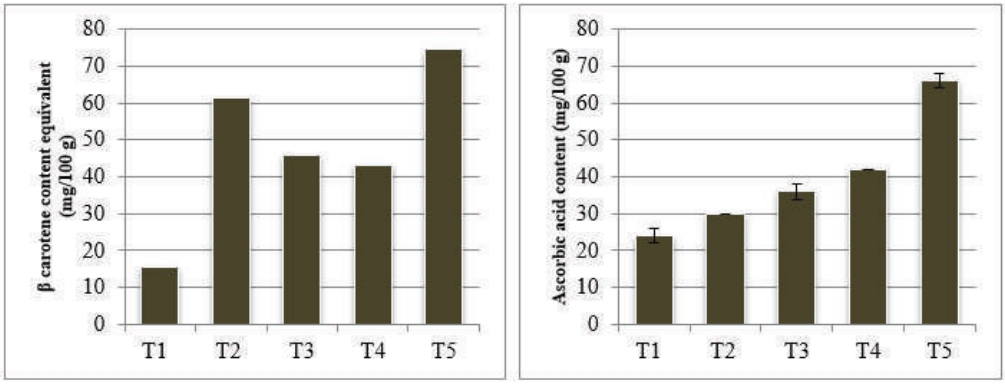

Fig. 6. Total carotenoids (β carotene equivalent) and ascorbic acid content of leaves as influenced by substrates [Soil: VC (v/v):: T_1 (1:0), T_2(0:1), T_3(1:1), T_4(2:1), T_5(1:2)]

Conclusion

The present study exhibited the supplementation of vermicompost with soil to be effective in profusely elevating the morphological parameters such as leaf length, leaf width, number of leaves, moisture and biochemical parameters *viz*. photosynthetic pigments, ascorbic acid content and β-carotene content.

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Phenotyping and Characterization of Pointed Gourd (*Trichosanthes dioica* Roxb.) Genotypes

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Abstract

In contrast to the field crops, quality is as important as yield in vegetable crops. Keeping this in view, the present investigation was carried out during 2021-22 with the objective of assessing the diversity and novelty of pointed gourd genotypes at Bidhan Chandra Krishi Viswavidyalaya, Kalyani, West Bengal, India. Qualitative data based on vegetative and reproductive characters was collected for 18 parameters of 34 distinct genotypes as per the DUS (distinctiveness, uniformity and stability) guidelines developed for the crop. The results revealed a high level of variability and large diversity among the genotypes for majority of the qualitative traits.

Key words: *DUS characterization, morphology, qualitative characters*

Introduction

Vegetables are important components of Indian agriculture and food security because of their short growing season, higher yield, nutrient content, commercial viability, and potential to generate on-and off-farm employment (Kumar, 2020). Pointed gourd (2n=2x=22) is a dioecious, perennial, nutritious, remunerative vegetable belonging to the family Cucurbitaceae. The total cultivated area under the crop in India is about 20,000 ha with the production of 3,25,000 MT per annum (Anon., 2019). In West Bengal, it is grown in almost all the districts and fetches high economical returns. It is known as the 'King of Gourds' as it is a rich source of different nutrients and vitamins. Its roots have purgative property whereas green fruits and succulent shoots exhibit laxative property (Rahman et al. 2008). Khatua et al. (2016) described the leaves to be useful in treatment of oedema, baldness, fever and heart.

The characterization of germplasm provides information on the characteristics possessed by each genotype, ensuring maximal use of the germplasm collection by end users (Reddy et al., 2016). Morphological features are the oldest and most commonly used genetic markers (Bretting and Widrlechner, 1995). Morphological characterization is the initial stage in the description, classification, and arrangement of germplasm collections besides identification of unique ones for future use (Torkpo et al., 2006; Arslanoglu et al., 2011). Information on characterization of pointed gourd genotypes and germplasm is scanty and hence, the current study was conducted with an aim to characterize, appraise, and compare the precise morphological aspects of pointed gourd genotypes to determine their variability and uniqueness. The main aim of the research work was to get acquainted with the desirable morphological descriptor states to be used as potential breeding traits for designing farmer-driven pointed gourd varieties by the breeders.

Material and methods

Description of study area

The experimental study was done at 'C' Block farm of Bidhan Chandra Krishi Viswavidyalaya, Kalyani, West Bengal, India under the AICRP on Vegetable Crops in the summer season of 2021-22. The farm is situated at an elevation of 9.75 m above the mean sea level. Topographic situation of the experimental site comes under Gangetic alluvial plains of West Bengal.

Experimental material and design

Thirty four genotypes of pointed gourd collected from different parts of western Uttar Pradesh, Jharkhand, West Bengal and other parts of India (Table 1) were planted in randomized block design (RBD) with three replications each. Raised beds of 15-20 cm height, 6.0 m length and 1.0 m width accommodating twelve plants per plot were prepared. Stem cuttings of each genotype were planted during the 1st week of November, 2021 in 5 cm deep pits previously filled with well rotten cow dung manure. The spacing followed was 0.90 m and 0.60 m between rows and plants, respectively.

Table 1. Brief description of the pointed gourd genotypes

Sl. No.	Notation	Sources of Material
1	BAUPG-I	Department of Horticulture, BAU, Ranchi, Jharkhand
2	BAUPG-II	Department of Horticulture, BAU, Ranchi, Jharkhand
3	BAUPG-III	Department of Horticulture, BAU, Ranchi, Jharkhand
4	BAUPG-IV	Department of Horticulture, BAU, Ranchi, Jharkhand
5	BCPG-1	Department of Horticulture, BCKV, West Bengal
6	BCPG-3	Department of Horticulture, BCKV, West Bengal
7	BCPG-4	Department of Horticulture, BCKV, West Bengal
8	BCPG-5	Department of Horticulture, BCKV, West Bengal
9	BCPG-6	Department of Horticulture, BCKV, West Bengal
10	BCPG-16	Department of Horticulture, BCKV, West Bengal
11	BCPG-17	Department of Horticulture, BCKV, West Bengal
12	BCPG-22	Department of Horticulture, BCKV, West Bengal
13	BCPG-23	Department of Horticulture, BCKV, West Bengal
14	BCPG-24	Department of Horticulture, BCKV, West Bengal
15	BCPG-25	Department of Horticulture, BCKV, West Bengal
16	BCPG-26	Department of Horticulture, BCKV, West Bengal
17	BCPG-27	Department of Horticulture, BCKV, West Bengal
18	BCPG-29	Department of Horticulture, BCKV, West Bengal
19	BCPG-30	Department of Horticulture, BCKV, West Bengal
20	BCPG-31	Department of Horticulture, BCKV, West Bengal
21	BCPG-34	Department of Horticulture, BCKV, West Bengal
22	BCPG-35	Department of Horticulture, BCKV, West Bengal
23	BCPG-36	Department of Horticulture, BCKV, West Bengal
24	BCPG-37	Department of Horticulture, BCKV, West Bengal
25	BCPG-38	Department of Horticulture, BCKV, West Bengal
26	SwarnaAlaukik	ICAR Research Complex for Eastern Region Ranchi, Jharkhand
27	SwarnaRekha	ICAR Research Complex for Eastern Region Ranchi, Jharkhand
28	KashiAlankar	ICAR- Indian Institute of Vegetable Research, Varanasi, Uttar Pradesh
29	KashiSuphal	ICAR- Indian Institute of Vegetable Research, Varanasi, Uttar Pradesh
30	Tripura Local	College of Agriculture, Tripura
31	NP-260	Dept. of Horticulture, Acharya Narendra Deva University of Agriculture and Technology, Uttar Pradesh
32	NP-520	Dept. of Horticulture, Acharya Narendra Deva University of Agriculture and Technology, Uttar Pradesh
33	Rajendra Parwal-1	Rajendra Agriculture University, Samastipur, Bihar
34	Rajendra Parwal-2	Rajendra Agriculture University, Samastipur, Bihar
35	Male	Department of Horticulture, BCKV, West Bengal

Qualitative observations recorded

Qualitative data was collected based on vegetative and reproductive characteristics that corresponded to the distinctiveness, uniformity, stability (DUS) test guidelines of pointed gourd developed by Protection of Plant Varieties and Farmers Rights Authority (PPV&FRA), Government of India (Table 2). For the assessment of uniformity of characteristics on the plot as a whole (visual assessment by a single observation of a group of plants or parts of plant), 30 plants are considered for observations and other observations were made an all plants in the test. Royal Horticulture Society (RHS) (sixth revised edition) colour chart was used for the assessment of characters such as leaf colour, fruit colour *etc*. Data for nine characteristics were collected by visual assessment by observation of individual plant or parts of plants (VG) whereas data for eight characteristics were collected via visual assessment by a single observation of a group of plants or parts of plants (VS). One character was measured by a number of individual plant or parts of plants (MS).Correlation between different characters were studied by using IndoStat software.

Table 2. Qualitative descriptors used in morphological characterization of pointed gourd genotypes

S. No.	Character code	Character measured	Descriptor states and codes	Notes	Stage of observation	Type of Assessment
1	SS	Shape of stem	Round (R)	3	10	VS
			Angular (A)	5		
2	SP	Stem pubescence nature	Sparse (S)	3	20	VG
			Dense (D)	7		
3	LS	Leaf shape	Auriculate (A)	3	10	VS
			Cordate (C)	5		
4	LP	Leaf pubescence nature	Absent (A)	1	20	VS
			Present (P)	9		
5	LL	Leaf lobes	Absent (A)	1	20	VS
			Present (P)	9		
6	DLL	Depth of leaf lobing	Shallow (S)	3	20	VS
			Medium (M)	5		
			Deep (D)	7		
7	LM	Leaf margin	Entire (E)	3	10	VS
			Undulated (U)	5		
			Lobed (L)	7		
8	LC	Leaf colour	Light green (LG)	1	30	VG
			Green (G)	2		
			Dark green (DG)	3		
9	LT	Leaf tip	Blunt (B)			VS
			Pointed (P)			
10	FS	Fruit shape	Club	1	30	VS
			Cylindrical	2		
			Oval	3		
			Spindle	4		
			Elongated spindle	5		
			Ovate	6		
			Spheroid	7		
			Spindle tapering	8		
11	FSC	Fruit skin primary colour	Light green (138C)	1	30	VG
			Green (138A)	2		
			Dark green (N137A)	3		

S. No.	Character code	Character measured	Descriptor states and codes	Notes	Stage of observation	Type of Assessment
12	FST	Fruit surface striped pattern	Uniform	1	30	VG
			Mottled	2		
			Striped	3		
13	FG	Fruit glossiness	Non-glossy	1	30	VG
			Glossy	9		
14	FPH	Fruit pericarp hardness	Soft	3	30	VG
			Hard	5		
15	FFC	Fruit flesh colour	White		30	VG
			Creamy white			
16	FBES	Blossom end fruit shape	Depressed	1	30	VS
			Flatten	3		
			Round	5		
			Pointed	7		
17	S	Seediness	Absent	1	40	MS
			Present	9		
18	FPA	Fruit peduncle attachment	Soft (S)	3	30	VG
			Hard(H)	5		

VG: Visual assessment by a single observation of a group of plants or parts of plants; VS: Visual assessment by observations of individual plant or parts of plants, MS: Measurement of a number of individual plant or parts of plants.

Results and discussion

a. Characterization of pointed gourd genotypes

Stem and leaf characters

A wide range of variations were recorded for stem and leaf characters (Table 3a) of the germplasm under study. Majority of the genotypes (70.58%) had sparse pubescence on stem, while 29.4% genotypes had dense pubescence. In case of stem shape, all the 34 genotypes were found to have angular stem (Fig. 1a). Similar findings related to stem pubescence and stem shape were reported by Sharma (2015). Leaf margin shape was categorized as cordate and auriculate. While 50% of the genotypes had cordate shaped leaves, remaining 50% had auriculate leaves. Majority of the genotypes (88.23%) recorded undulated leaf margin and the remaining recorded entire (2.94%) and lobed (8.82%) margins. Leaf colours of different genotypes were divided into three groups; light green (20.58%), deep green (29.41%) and green (50%).

Fig.1. Stem shape (a) and leaf blade-depth of lobing (b)

Table 3a. Qualitative morphological characteristics of pointed gourd genotypes (Stem and leaf)

Sl. No.	Genotypes	SS	SP	LS	LP	LL	DLL	LM	LC	LT
1	BCPG-1	A	S	A	P	P	S	UN	DG	P
2	BCPG-3	A	S	A	P	P	S	UN	G	P
3	BCPG-4	A	D	A	P	P	D	UN	DG	B
4	BCPG-5	A	S	C	P	P	S	UN	G	P
5	BCPG-6	A	S	C	P	P	S	UN	G	B
6	BCPG-16	A	S	A	P	P	S	UN	LG	P
7	BCPG-17	A	S	C	P	P	D	UN	DG	P
8	BCPG-22	A	S	C	P	P	S	UN	LG	B
9	BCPG-23	A	D	C	P	P	S	UN	G	P
10	BCPG-24	A	S	C	P	P	S	UN	G	P
11	BCPG-25	A	S	A	P	P	S	UN	DG	P
12	BCPG-26	A	S	A	P	P	S	UN	G	P
13	BCPG-27	A	S	A	P	P	S	UN	G	P
14	BCPG-29	A	S	C	P	P	S	UN	DG	P
15	BCPG-30	A	S	A	P	P	S	UN	G	P
16	BCPG-31	A	S	C	P	P	S	UN	G	B
17	BCPG-34	A	S	A	P	P	D	UN	G	P
18	BCPG-35	A	S	A	P	P	M	UN	G	P
19	BCPG-36	A	S	C	P	P	S	UN	G	P
20	BCPG-37	A	D	C	P	P	D	UN	LG	B
21	BCPG-38	A	S	C	P	P	S	UN	G	B
22	Swarna Alaukik	A	D	C	P	P	S	L	DG	B
23	Swarna Rekha	A	D	C	P	P	S	E	LG	P
24	Kashi Alankar	A	D	C	P	P	M	UN	G	P
25	Kashi Suphal	A	D	A	P	P	S	UN	DG	P
26	Tripura Local	A	S	A	P	P	S	UN	DG	P
27	NP-260	A	D	A	P	P	S	L	G	P
28	NP-520	A	S	C	P	P	S	L	DG	B
29	Rajendra Parwal-1	A	S	A	P	P	S	UN	G	P
30	Rajendra Parwal-2	A	D	A	P	P	S	UN	LG	P
31	BAUPG-I	A	S	C	P	P	M	UN	G	P
32	BAUPG-II	A	S	A	P	P	S	UN	LG	P
33	BAUPG-III	A	S	A	P	P	S	UN	G	P
34	BAUPG-IV	A	D	C	P	P	D	UN	LG	P
Descriptor		A	S	A	P	P	S	UN	G	P
Number of cultivars		34	24	17	34	34	26	30	17	26
Percent of cultivars		100	70.58	50	100	100	76.47	88.23	50	76.47
Descriptor			D	C			M	E	LG	B
No. of cultivars			10	17			3	1	7	8
Percent of cultivars			29.41	50			8.82	2.94	20.58	23.52
Descriptor							D	L	DG	
No. of cultivars							5	3	10	
Percent of cultivars							14.70	8.82	29.41	

SS: stem shape; SP: stem pubescence nature; LS: leaf shape; LP: leaf pubescence nature; LL: leaf lobes; DLL: depth of leaf lobing; LM: leaf margin; LC: leaf colour; LT: leaf tip. Descriptor Codes for different characters are as detailed in Table 2.

Pointed apex leaves were produced by 76.47% of the genotypes. Leaf lobes were present in all the genotypes. The depth of leaf lobbing (Fig. 1b) was found to be shallow (76.47%), medium (8.82%) and deep (14.70%). Deeply lobed leaf trait meant less surface area per leaf. This finding related to leaf margin, leaf colour and leaf end nature had a similarity with Ara et al. (2012).

Fig.2. Fruit shape (a) and fruit surface colour pattern(b)

Fruit characters

Fruit characters of different genotypes under study showed large range of deviation and were categorized as per DUS guidelines (Table 2, 3b). Fruit shapes of different genotypes were categorized into seven groups namely club, cylindrical, oval, spindle, spindle tapering, elongated spindle and spheroid. Maximum frequency of genotypes was observed in case of spindle tapering (47.05), followed by club shaped (26.47%), cylindrical (11.76%), ovate (5.88%), spindle (5.88%) and elongated spindle (2.94%) (Fig. 2a). Previously, Hazra et. al. (1998) had characterized sixty eight female clones of pointed gourd, which were categorized under four groups based on fruit shape. For surface colour of fruits at marketable stage, the results revealed that 55.88% of genotypes exhibited dark green fruit surface followed by 35.29%(green) and 8.82% (light green) (Fig. 2b). A similar range of variations in fruit surface colour has also been reported by Kumar and Singh (2012). Pattern of fruit stripes is also an important morphological parameter in distinguishing two or more genotypes. For this character, all the studied genotypes were divided into three groups namely uniform, mottled and stripped. Striped pattern was found in higher frequency of genotypes (76.47%) than mottled (17.64%) and uniform (5.89%). Unique genetic identity of the individual genotypes is responsible for such type of variations. Similar findings were also recorded by Ara et. al. (2012), Ghosh (2000) and Sharma (2015).

Fruit glossiness is one of the important indices which determine the market value of pointed gourd. The present study revealed that twenty seven genotypes (79.47%) had glossy and seven (20.58%) had non-glossy fruit surface (Fig. 3). The result was supported by findings of Ghosh (2000). As per pericarp hardness of the fruit at marketable stage, all the genotypes were grouped into two categories; soft and hard. Soft pericarp found higher frequency (52.95%) over hard pericarp (47.05%) and it had conformity with the findings of Ghosh (2000).

Fig.3. Fruit glossiness

Table 3b. Qualitative morphological characteristics of pointed gourd genotypes (Fruit)

Sl.No.	Genotypes	FS	FSC	FST	FG	FPH	FFC	FBES	FS	FPA
1	BCPG-1	O	DG	S	G	S	W	F	P	S
2	BCPG-3	C	G	M	G	H	W	R	P	H
3	BCPG-4	CS	DG	S	NG	H	CW	F	P	H
4	BCPG-5	CS	DG	S	NG	H	CW	F	P	H
5	BCPG-6	CS	G	S	G	H	W	F	P	H
6	BCPG-16	ST	G	S	G	S	W	P	P	S
7	BCPG-17	O	DG	M	G	S	W	P	P	S
8	BCPG-22	ST	DG	S	G	S	CW	F	P	H
9	BCPG-23	C	DG	S	NG	S	CW	F	P	H
10	BCPG-24	ST	G	S	G	S	CW	F	P	S
11	BCPG-25	ST	DG	S	G	H	W	P	P	S
12	BCPG-26	ST	DG	S	G	H	W	P	P	S
13	BCPG-27	CS	G	S	G	H	W	P	P	S
14	BCPG-29	CS	DG	S	G	H	CW	F	P	S
15	BCPG-30	CS	DG	S	G	H	CW	P	P	S
16	BCPG-31	ST	DG	S	G	H	CW	R	P	S
17	BCPG-34	ST	DG	S	G	H	CW	R	P	S
18	BCPG-35	S	DG	S	NG	S	CW	R	P	S
19	BCPG-36	ST	DG	S	NG	H	W	P	P	S
20	BCPG-37	CS	G	S	G	S	W	P	P	S
21	BCPG-38	ST	DG	M	G	S	W	R	P	H
22	Swarna Alaukik	ST	G	U	G	S	CW	R	P	H
23	Swarna Rekha	ST	G	M	G	S	CW	R	P	H
24	Kashi Alankar	ST	DG	S	NG	H	CW	F	P	H
25	Kashi Suphal	ST	G	S	G	H	W	P	P	S
26	Tripura Local	ST	G	S	G	H	W	P	P	S
27	NP-260	CS	DG	S	G	H	W	P	P	S
28	NP-520	ST	LG	M	G	S	CW	F	P	S
29	Rajendra Parwal-1	CS	DG	S	G	S	CW	F	P	S
30	Rajendra Parwal-2	ST	G	S	G	S	CW	P	P	S
31	BAUPG-I	S	G	S	G	S	CW	P	P	S
32	BAUPG-II	C	LG	U	G	S	CW	R	P	S
33	BAUPG-III	ES	DG	S	NG	S	CW	P	P	S
34	BAUPG-IV	C	LG	M	G	S	CW	F	P	S
Descriptor		O	DG	S	G	S	CW	F	P	S
No. of cultivars		2	19	26	27	18	21	12	34	24
Percent of cultivars		5.88	55.88	76.47	79.41	52.94	61.76	35.29	100	70.58
Descriptor		C	LG	M	NG	H	W	R		H
No. of cultivars		4	3	6	7	16	13	8		10
Percent of cultivars		11.76	8.82	17.64	20.58	47.05	38.23	23.52		29.41

	FS	FSC	FST	FG	FPH	FFC	FBES	FS	FPA
Descriptor	CS	G	U				P		
No. of cultivars	9	12	2				14		
Percent of cultivars	26.47	35.29	5.88				41.17		
Descriptor	ST								
No. of cultivars	16								
Percent of cultivars	47.05								
Descriptor	S								
No. of cultivars	2								
Percent of cultivars	5.88								
Descriptor	ES								
No. of cultivars	1								
Percent of cultivars	2.94								

FS: Fruit shape; FSC: Fruit skin primary colour; FST: Fruit surface striped pattern; FG: Fruit glossiness; FPH: Fruit pericarp hardness; FFC: Fruit flesh colour; FBES: Blossom end fruit shape; S: Seediness; FPA: Fruit peduncle attachment. Descriptor Codes for different characters are as detailed in Table 2.

Flesh colour of the fruit varied from white to creamy white and most of the genotypes were categorized under creamy white flesh colour category (61.76%). In the present study, fruit shape varied from flattened (12 genotypes) to round (8 genotypes) and pointed (14 genotypes). Fruit peduncle attachment is also an important qualitative character. Twenty four genotypes showed soft fruit attachment with peduncle and only ten genotypes showed hard attachment. Seeded fruits were produced by all the genotypes under study and none of the genotypes was found to bear seedless fruits.

Conclusion

Vegetable product innovation is necessary to maintain the interest of today's consumers. Unlike field crops, quality generally dominates yield with vegetable crops. Market acceptability is required for farmers to survive as well as increase of shelf-life is also very important. Now pointed gourd is becoming popular crop among cucurbits crops for its medicinal value. Thus, quality typically trumps productivity. Vegetable breeding programmes aim to create a new variety with exceptional combinations of desirable horticultural traits. Along with fruit yield, there are fruit attributes that influence pointed gourd's productivity and marketability. The economic value of pointed gourd depends on both fruit output and quality, which is a combination of horticultural features. The pointed gourd genotype was linked to fruit greenness, length, weight, seediness and glossiness. So, it is traded based on its quality and size.

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Sensory Quality of Moringa Leaf Powder as Affected by Different Drying Methods and Packaging Material

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Abstract

The present experiment was conducted at Post Harvest Technology Laboratory, College of Horticulture, Dr. Y.S.R. Horticultural University, Andhra Pradesh in a Completely Randomized Factorial Design with two factors at unequal levels, replicated thrice. Moringa leaves were dried by using different drying methods *viz.* sun drying, solar drying, tray drying, vacuum drying and freeze drying. These dried powders were packed in three different packaging materials *viz.* PET bottles, aluminium pouches and LDPE (200 gauge) pouches to know their suitability in retention of quality. Using the moringa leaf powder obtained from different drying methods, moringa tea was prepared and evaluated for sensory qualities at 30 days interval up to 90 days of storage period. Sensory scores were obtained for colour, aroma, taste, mouthfeel and overall acceptability. Moringa tea prepared from freeze dried leaf powder (D_5) recorded highest acceptability scores for colour (6.67), aroma (7.12), taste (7.34), mouthfeel (7.18) and overall acceptability (7.47), whereas the lowest score (6.30) for astringency was recorded in moringa tea prepared from sun dried (D_1) leaf powder.

Key words: *Moringa tea, Organoleptic evaluation, overall acceptability, storage*

Introduction

Drumstick (*Moringa oleifera*) is an underexploited perennial vegetable species of Moringaceae family, native to the Sub-Himalayan tracts of India, Pakistan, Bangladesh and Afghanistan (Makkar and Becker, 1997). This fast growing tree is also known as the moringa, horseradish tree, benzolive tree, or ben oil tree. It is a perennial softwood tree and it has been suggested for medicinal and industrial uses. Moringa is one of the world's most nutritious crops and its leaves have been reported to possess various bioactive compounds such as phenols, flavonoids, tannins, saponins *etc*. (Vergara-Jimenez et al*.,* 2017). Moringa leaf products especially leaf powder is gaining popularity in the recent past due to outstanding nutritive value and traditional preference. Moringa leaves help in protecting the liver, nourishing skin and hair, fighting against bacterial diseases and are reported to have high antioxidant capacity (Siddhuraju and Becker, 2003). With the above background, an investigation on different drying methods and packaging materials on the sensory quality of moringa tea was taken up.

Materials and methods

The present investigation was carried out during November 2020 to April 2021 at Post Harvest Laboratory, College of Horticulture, Dr. Y.S.R Horticultural University, Venkataramannagudem, Andhra Pradesh. The experiment was conducted in Completely Randomized Factorial Design with two factors at unequal levels, each replicated thrice. Moringa variety PKM-1 was used for the experimental studies. Leaves were dried using different methods *viz.* sun drying (D_1), solar drying (D_2), tray drying (D_3), vacuum drying (D_4) and freeze drying (D) (Fig. 1). The produce obtained from these treatments were packed using three packaging materials namely PET bottles (P_1), Aluminium pouches (P_2) and LDPE (200 gauge) polybags (P_3). The packed product was stored up to 90 days at ambient temperature.

Using the moringa leaf powder obtained from different drying methods, moringa tea was prepared using honey and lemon and evaluated for sensory qualities. Sensory scores were obtained for colour, aroma, taste, mouthfeel and overall acceptability. The scores were provided based on hedonic scale 9- like extremely, 8- like very much, 7-

like moderately, 6- like slightly, 5- neither like or dislike, 4- dislike slightly, 3- dislike moderately, 2- dislike very much, 1- dislike extremely (Ranganna, 1995). The data were analysed using the standard statistical procedures for factorial experiments.

Results and discussion

Colour

Drying methods and packaging material had profound influence on the colour of the tea prepared from moringa leaf powder at 0, 30, 60 and 90 days after storage (Table 1). The freeze dried moringa leaf powder (D_5) had the highest scores for colour (8.11) and significant reduction in colour was observed from initial day of storage to 90 days after storage (6.67). Better retention of colour may be attributed to the low temperatures involved in freeze drying process, when compared with other drying methods studied. This was followed by vacuum dried leaf powder (D_4) in which the initial score was 7.55 and it was reduced to 6.32 after 90 days of storage. In this case also, lower temperature of 40 ºC was employed during drying process and hence better retention of colour was as expected. The least acceptable colour was recorded in sun dried moringa leaf powder (D_1) from initial day of storage (6.44). Score in this case was reduced to 5.35 after 90 days of storage. The results are in accordance with earlier reports by Magdalini et al. (2008) in dehydrated products and Sravankumar et al. (2014) in hibiscus leaves. On the contrary, tea prepared from other drying methods received lower score from the judges due to the fading of colour as a result of high temperatures employed in drying process.

Table 1. Effect of different drying methods and packaging materials on colour of moringa tea over the storage period of 90 days

Drying methods (D)	Packaging material (P) Days of storage															
	Initial(0 day)				30days				60days				90days			
	P_1	P_2	P_3	Mean	P_1	P_2	P_3	Mean	P_1	P_2	P_3	Mean	P_1	P_2	P_3	Mean
D_1	6.20	7.21	5.93	**6.44**	5.85	7.03	5.76	**6.21**	5.36	6.43	5.33	**5.71**	5.00	6.16	4.90	**5.35**
D_2	7.46	7.92	7.22	**7.53**	7.13	7.36	7.06	**7.18**	6.70	7.06	6.23	**6.66**	6.16	6.40	6.00	**6.18**
D_3	7.35	7.59	6.76	**7.24**	6.40	7.53	6.13	**6.68**	5.93	6.90	5.83	**6.22**	5.73	6.16	5.43	**5.77**
D_4	7.43	7.83	7.40	**7.55**	7.16	7.66	6.86	**7.23**	6.43	7.20	6.33	**6.65**	6.10	6.80	6.06	**6.32**
D_5	8.33	8.33	7.66	**8.11**	7.76	8.33	7.50	**7.86**	7.40	7.60	7.20	**7.40**	6.80	6.92	6.30	**6.67**
Mean	**7.35**	**7.78**	**6.99**	**7.37**	**6.86**	**7.58**	**6.66**	**7.03**	**6.36**	**7.04**	**6.18**	**6.52**	**5.96**	**6.49**	**5.74**	**6.05**
Comparing means	**SEm±**		**CD@5%**		**SEm±**		**CD@5%**		**SEm±**		**CD@5%**		**SEm±**		**CD@5%**	
(D)	**0.226**		**0.773**		**0.259**		**0.753**		**0.188**		**0.546**		**0.138**		**0.401**	
(P)	**0.206**		**NS**		**0.201**		**0.583**		**0.146**		**0.423**		**0.107**		**0.311**	
Interaction (D×P)	**0.461**		**NS**		**0.449**		**0.301**		**0.326**		**0.223**		**0.240**		**0.121**	

Drying methods: D_1: Sun drying, D_2: Solar drying, D_3: Tray drying, D_4: Vacuum drying, D_5: Freeze drying Packaging materials: P_1:PET bottle, P_2: Aluminium pouch, P_3: LDPE (200 gauge)

Among packaging materials at 30, 60 and 90 days after storage, the highest colour score was recorded in leaf powder packed in aluminium pouches (P_2) from 30 days (7.58), which reduced 6.49 after 90 days of storage. It was followed by leaf powder packed in PET bottles (P_1) from 30 days (6.86), which reduced to 5.96 after 90 days of storage. The lowest colour score was recorded in leaf powder packed in LDPE 200 gauge polybags at 30 days (6.66) and 90 days after storage (5.74). During the storage, the colour scores decreased, irrespective of packaging material due to exposure of the product to light, when stored at ambient condition.

Aroma

Significant differences in scores of aroma were recorded among drying and packaging treatments studied at 0, 30, 60 and 90 days of storage (Table 2). On all the days of storage, the highest aroma score was recorded in

freeze dried moringa leaf powder (D_5). In the freshly dried produce, the score of 8.00 was recorded in this treatment, which reduced with storage duration to 7.12 after 90 days. These values were on par with vacuum dried leaf powder (D_4) in which the aroma score of 7.77 was recorded at 0 days of storage and it was reduced to 6.98 after 90 days. Like the scores observed for colour parameter, least acceptable score for aroma was recorded in sun dried leaf powder (D_1) on the initial day of storage (6.57). The score in sun dried sample dropped to 6.05 after 90 days of storage. No significant difference was observed among packaging materials till the end of storage period. The interaction effect was also found to be non-significant during the storage.

Table 2.Effect of different drying methods and packaging materials on aroma of moringa tea over the storage period of 90 days

Drying methods (D)	Packaging material (P)															
	Days of storage															
	Initial (0 day)				Initial (30 day)				Initial (60 day)				Initial (90 day)			
	P_1	P_2	P_3	Mean	P_1	P_2	P_3	Mean	P_1	P_2	P_3	Mean	P_1	P_2	P_3	Mean
D_1	7.35	6.30	6.06	**6.57**	7.21	6.20	5.93	**6.44**	6.80	6.10	5.83	**6.24**	6.63	5.90	5.63	**6.05**
D_2	7.63	8.06	7.30	**7.66**	7.46	7.92	7.22	**7.53**	7.20	7.70	7.02	**7.31**	7.00	7.12	6.71	**6.94**
D_3	7.76	6.96	7.43	**7.38**	7.59	6.76	7.35	**7.24**	7.33	6.53	7.15	**7.00**	7.03	6.26	6.84	**6.71**
D_4	7.66	7.66	8.00	**7.77**	7.43	7.40	7.83	**7.55**	7.06	7.16	7.46	**7.23**	6.93	6.90	7.13	**6.98**
D_5	7.33	8.33	8.33	**8.00**	7.20	8.13	8.13	**7.82**	6.83	7.83	7.83	**7.50**	6.50	7.43	7.43	**7.12**
Mean	**7.55**	**7.46**	**7.42**	**7.47**	**7.38**	**7.28**	**7.29**	**7.31**	**7.04**	**7.06**	**7.06**	**7.05**	**6.82**	**6.72**	**6.75**	**6.76**
Comparing means	**SEm±**		**CD@5%**		**SEm±**		**CD@5%**		**SEm±**		**CD@5%**		**SEm±**		**CD@5%**	
(D)	**0.268**		**0.779**		**0.267**		**0.775**		**0.259**		**0.752**		**0.240**		**0.697**	
(P)	**0.208**		**NS**		**0.207**		**NS**		**0.201**		**NS**		**0.186**		**NS**	
Interaction (D×P)	**0.465**		**NS**		**0.462**		**NS**		**0.449**		**NS**		**0.416**		**NS**	

Drying methods: D_1: Sun drying, D_2: Solar drying, D_3: Tray drying, D_4: Vacuum drying, D_5: Freeze drying Packaging materials: P_1: PET bottle, P_2: Aluminium pouch, P_3: LDPE (200 gauge)

Taste

The results pertaining to effect of drying methods and packaging material on taste of the moringa tea at initial, 30, 60 and 90 days after storage are presented in Table 3. Irrespective of the drying/ packaging treatment, the scores for taste decreased with storage duration. On all the days of storage, the highest taste score was recorded in freeze dried moringa leaf powder (D_5) on the initial day of storage (7.88) and with passage of time, the scores were reduced to 7.34 after 90 days of storage. This treatment was on par with vacuum dried leaf powder (D_4) in which the scores of 7.72 and 7.01 were recorded after 0 and 90 days of storage, respectively. The least acceptable score was observed in tray dried (D_3) leaf powder in which mean score of 6.70 was recorded on the initial day of storage.

In case of packaging material, the highest taste score was recorded in leaf powder packed in aluminium pouches (P_2) after 30 days (7.47), which reduced to 7.10 after 90 days of storage. This was followed by powder packed in PET bottles (P_1) in which scores of 7.09 and 6.74 were recorded after 30 and 90 days of storage, respectively. The lowest taste scores of 6.61 and 6.27 were recorded in leaf powder packed in LDPE polybags (P_3) after 30 and 90 days, respectively. Interaction effect was found to be non-significant on all the days of storage. The taste was superior in the freeze dried samples which may be due to the superior aroma and better retention of all the nutrients.

Mouthfeel

Mouthfeel of the moringa tea prepared from leaves dried using different drying methods and packaging material was significantly influenced during storage (Table 4). The mean acceptability scores for mouthfeel decreased steadily during the storage period from 7.44 (30 days) to 6.76 (90 days). The highest score for mouthfeel

was recorded in freeze dried moringa leaf powder (D_5) on the initial day of storage (8.07), which dropped to 7.18 at 90 days after storage. This treatment did not differ significantly with vacuum drying (D_4). In vacuum dying, the mean score for mouth feel reduced from 7.77 to 7.23 after 0 and 90 days of storage. The least acceptable score was recorded in sun dried sample (D_1) on initial day of storage (6.77) and 90 days after storage (6.13). Among different packaging materials no significant difference was observed till the end of storage. The interaction effect was also found to be non-significant on all the days of storage.

Table 3. Effect of different drying methods and packaging materials on taste of moringa tea over the storage period of 90 days

Drying methods (D)	Packaging materials (P)															
	Days of storage															
	Initial (0 day)				30 days				60 days				90 days			
	P_1	P_2	P_3	Mean	P_1	P_2	P_3	Mean	P_1	P_2	P_3	Mean	P_1	P_2	P_3	Mean
D_1	7.50	6.46	7.06	**7.01**	6.83	7.23	6.20	**6.75**	6.63	7.10	6.06	**6.60**	6.50	6.93	5.90	**6.44**
D_2	7.00	6.96	7.66	**7.21**	6.80	7.46	6.80	**7.02**	6.60	7.20	6.56	**6.78**	6.43	7.00	6.36	**6.60**
D_3	6.80	7.10	6.20	**6.70**	6.50	6.86	5.96	**6.44**	6.31	6.70	5.80	**6.27**	6.24	6.56	5.63	**6.14**
D_4	6.83	8.33	8.00	**7.72**	7.60	8.00	6.63	**7.41**	7.36	7.73	6.43	**7.17**	7.23	7.53	6.26	**7.01**
D_5	8.00	8.00	7.66	**7.88**	7.73	7.81	7.49	**7.67**	7.53	7.64	7.38	**7.52**	7.33	7.47	7.21	**7.34**
Mean	**7.22**	**7.37**	**7.32**	**7.30**	**7.09**	**7.47**	**6.61**	**7.05**	**6.89**	**7.27**	**6.44**	**6.86**	**6.74**	**7.10**	**6.27**	**6.70**

Comparing means	SEm±	CD@5%	SEm±	CD@5%	SEm±	CD@5%	SEm±	CD@5%
(D)	0.265	0.770	0.262	0.761	0.261	0.757	0.262	0.760
(P)	0.205	NS	0.203	0.590	0.202	0.587	0.203	0.589
Interaction (D×P)	0.450	NS	0.454	NS	0.452	NS	0.454	NS

Drying methods: D_1: Sun drying, D_2: Solar drying, D_3: Tray drying, D_4: Vacuum drying, D_5: Freeze drying Packaging materials: P_1: PET bottle, P_2: Aluminium pouch, P_3: LDPE (200 gauge)

Table 4. Effect of different drying methods and packaging materials on astringency of moringa tea over the storage period of 90 days

Drying methods (D)	Packaging materials (P)															
	Days of storage															
	Initial (0 day)				Initial (30 day)				Initial (60 day)				Initial (90 day)			
	P_1	P_2	P_3	Mean	P_1	P_2	P_3	Mean	P_1	P_2	P_3	Mean	P_1	P_2	P_3	Mean
D_1	7.21	6.36	6.40	**6.66**	7.11	6.26	6.26	**6.54**	6.96	6.16	6.10	**6.41**	6.86	6.03	6.00	**6.30**
D_2	7.63	6.83	7.49	**7.31**	7.52	6.73	7.30	**7.18**	7.30	6.60	7.13	**7.01**	7.16	6.46	7.00	**6.87**
D_3	7.46	7.53	7.96	**7.65**	7.30	7.53	7.80	**7.54**	7.16	7.33	7.63	**7.37**	7.06	7.23	7.53	**7.27**
D_4	7.43	8.15	8.15	**7.91**	7.26	8.01	8.04	**7.77**	7.06	7.78	7.80	**7.55**	6.96	7.40	7.66	**7.34**
D_5	7.50	8.09	7.22	**7.60**	7.40	7.93	7.03	**7.45**	7.13	7.73	6.83	**7.23**	7.03	7.63	6.73	**7.13**
Mean	**7.44**	**7.39**	**7.44**	**7.42**	**7.32**	**7.29**	**7.28**	**7.30**	**7.12**	**7.12**	**7.10**	**7.11**	**7.02**	**6.95**	**6.98**	**6.98**

Comparing means	SEm±	CD@5%	SEm±	CD@5%	SEm±	CD@5%	SEm±	CD@5%
(D)	0.237	0.687	0.234	0.678	0.234	0.680	0.240	0.695
(P)	0.183	NS	0.181	NS	0.181	NS	0.186	NS
Interaction (D×P)	0.410	NS	0.405	NS	0.406	NS	0.415	NS

Drying methods: D_1: Sun drying, D_2: Solar drying, D_3: Tray drying, D_4: Vacuum drying, D_5: Freeze drying Packaging materials: P_1: PET bottle, P_2: Aluminium pouch, P_3: LDPE (200 gauge)

Table 5. Effects of different drying methods and packaging materials on mouth feel of moringa tea over the storage period of 90 days

Drying methods (D)	Packaging materials (P)															
	Days of storage															
	Initial (0 day)				Initial (30 day)				Initial (60 day)				Initial (90 day)			
	P_1	P_2	P_3	Mean	P_1	P_2	P_3	Mean	P_1	P_2	P_3	Mean	P_1	P_2	P_3	Mean
D_1	7.66	6.66	6.00	**6.77**	7.40	6.43	5.85	**6.56**	7.30	6.26	5.71	**6.42**	6.86	5.96	5.57	**6.13**
D_2	7.50	8.16	7.66	**7.77**	7.33	7.96	7.46	**7.58**	7.16	7.81	7.32	**7.43**	6.96	7.62	7.10	**7.23**
D_3	7.83	6.40	6.66	**6.96**	7.60	6.13	6.40	**6.71**	7.13	5.96	6.68	**6.59**	6.76	5.76	6.28	**6.27**
D_4	7.33	8.00	7.66	**7.66**	6.80	7.73	7.36	**7.30**	6.60	7.63	7.16	**7.13**	6.30	7.43	7.26	**7.00**
D_5	7.50	8.40	8.33	**8.07**	7.20	8.10	8.10	**7.80**	7.00	7.93	7.90	**7.61**	6.46	7.66	7.43	**7.18**
Mean	**7.56**	**7.52**	**7.26**	**7.44**	**7.26**	**7.27**	**7.03**	**7.19**	**7.04**	**7.12**	**6.95**	**7.03**	**6.67**	**6.89**	**6.73**	**6.76**
Comparing means	**SEm±**		**CD@5%**		**SEm±**		**CD@5%**		**SEm±**		**CD@5%**		**SEm±**		**CD@5%**	
(D)	**0.323**		**0.937**		**0.300**		**0.872**		**0.284**		**0.825**		**0.286**		**0.831**	
(P)	**0.250**		**NS**		**0.233**		**NS**		**0.220**		**NS**		**0.222**		**NS**	
Interaction (D×P)	**0.259**		**NS**		**0.520**		**NS**		**0.492**		**NS**		**0.496**		**NS**	

Drying methods: D_1: Sun drying, D_2: Solar drying, D_3: Tray drying, D_4: Vacuum drying, D_5: Freeze drying Packaging materials: P_1: PET bottle, P_2: Aluminium pouch, P_3: LDPE (200 gauge)

Table 6. Effect of different drying methods and packaging materials on overall acceptability of moringa tea over the storage period of 90 days

Drying methods (D)	Packaging materials (P)															
	Days of storage															
	Initial (0 day)				Initial (30 day)				Initial (60 day)				Initial (90 day)			
	P1	P2	P3	Mean	P1	P2	P3	Mean	P1	P2	P3	Mean	P1	P2	P3	Mean
D_1	7.35	6.96	6.40	**6.90**	7.18	6.79	6.16	**6.71**	7.00	6.50	6.01	**6.50**	6.93	6.40	5.83	**6.38**
D_2	7.63	8.06	7.36	**7.68**	7.40	7.89	7.27	**7.52**	7.18	7.63	7.13	**7.31**	6.96	7.49	7.01	**7.15**
D_3	7.76	6.96	7.43	**7.38**	7.59	6.76	7.29	**7.21**	7.43	6.50	6.86	**6.93**	7.30	6.40	6.73	**6.81**
D_4	7.66	8.00	8.26	**7.97**	7.53	7.76	8.02	**7.77**	7.16	7.50	7.74	**7.47**	7.03	7.33	7.63	**7.33**
D_5	7.66	8.66	8.33	**8.22**	7.26	8.46	8.10	**7.94**	6.83	8.10	7.80	**7.57**	6.73	8.00	7.70	**7.47**
Mean	**7.61**	**7.73**	**7.56**	**7.63**	**7.39**	**7.53**	**7.37**	**7.43**	**7.12**	**7.24**	**7.11**	**7.15**	**6.99**	**7.12**	**6.98**	**7.03**
Comparing means	**SEm±**		**CD@5%**		**SEm±**		**CD@5%**		**SEm±**		**CD@5%**		**SEm±**		**CD@5%**	
(D)	**0.235**		**0.681**		**0.227**		**0.658**		**0.222**		**0.644**		**0.220**		**0.642**	
(P)	**0.182**		**NS**		**0.176**		**NS**		**0.172**		**NS**		**0.171**		**NS**	
Interaction (D×P)	**0.407**		**NS**		**0.393**		**NS**		**0.384**		**NS**		**0.383**		**NS**	

Drying methods: D_1: Sun drying, D_2: Solar drying, D_3: Tray drying, D_4: Vacuum drying, D_5: Freeze drying Packaging materials: P_1: PET bottle, P_2: Aluminium pouch, P_3: LDPE (200 gauge)

Astringency

The data pertaining to effect of drying methods and packaging material on astringency scores of moringa tea at initial, 30, 60 and 90 days after storage are presented in Table 5. The least score for astringency was recorded in sun dried moringa leaf powder (D_1) from initial day of storage (6.66) to 90 days after storage (6.30). The highest score was recorded in vacuum dried moringa leaf powder (D_4) from initial day of storage (7.91) to 90 days after storage (7.34). This was on par with tray dried sample (D_3) with mean scores of 7.65 and 7.27 scores at 0 and 90 days, respectively followed by freeze dried leaf powder (D_5). No significant difference in astringency scores among packaging materials and the interaction effect was recorded during 90 days of storage.

Overall acceptability

Data related to the effect of different drying methods and packaging material on overall acceptability scores of moringa tea during storage period has been presented in Table 5. Throughout the storage period, the highest score of overall acceptability was recorded in moringa tea prepared from freeze dried sample (D_5). On the initial day of storage the score in freeze dried sample was 8.22, which reduce to 7.47 after 90 days of storage. No significant difference in overall acceptability scores among the packaging materials and the interaction effect was recorded during the storage period. Overall acceptability score had decreased during storage due to change in chemical composition of the product and loss of colour and flavour. These kind of observations were recorded by Singh et al. (2006).

The highest overall acceptability recorded for moringa tea prepared from freeze dried leaf powder might be due to the retention of more nutrient content, balance of the taste, aroma and colour, mouth feel and astringency. The main advantage of freeze drying is that it results in products that appear almost like the fresh. Physically, retention of original texture, structure and highly volatile components (responsible for aroma) has been reported in freeze dried food products (Chen et al. 2000). The least acceptable score (6.90) was recorded in sun dried leaf powder (D_1) on the initial day of storage, which dropped to 6.38 after 90 days of storage. Lower acceptability score was recorded in moringa tea prepared from sun dried leaf powder which might be due to its less acceptable taste and aroma.

Conclusion

Organoleptic evaluation of moringa tea prepared from leaves dried using different drying methods and stored in different packaging material was carried out. Moringa tea prepared from freeze dried leaf powder (D_5) recorded the highest acceptability scores for colour (6.67), aroma (7.12), taste (7.34), mouthfeel (7.18) and overall acceptability (7.47) whereas the lowest score (6.30) for astringency was recorded in moringa tea prepared from sun dried (D_1) leaf powder. The most effective method for drying moringa leaves was freeze drying. An overall recommendation of drying of moringa leaves by the freeze drying method based on the results is not possible due to requirement of high cost and skill. The initial cost of equipment, electrical energy consumption and equipment maintenance are relatively higher than those for other drying methods. Alternatively, cost effective method of drying moringa leaves in vacuum drier and packing in LDPE 200 gauge polybags is recommended.

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Seed Germination Studies in Andaman Kokum (*Garcinia dhanikhariensis* S.K. Srivastava): An Endemic Species from Bay Islands, India

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Abstract

Garcinia dhanikhariensis is an endemic species distributed in the warm and humid tropical South Andaman Island in the Bay of Bengal. Natural populations of this species are dwindling due to anthropogenic activities and natural calamities. Lack of awareness among the local people about its potential uses is adding to the gravity of the situation. Considering the endemic nature and very low natural population of the species, urgent efforts on conservation are required. Effect of retention of seed coat (presence/ absence), seed division (whole/ fragmented) and seed size (small/ large) was studied on seed germination and seedling growth parameters. Results revealed that removal of seed coat hastened the germination process and 95.9% germination was noticed in such seeds. Division of seeds into two pieces gave rise to plants from both the segments, although with variable success. Higher germination percentage of 70.7% was obtained when large seeds were used for sowing. Seedling growth parameters were also influenced due to the studied treatments. Thus, the present findings could be helpful in large scale multiplication of this endemic genetic resource of the fragile island ecosystem.

Key words: *Seed coat removal, seed division, seed size, seedling vigour*

Introduction

Garcinia dhanikhariensis S.K. Srivastava is an endemic species of the Clusiaceae family distributed in the Andaman Islands in the Bay of Bengal (Srivastava, 1994). Limited natural populations of this species are found scattered around the Dhanikhari, Chouldari, Lal Pahad and Kalatang villages of South Andaman Island. Locally it is known as *Lal Kau Phal* while it was christened as Andaman Kokumowing to some similarities of the species with *Garcinia indica* of the Western Ghats (Bohra et al., 2021a). The tree grows slender with narrow canopy. Fruits are generally ready to harvest during January-February and the harvesting continues until May (Bohra et al., 2021a). Traditionally, only a few people in and around the area are aware about the species and they consume the sour sweet pulp of the fruits, while rind is largely discarded.

Even though the species was discovered in 1994, it remained largely understudied till the recent past. Systematic studies were initiated at ICAR-Central Island Agricultural Research Institute, Port Blair since 2016 to conserve, characterize and utilize this species (Bohra et al., 2021a). Rind of the species has been identified as a natural source of anthocyanins, and antioxidant with potential as a natural colorant, and pharmaceutically important hydroxycitric acid (Bohra and Waman, 2022). Further, seeds of the species have been identified as a potential source of fatty acids *viz*. stearic acid and oleic acid of industrial significance (Bohra et al., 2021b). Considering these characteristics, the species has been identified as a novel crop for commercial cultivation in the warm humid tropical regions of Andaman Islands (Bohra et al., 2021a).

Even though the species has potential for commercial utilization in the islands, lack of planting material is a major issue in promoting it. As the species is endemic and only a few natural populations are available, development of efficient multiplication protocol is important. Seed germination is one of the easy yet efficient means for mass multiplication of species, especially the endemic ones. However, a number of aspects need to be studied to have a reliable mass multiplication protocol. Presence of prolonged dormancy (8-11 months) has been reported

in species of *Garcinia* (Liu et al., 2005). In earlier study, we studied the effect of seed soaking treatments and seed source for obtaining superior germination in this species (Bohra et al., 2021).

Earlier studies involving different *Garcinia* species suggested that removal of seed coat (Joshi et al., 2006; Liu et al., 2005; Cardoso et al., 2021) and seed size (Florent et al., 2021) significantly influenced the seed germination and subsequent plant growth. *Garcinia* type germination, which is characterized by primary root and shoot emergence from opposite ends of the seed (de Vogel, 1980), has also been reported in this species recently (Bohra et al., 2021). In Malabar tamarind (*G. gummi-gutta*), use of seed fragments was found to give rise to seedlings from each segment of the seed (Joshi et al., 2006). This could be of great practical help in multiplication of endemic species such as Andaman Kokum, where the seed availability is rare. Considering these aspects, the present study was undertaken with three objectives: 1. to study the effect of seed coat removal on seed germination and seedling growth, 2. to explore the possibility of using seed pieces for propagation and 3. to study the effect of seed size on seed germination and seedling growth.

Materials and methods

Collection of seed samples

Fully ripe fruits of *Garcinia dhanikhariensis* S.K. Srivastava were collected from an identified collection (GDH/SA/RUP) from Lal Pahad village of South Andaman Island during 2021. Seeds were extracted manually from the fruits and washed with water to remove the adhering pulp. After washing, seeds were divided into three experiments as detailed below. After appropriate treatments, the seeds were sown in pro-trays (49 cavities) filled with soil + vermicompost (1:1, v/v) as substrate. Each treatment was replicated thrice and there were 49 seeds in each replication.

Experiment 1: Effect of removal of seed coat

In this experiment, washed seeds were divided into two lots. In first lot, seed coats were manually removed from the seeds and used for sowing (T_1), while in the second lot, seed coat was kept intact (T_2).

Experiment 2: Effect of seed division

In order to know the effect of division of seeds, seed coats were removed manually from all the seeds. Seeds in the first lot were cut into two pieces (T_1) before sowing while seeds in other lot were used intact for sowing in the aforesaid medium (T_2).

Experiment 3: Effect of size of seeds

Seeds after washing were air dried and then sorted into two groups based on seed weight. Individual seeds were weighed using analytical balance and those weighing less than 0.5 g were graded as small sized seeds (T_1), while those weighing more than 0.5 g were graded as large sized seeds (T_2). Such graded seeds were sown in the substrate as mentioned above.

Record of observations

Number of seeds germinating every day was counted manually. Seedlings with minimum 2 mm of epicotyls elongation above the substrate were considered as germinated. Germination percentage was determined at 30, 60, 90 and 120 days after sowing. Seedling growth parameters were recorded at the end of experiment (120 days) using 20 seedlings in each treatment. Parameters such as seedling length (cm), shoot length (cm), root length (cm), number of roots per seedling, length of longest root (cm), number of leaves per seedling, leaf length (cm), leaf width (cm) and collar thickness (cm) were recorded using standard procedures.

Mean time taken for initiation of germination (days) was determined as time taken for start of germination in each replication / number of replications, whereas mean time taken for completion of germination (days) was calculated as time taken for completion of germination in each replication / number of replications. Seedling vigour index was calculated as mean seedling length × cumulative germination percentage (Abdul Baki and Anderson, 1973).

Data analysis

Collected data from various experiments was subjected to *t*-test using Web Agri Stat Package 2.0 (ICAR-CCARI, Ela, Goa).

Results and discussion

Standardization of seed germination technique is one of the basic steps for mass multiplication of any species. Standardized nursery techniques could help in habitat enrichment activities in species which are endemic or facing conservation issues, whereas for commercial/ potential crops, it helps in providing rootstocks/ propagules for area expansion activities. Andaman Kokum belongs to both the categories as it is endemic and also has potential for cultivation as a novel crop (Bohra et al., 2021). Hence, series of experiments were undertaken to standardize its mass multiplication protocol.

Experiment 1: Effect of removal of seed coat

Effect of removal of seed coat on germination percentage and characteristics was studied. Germination percentage over a period of 120 days showed significant influence of the treatment. After 30 days, no germination was noticed in seeds wherein seed coat was retained (Fig. 1), while 19.05% seeds could germinate when the coat was removed. After 60, 90 and 120 DAS also, this treatment showed significantly higher germination of 89.80, 95.24 and 96.60%, respectively as against 36.73, 74.15 and 82.31%, respectively in seeds wherein seed coat was retained. Presence of seed coat induced dormancy has also been reported in *G. gummi-gutta, G. brasiliensis, etc.* (Joshi et al., 2006; Cardoso et al., 2021).

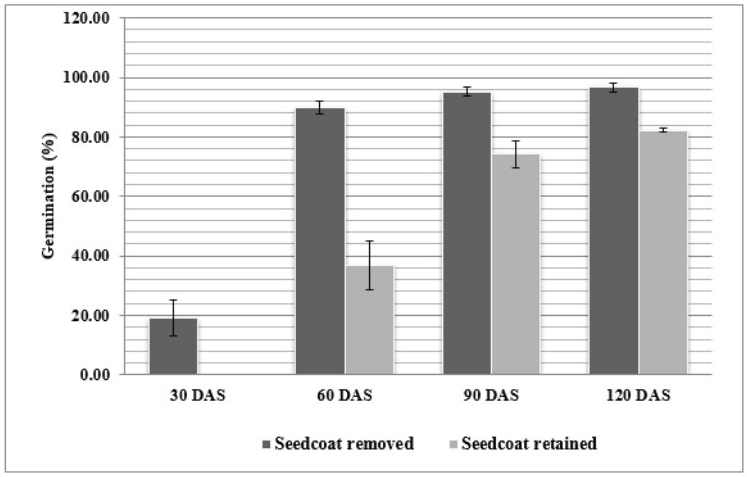
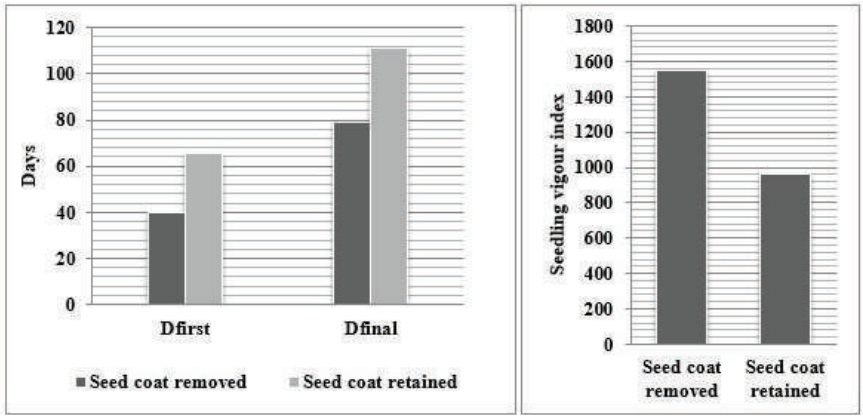


Fig. 1. Effect of seed coat removal on germination percentage over the period of 120 DAS

Removal of seed coat quickened the initiation of germination (Fig. 2a) and about 39.71 days were required in this treatment as against 65.68 days for seeds in which seed coat was retained. This finding is in conformity with earlier report on *G. kola* (Matig et al., 2007). Similarly, the completion of germination (Fig. 2b) was also achieved within 79.33 days when seed coat was removed as against 111.33 days in seeds, where it was not removed. Imbibition of water is the prime step in seed germination process and removal of this barrier is known to quicken the moisture absorbance in the seed (Cardoso et al., 2021) and hence, the results obtained here could be expected. Thick seed coat (testa) has been considered as one of the major hindrances in germination of *Garcinia* species (Liu et al., 2005). Presence of hydrophobic suberin layer has been reported in the seeds of *G. prainiana*, which serves as a mechanical barrier to movement of air, restricts imbibition process and emergence of radicle (Normah et al., 2016). Seed coat removal has been reported to hasten the germination process in *G. gummi-gutta,* wherein Joshi et al. (2006) reported germination time of three weeks as against four months in intact seeds. Removal of seed coat could significantly reduce the mean germination time from 120 days to just 13 days in *G. cowa* (Liu et al., 2005).



(a) (b)

Fig. 2. Effect of seed coat removal on (a) days for first and final germination and (b) seedling vigour index

The *t*-test analysis was carried out for the seedling growth parameters. Highly significant superiority of seedling characteristics such as seedling length (16.14 cm), shoot length (6.68 cm), root length (9.46 cm), number of roots per seedling (18.90), number of leaves per seedling (12.80) and leaf length (5.56 cm) was observed in the treatment in which seed coat was removed. Leaf width was significantly superior in this treatment (1.06 cm) over seeds receiving seed coat retention treatment (0.83 cm) (Table 1, Fig. 3). Length of longest root and petiole length showed no significant differences between the treatments. Seedling vigour index is one of the important seedling characteristic, which could determine the further establishment under field conditions. In present study, higher SVI of 1547.7 was observed in seeds in which seed coat was removed as against 965.25 in treatment wherein seed coat was retained.

Table 1. Seedling morphological parameters as influenced by retention or removal of seed coat in *G. dhanikhariensis*

Parameters	Seed coat removed	Seed coat retained	t- test
Seedling length (cm)	16.135	11.535	**
Shoot length (cm)	6.680	4.970	**
Root length (cm)	9.455	6.415	**
Number of roots per seedling	18.900	9.350	**
Length of longest root (cm)	4.030	2.480	NS
Number of leaves /Seedling	12.800	11.200	**
Leaf length (cm)	5.560	4.140	**
Leaf width (cm)	1.055	0.830	*
Petiole length (cm)	0.205	0.185	NS

NS: non significant; **: significant at 1% level of significance, *: significant at 5% level of significance

As the germination started earlier in the seeds wherein seed coat was removed, the regenerated seedlings got more time to grow than those which germinated later and hence, superior seedling growth characteristics were observed in this treatment. As the SVI is a product of seedling length and percent germination, higher SVI in this treatment could be justified. These findings are in accordance with the earlier reports on endemic wild banana of the islands (Bohra et al., 2020).

Fig. 3. Seedlings obtained from seeds without seed coat (left) and seeds with seed coat intact

Experiment 2: Effect of seed division

Occurrence of *Garcinia* type germination has been reported in *G. dhanikhariensis* (Bohra et al., 2021) and hence, to explore the possibility of use of fragmented seeds for propagation, the present study was carried out. Germination was significantly influenced by division treatment (Fig. 4). In general, fragmented seeds showed higher germination percentage than that observed in intact seeds throughout the study period. As high as 84.35% seeds showed germination at 120 DAS in seed fragmented treatment as against 55.78% germination in intact seeds. Joshi et al. (2006) have reported that even small segments of seeds could give rise to seedlings in Malabar tamarind, which they proposed as a possible strategy for exploiting mammalian frugivory for seed dispersal. However, in the present study, only 7.48% of the fragmented seeds gave rise to two plants, one from each segment, while the intact seed gave single seedling per seed.

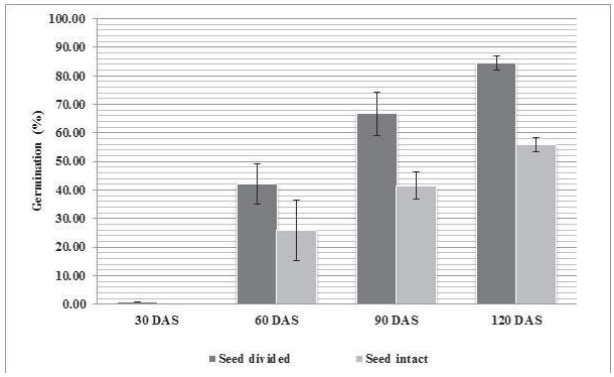


Fig. 4. Effect of seed division on germination percentage over the period of 120 DAS

Results revealed that fragmented seeds germinated (Fig. 5a) earlier (55.97 days) than the intact seeds (67.87 days), while the completion of germination took almost similar time (110.0 and 111.0 days) in both the treatments (Fig. 5b). None of the seedling growth parameters were influenced by the division treatments (Table 2, Fig. 6). Seedling vigour index was found to be higher (700.55) in fragmented seeds than the intact ones (577.71).

Experiment 3: Effect of size of seeds

Effect of size of seed on germination characteristics was studied, which showed significant differences among the treatments. Irrespective of the seed size, no germination was noticed at 30 DAS, which increased with passage of time (Fig. 7). Use of large sized seeds resulted in significantly higher germination of 7.48, 47.62 and 65.99% after 60, 90 and 120 DAS, respectively as against 4.08, 21.09 and 44.22%, respectively in the small seeds.

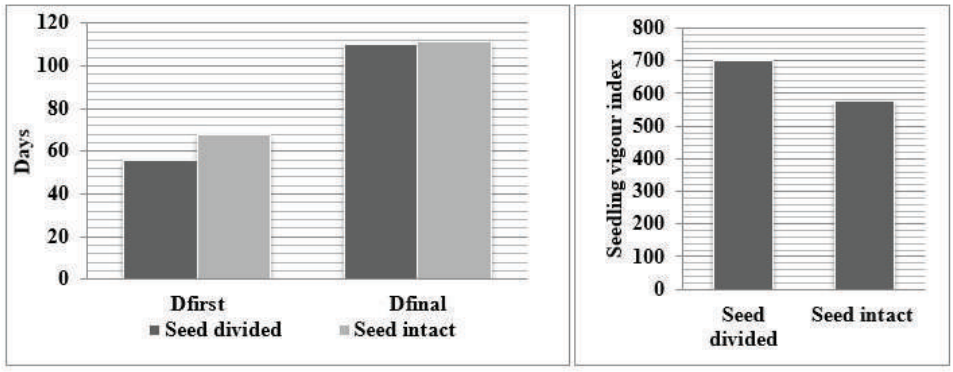


Fig. 5. Effect of seed division on (a) days for first and final germination and (b) seedling vigour index

Table 2. Seedling morphological parameters as influenced by seed division or non-division in *G. dhanikhariensis*

Parameters	Seed fragmented	Seed intact	t- test
Seedling length (cm)	9.715	10.355	NS
Shoot length (cm)	4.785	5.320	NS
Root length (cm)	4.930	5.020	NS
Number of roots per seedling	11.450	9.800	NS
Length of longest root (cm)	2.760	2.280	NS
Number of leaves /Seedling	11.500	10.900	NS
Leaf length (cm)	4.390	4.430	NS
Leaf width (cm)	0.800	0.795	NS
Petiole length (cm)	0.215	0.180	NS

NS: non significant; **: significant at 1% level of significance, *: significant at 5% level of significance

Fig. 6. Seedlings derived from seed segments (left) and intact seeds (right)

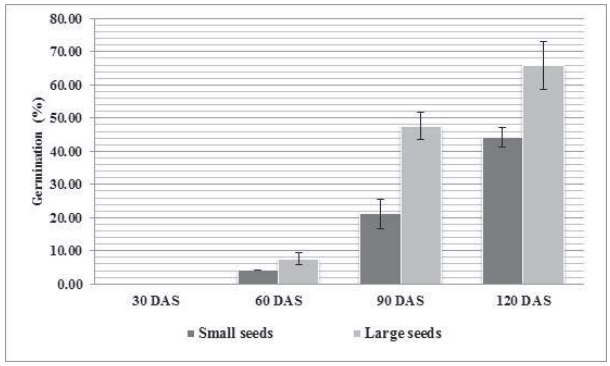


Fig. 7. Effect of seed size on germination percentage over the period of 120 DAS

Results suggested that the initiation (Fig. 8a) and completion (Fig. 8b) of germination process took marginally lesser time in large seeds (84.61 days and 117.33 days, respectively) than that observed in seeds of small size (88.58 days and 118.67 days, respectively). Seedling growth parameters were influenced due to the seed size used for experimentation (Table 3). Use of large sized seeds resulted in highly significant superiority for seedling length (9.76 cm) and root length (5.21 cm), when compared with use of small seeds (7.935 cm and 3.835 cm, respectively). Shoot length (4.55 cm), number of leaves per seedling (10.60) and leaf width (0.76 cm) were significantly superior in seedlings raised from large seeds; whereas other parameters were not influenced by the size of seeds.

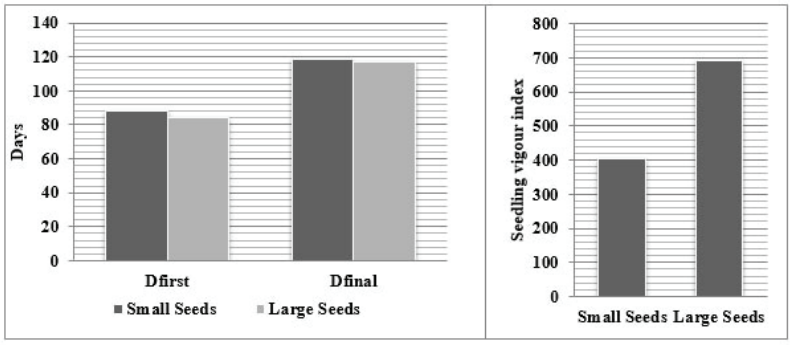


Fig. 8. Effect of seed size on (a) days for first and final germination and (b) seedling vigour index

Table 3. Seedling morphological parameters as influenced by seed size in *G. dhanikhariensis*

Parameters	Small seed	Large seed	t- test
Seedling length (cm)	7.935	9.760	**
Shoot length (cm)	4.115	4.550	*
Root length (cm)	3.835	5.210	**
Number of roots per seedling	6.650	6.300	NS
Length of longest root (cm)	1.495	1.325	NS
Number of leaves /Seedling	9.600	10.600	*
Leaf length (cm)	3.365	3.635	NS
Leaf width (cm)	0.650	0.755	*
Petiole length (cm)	0.155	0.165	NS

NS: non significant; **: significant at 1% level of significance, *: significant at 5% level of significance

SVI was observed to be higher (690.42) in seedlings regenerated from large seeds than those from smaller ones (404.84). Seed size is known to vary within a fruit and also within different genotypes/ collections of a species (Okonkwo et al., 2020). Seed size determines the amount of stored reserves and moisture content available in the seeds (Normah et al., 2016, Florent et al., 2021). Principal component analysis suggested that seed sizes in *G. kola* are positively correlated with germination and growth parameters (Agwu et al., 2018). Similar results have been reported in *G. mangostana* and thus, use of large sized seeds has been recommended for obtaining superior quality seedlings (Florent et al., 2021). Maturity and favourable physiological predisposition of the large seeds might have resulted in improvement of the germination characteristics.

Conclusion

The present study helped in understanding various germination features of this lesser known endemic species. Removal of seed coat before sowing and large sized seeds facilitated improving the germination percentage, seedling vigour and germination characteristics of the regenerated seedlings. Further, use of fragmented seeds improved the germination process apart from improving the possibility of getting two seedlings from each segment. These standardized nursery practices could be of great practical utility for mass multiplication of this endemic species.

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Short Communication

Observations on Weed Flora of Medicinal Significance in Oil Palm Plantations

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Oil palm is one of the important plantation crops grown in Andhra Pradesh state of India. It occupies nearly 1.72 lakh hectare area in coastal parts of the state. In oil palm plantations, inter-culture is not practicable for initial up to four years after planting as the roots are arranged like a mat in the upper soil layer. During rainy season, weed flora naturally germinate in the inter row spaces of oil palm plantation. Medicinal weeds of the Asteraceae, Amaranthaceae and Poaceae families have been reported from such plantations and most of these have been used for treatment of various ailments such as dysentery, wounds and skin diseases (Debabrata et al., 2014).

Weeds are known to have considerable medicinal utility especially in the folk medicines and these plants have been the only source of medicines in remote villages of India, where modern facilities or awareness about the modern medicine is lacking (Pattnaik and Mohapatra, 2010). In this context, a preliminary study was undertaken, where data on weed density and weed dry weight were recorded for estimating the yield potential of medicinally important weed species in oil palm plantations.

The present experimental site was located at Horticultural Research Station (16.81 °N, 81.03 °E) Vijayarai, Eluru District of Andhra Pradesh. Oil palm plantation was established in 2011 with a spacing of 9 m × 9 m × 9 m and the data on weed flora was recorded during 2017. The weeds were germinated in field after the receipt of first monsoon shower in first fortnight of June and the observations at 30 d after germination of weeds were recorded. Later observations were taken at 60, 90 and 120 days after weeds emergence. The data was recorded in 1 m^2 quadrant from three random spots. Weed species were counted, separated and dried in open sun for one week before recording dry weights.

Perusal of data revealed that weed density was higher (113.5) at 30 days after germination. At 120 d after germination, weed density reduced due to death of some plant stands because of inter and intra weed species competition for space and other resources. Dry weight of weeds was very low at 30 days after weed emergence because they were slender with very less dry-matter accumulation. With increase in duration, the dry weight increased up to 90 days after germination. Dry weight of weeds (168.6 g) was recorded the maximum at 90 days after germination. Weed dry weight reduced at 120 days after germination due to leaf fall and also diversion of dry matter to seed production. In India, more than 43% of the total flowering plants are reported to be of medicinal importance (Puspagandhan, 1995 and Raut et al., 2012). In 3-4 months duration, up to 1,079 kg/ha of weed dry weight was harvested, which has medicinal values. These observations could open up opportunities for identifying potential weed species of medicinal importance in the existing oil palm plantations.

Table 1. Information on weeds and their medicinal uses

Name	Plant part used	Medicinal importance
Ageratum conyzoides	Whole plant	Kidney stones, cuts, Epilepsy and wounds
Commelina benghalensis	Whole plant	Leprosy, sore throat, opthalmia, burns, pain and inflammation and also used as emollient and laxative.
Convolvulus arvensis	Roots	Diuretic, laxative and purgative
Cynodon dactylon	Leaf and Root	Nasal bleeding and Dysentery and astringent
Cyperus rotundus	Whole plant	Epilepsy, Dysentery anti inflammatory, pain killer and anti oxidant
Spermacoce hispida	Whole plant	Ring worm and Eczema
Tridax procumbens	Leaf	Wounds

Table 2. Weed species density/m^2 and dry weight at different time intervals during *kharif* in oil palm plantation

Weed species	Days after weeds germination								Total weed dry weight (kg/ha)
	30		60		90		120		
	Mean weed spp. density/m^2	Mean weed dry weight (g /m^2)	Mean weed spp. density/ m^2	Mean weed dry weight (g /m^2)	Mean weed spp. density/ m^2	Mean weed dry weight (g /m^2)	Mean weed spp. density/ m^2	Mean weed dry weight (g /m^2)	
Ageratum conyzoides	27.0	5.2	24.0	11.8	30.0	15.6	15.0	17.8	126.0
Commelina benghalensis	21.5	9.4	25.5	20.2	17.0	25.6	13.0	30.1	213.3
Convolvulus arvensis	5.0	1.5	3.0	2.6	4.0	4.2	6.0	7.8	40.3
Cynodon dactylon	15.0	1.0	8.5	2.2	8.0	15.0	5.0	9.0	68.0
Cyperus rotundus	30.0	4.8	10.5	12.0	16.0	26.0	8.0	15.0	144.5
Spermacoce hispida	9.0	5.9	6.0	17.2	8.0	22.0	11.0	20.8	164.8
Tridax procumbens	6.0	8.8	4.0	30.2	5.0	60.2	3.0	30.0	323.0
Total	**113.5**	**36.6**	**81.5**	**96.2**	**88.0**	**168.6**	**61.0**	**130.5**	**1079.0**

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