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Noni (*Morinda citrifolia L.*) production technologies – A global review

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Abstract : *Morinda citrifolia* L., popularly known as Indian Noni or Indian mulberry belonging to family *Rubiaceae,* is an ever green small tree which flowers and fruits throughout the year and naturally spreads in the tropical regions of the world. The species is generally found from sea level to 400m above m.s.l, although it adapts better to coastal regions. Noni, often found growing along lava flows is an underutilized plant and not known to many people including botanists in spite of the fact that this miracle plant has more than 150 nutraceuticals which are useful for health and wellness of people. In India it is widely grown under natural conditions in Andaman and Nicobar Islands. It is seen throughout the coastal region along fences and road sides due to its wider adaptability to hardy environment. In the main land of India, it is found along the coastal areas of Kerala, Karnataka, Tamil Nadu and many other places. Systematic cultivation in large area is very much limited and the bulk of the fruit supply comes from natural wild growth.

A global review made has indicated that Noni is relatively easy to propagate from seeds. Stem, or rooted cuttings and air layering need to be standardized. The preferred methods of propagation are by seeds and cuttings made from stem verticals. Micro propagation using tissue culture is the other possibility of multiplication of planting material. Seed germination technique and use of hormones for rooting have been attempted but large scale technology standardization is yet to be done. Nutrient and water management studies are in infant stage only. Organic cultivation is advocated but still cost effective technology development is also in infant stage. Noni growing in natural ecosystem did not have much pest problems, but became susceptible to a wide spectrum of insect pests, pathogens and nematodes when domesticated in a monoculture as experienced in Hawaii and other Pacific Islands. The production technologies for this crop including widening germplasm, developing high yielding varieties and hybrids, standardization of nursery techniques, nutrient and water management, cropping system, pruning and training, standardization of harvest and post harvest technologies need to be addressed for its successful cultivation.

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Introduction

Noni (Morinda citrifolia L.) loves tropical humid island/coastal/marine climate for its natural growth and development including of flowering and fruit setting round the year (Nelson, 2002**).** It is an ever green small tree bearing flowers and fruits throughout the year and belongs to family ***Rubiaceae***. It grows in tropical regions of the world from sea level to 400 m above m.s.l, although it adapts better to coastal regions. The tropical humid climate is very much suitable for its cultivation (Singh *et al*., 2005). It is believed that about 2000 years ago, Polynesian sailors took this plant from South East Asia and used for many centuries as food and medicine. It is often found growing along lava flows. Bulk of the crop is wild growing in hardy environment and adopts to acidic, alkaline and saline soil conditions (Cambie and Ash, 1994). The genus *Morinda* is present worldwide predominantly in tropical countries. It occurs in Africa, Australia, Barbados, Cambodia, Caribbean, Cayman Islands, Cuba, Dominican Republic, El Salvador, Fiji, Florida, French West Indies, Guadeloupe, Guam, Haiti, Hawaii, India, Jamaica, Java, Laos, Malaysia, Marquesas Islands, Philippines, Polynesia, Puerto Rico, Raratonga, Samoa, Seychelles, Solomon Islands, Southeast Asia, St. Croix, Surinam, Tahiti, Thailand, Tonga, Trinidad and Tobago and Vietnam (Rethinam and Sivaraman, 2007).

It is seen throughout the coastal region along fences and road sides due to its wider adaptability to hardy environment. In the main land of India, it is found along the coastal areas of Kerala, Karnataka, Tamil Nadu and many other places. The fruits collected from these wild trees are used for medicinal purposes and as food supplement. Recently, farmers in India have started taking up the cultivation of Noni as irrigated crop since this crop has the capacity to change the socio economic status of the farmers through its high yield and returns. In Andaman and Nicobar Islands after Tsunami, this crop has been projected as livelyhood security crop for Bay Islands (Srivastava and Singh, 2008) and all efforts are being made to popularise this crop as small holders crop (Subash Chand *et al*., 2008). The economic appraisal had also been made to show the profitability of this crop (Subash Chand *et a*l., 2007) and technological interventions for enhancing the productivity have been identified and implemented (Srivastava and Singh, 2007). The agro climatic requirements and production potential for Noni were also worked out for Andaman and Nicobar Islands (Singh et al 2006).

Morton (1992) reported that the fruits of this tree have a history of use in the pharmacopoeias of pharmaceutical units in the Universe because it has more than 150 nutraceuticals, several vitamins, minerals, micro and macro nutrients that help the body in various ways from cellular level to organ level.

Noni is one of the important traditional folk medicinal plants that has been used for over 2000 years in Polynesia. It is reported to have a broad range of therapeutic and nutritional value. The ancestors of Polynesians are believed to have brought many plants with them, as they migrated from Southeast Asia about 2000 years ago (Gerlach, 1996; Tabrah and Eveleth, 1966). Of the 12 most common plants they brought, Noni was the second most popular plant used in herbal remedies to treat various common diseases and to maintain overall good health (Krauss, 1993; Gerlach, 1996). While a lot of information are available on medicinal and nutraceuticals effect of Noni, not much literature are available on cultivation side. More than 200 commercial entities across the globe sell these products and enjoy enormous market share. A Review of agricultural research, nutritional and therapeutic properties of the Noni fruit (*Morinda citrifolia* L. was made by Mathivanan *et al.* (2005), Blanco *et al.* (2006), Rethinam and Sivaraman (2007) and Rethinam (2008).

The importance of Noni in the livelihood security, economic appraisal as well as popularization on Noni is reported by Subash Chand *et al.*, 2007 and Subash Chand *et al.*, 2008. It is now cultivated in Andhra Pradesh, Andaman and Nicobar Islands, Karnataka, Kerala, Chattisgarh, Gujarat, Meghalaya, Madhya Pradesh, Orissa, Punjab, Rajasthan, Tamil Nadu and West Bengal covering more than 1,850 acres under organic contract farming.

2. Noni Production

2.1. Germplasm and Varieties

The genus *Morinda* is distributed world over and the presence of as many as 80 different species are reported (Johanssen, 1994; Mc Clatch, 2002). Johansson (1994) recorded presence of 19 species in New Caledona. Mabberley (1997) studied the genus *Morinda* extensively and renamed *Morinda tinctoria* as *Morinda pubescens*. Survey of *Morinda* in South India indicated that 12 different species or varieties of *Morinda* are distributed throughout Tamil Nadu and Kerala (Singh *et al.*, 2006a). Two accessions viz., IC 524021 & IC 524022 tolerant to salty soils and salt spray were identified (Veena Gupta *et al.*, 2007). The genetic diversity and analysis of *Morinda citrifolia* L. was done by Singh *et al.*, (2008a).

The species *M. tinctoria* is present abundantly in most parts of Tamil Nadu and in some parts of Kerala. *M. citrifolia* is not recorded in the study area of Tamil Nadu where as, it is widely found across Andaman and Nicobar islands. The accessions collected in all the14 localities of Andaman and Nicobar Islands were fingerprinted using 48 random amplified polymorphic

DNA (RAPD) markers (Singh *et al*., 2008b). This crop is sparsely distributed in most parts of Kerala especially coastal region and also in the Mangalore area of Karnataka (Veena Gupta and Sharma, 2008). Recently an unidentified *Morinda* species with large and leathery leaves in the Dhandakaranya forest area of Malkanagiri district in Orissa was reported (Singh *et al*., 2007a). The presence of *Morinda sp*. was also reported in the semi –arid ecosystem of Gujarat (Singh *et al*., 2008c).

As far as varieties are concerned world over, the situation remains same and no variety has been released so far. Seeds from the existing wild plants are collected, nurseries are raised and seedlings are distributed for planting. It is time now for the selection, evaluation and identification of high yielding varieties from available germplasm.

Morphological Description

Morinda citrifolia L., a perennial bush or tree growing to a height of 3 to 10 m tall with a girth of 13 cm or more, has eliptical leaves and small tubular white flowers grouped together and inserted on a peduncle. The petioled leaves ring like mark on the stalks and the corolla is greenish white (Elkins, 1997; Dixon *et al*., 1999; Cardon, 2003). Sapwood is yellow brown and soft. The bark is grey or brown, smooth to slightly rough.

2.2. Planting Material Production

Noni is relatively easy to propagate from seeds, stem or rooted cuttings and air layering. The preferred methods of propagation are by seeds and cuttings made from stem verticals (Nelson, 2001). It was also found out that vegetative propagated planting materials maintain uniformity and productivity (Wagner *et al*., 1999; Nelson, 2001; Hartmann *et al*., 2002). Micro propagation using tissue culture is the other possibility of multiplication of planting material.

2.2.1. Seeds

It is the best to collect *Morinda* fruits after they are fallen from the tree for seed purpose (Nelson, 2005). Seeds are extracted from the fruits and sowing can be done immediately after extraction. The seeds possess woody water coat which enables the germination (Wagner *et al*., 1999). The seed requires scarification to reduce the time to germinate and to maintain uniformity. To scarify, the seed is to cut, scratch or soften its outer coat to allow ready penetration of water and air. Unscarified seed usually takes at least 60 days and usually much longer (up to six months or more) for germination than scarified seed (3 to 4 weeks) depending upon the condition. Scarifying can be done by a blending machine, nail cutter etc., (Nelson, 2005). Seeds can

be planted in the seedling flats, trays, or directly in the light medium containers immediately after extraction from fruits and requires hot and wet conditions for optimum germination. Under green house condition, raising seedling in the warmest part of land provides better environment for better seed germination (Singh *et. al.*, 2007b). Noni seed has dormancy due to hard seed coat (water repellant) thus delaying germination and needs 43 days to germinate (Mathivanan *et al.*, 2005). Seeds after drying in shade for 3 or 4 days can be stored in air-tight containers at room temperature. Preliminary seed storage experiments have shown that seeds have low viability but are orthodox in nature (Veena Gupta and Sharma, 2008). However, detailed storage studies are yet to be taken up (Singh *et al.*, 2007b). The treatment with hot water at 40°C for a period of 24 h and a treatment with sulphuric acid at 50% concentration for 5 minutes were able to overcome the seed dormancy (Ponnaiyan and Vezhavendan, 2005). The highest germination of seeds was obtained where the seeds were nicked and then treated with gibberllic acid (GA) at 1000ppm for a period of 24 h (Ponnaiyan and Vezhavendan, 2005a). Seed treatment with hot water at 40°C combined with sea weed (*Ascophyllum nodosum*) extract Biozyme and the treatment with sulphuric acid 50% for 5 minute combined with Biozyme were able to break seed dormancy as well as give better health and vigour to the germinated seedlings (Muthu and Mathan, 2006). Seed germination studies of soaking seeds for 24 h with gibberllic acid (GA) at 800 ppm increased the germination percentage to 91.06 as against mere water treatment (51.4). The interaction of seed soaking and treatment by GA 800 ppm increased high percentage of seedlings and number of leaves (Singh and Rai, 2005 and Singh *et al.*, 2007c). Pre treatment of seeds with $NaHClO_3$ (5% available chlorine for 30 minutes) increased the germination up to 84%. However, the growth parameters were good in KNO_3 (150 ppm) (Sudha and Singh, 2007). Seasonal influence on seed germination was also reported by Singh *et al.* (2007d).

2.2.2. Vegetative Propagation

The importance of vegetative propagation is to get the best planting materials with the highest genetic quality (Nanda, 1970; Wright, 1975; Hartman and Kester, 1983). Singh and Rai (2005) and Singh *et al.* (2007c) have suggested the use of growth regulators like Naphthalene acetic acid (NAA), Indole butyric acid (IBA) for quick and better rooting in vegetative propagation. Vertical and lateral stem cuttings with sap flow at the time of cutting with vigorous growing points are the best suited for vegetative propagation. Vezhavedan and Ponnaiyan (2005) compared different types of cuttings *viz.*, tip, semi hard, and hard wood cuttings with different number of nodes (2, 3 and 4) and reported that hard wood cuttings with 4 nodes performed better and gave more success percentage and healthy planting

materials. It is better to avoid the cuttings without sap flow for vegetative propagation. It is also better to avoid hollow stem cuttings since the percentage of recoverable seedlings are low and take 5 days more than non hollow cuttings which took only 15 days and survival was 79.4% (Singh *et al.*, 2007c). In another study, Sudha and Singh (2007a) found that both in hollow and non hollow cuttings, IBA (6000 ppm) showed significantly higher rooting values than IBA (2000 ppm). Root initiation and percentage of sprouting were higher in non-hollow cuttings compared to hollow cuttings. In a preliminary study with single, double and triple node cuttings dipped with cow dung solution and IBA solution, encouraging results were obtained at Centre for Organic Indian Noni (COIN) which should be further studied.

Root hormones may help to promote the vegetative growth of cuttings. Soaking of the cuttings with 4000 ppm of IAA and NAA separately and in combination promoted root and shoot growth establishment besides increasing percentage of rooting, length and number of roots, length of the longest primary root (Singh *et al.*, 2007c). At high level of auxins, reduced root growth is recorded due to nutritional imbalances (Spiegel, 1954; Nanda *et al.*, 1974). The sprouting of cuttings under closed poly house was earlier (15 days) and survival of cuttings was 83.3% while in covered poly house it was 20 days with 60 % sprouting at 33.5ºC and 80% RH. The vegetatively propagated plants under open grew faster and put up 4 branches in 32 days and reached reproductive stage (Singh *et al.*, 2007a). Since vegetative propagated materials will have uniformity, this area of research needs immediate attention.

2.2.3. Micro propagation / Tissue Culture

The varying effects of cytokinin and auxin combinations were studied on *Morinda citrifolia* for effective *in vitro* induction of shoots from nodal explants and it clearly indicated that irrespective of basal media used (either MS or WP), it is the hormonal combinations which are very vital for the *in vitro* response, BAP alone for shoot initiation, kinetin along with BAP for multiple shoot formation. Calli with roots produce shoot(s) in BAP with IBA medium (Antony Selvaraj *et al.*, 2006). Further studies showed that the rooted plants have established well with 95-98.5% survival under green house conditions while hardening. Further, better growth with zero per cent mortality was observed at nursery stage (Subramani, 2006). Now, micro propagated plants have gone for field test. Presently cell culture study is being carried out (Subramani *et al.*, 2007 b). Studies on the cell suspension culture of *Morinda citrifolia* L. are under progress (Subramani, 2008). Basseth and Tramper (1995) have reported the use of non conventional media in *Morinda citrifolia* L. cell culture.

2.3. Plantation management

Information on plantation management like pruning and training and canopy management are lacking but the preliminary observational studies gave encouraging results which should be perused further.

2.3.1. Planting season

Ideal season for planting is from June to October. It can also be planted during January to March.

2.3.2. Pitting for planting

A pit of 0.6 x 0.6 x 0.6 m is dug a few days before planting and allowed to dry. The fit is filled with a mixture of top soil, 2 kg of compost or Farm Yard Manure, 2 kg of vermicompost and 1 kg of Neem cake along with *Pseudomonas* sp., *Trichoderma*, VAM and *Azosprillum*. Now the pit is ready for planting. Irrigation channels are to be formed or drip systems need to be installed (Rethinam, 2008; Singh *et al*., 2009).

2.3.3. Spacing

The recommended spacing is 4 x 4 m with a plant population of 700 plants per hectare (Singh *et al*., 2009).

2.3.4. Weed management

It is suggested to take up proper weed management till the plants are established and later adopt repeated mulching along with inter cropping with indigo, cow pea, horse gram or fodder crop (Peter, 2007). In Hawaii, weeds like Guinea grass and sensitive grass (*Mimosa pudica*) compete with Noni. It is also attacked by dodder (*Cuscuta sandwihensis*), a parasitic weed. Manual removal of weeds from the field is recommended (Nelson, 2001).

2.4. Nutrient management

Noni has a deep tap root system and extensive surface feeding root system. The tree may not compete well in a landscape with plants that have aggressive, surface-feeding lateral roots, like grasses. So it can absorb the fertilizers on the surface which is racked well coupled with irrigation. A fertilizer application of 10-20-20 kg NPK/ha will be sufficient (Nelson, 2005). The strategy to apply nutrients is similar to that of other fruit crops like citrus and coffee. Young non-fruiting plants are encouraged to produce lush vegetative growth with balanced fertilizers (14:14:14 or 16:16:16), whereas more mature and fruit bearing trees are encouraged to produce many large fruits by application of high phosphorus complex fertilizers such as 10:20:20 or 10: 45: 19 (Singh *et al*., 2009). Since organic cultivation is promoted in India, the

above nutrient dose may be given in the organic form from the approved organic sources. A tentative manurial application for Organic Noni Cultivation is suggested as shown below till the technology is developed scientifically.

At 6th month	2 kg. vermi compost or compost + ½ kg Neem cake.
At 12th month	2kg. vermi compost or compost + 1 kg neem cake + bio fertilizers.
At 18th month	2kg. vermi compost + 1kg neem cake
At 24th month	5kg. vermi compost + 5kg wood ash + 1kg neem cake + Bio fertilizers including *Trichoderma viride, Azatobacter,* Phosphobacteria, *Psuedomonas sp*. etc.
After 24 months	The above dose is repeated every 6 months (Rethinam and Sivaraman, 2007)

Noni is reported to display abnormal foliar symptoms for nitrogen, iron and phosphorous deficiencies like inter veinal chlorosis, scorching of leaf margins, leaf curling, purpling and marginal necrosis indicating various deficiency symptoms. The leaf nutrient content, uptake of nutrients at different stages of the crop, critical level of nutrients *etc.*, are to be assessed.

2.5. Irrigation management

Nelson (2005) reported that the plant can thrive well with moderate irrigation and can survive extended drought once established and matured. In dry condition when the plants are less than two to three years, irrigation once or more per week with 10 gallons of water per plant and for older plant more water with less frequency were suggested. However, over watering is not recommended. There is not much literature available on irrigation and quantity of water to be irrigated. However, in India irrigated Noni is advocated for getting better yield and hence an adhoc recommendation has been given as shown below.

Planting pits are to be irrigated completely to wet the soil for three consecutive days. After planting, drip irrigation everyday for half an hour to supply 4 liters of water/plant/day for first three months, 8 litres of water/plant/day for next nine months and there after 12 litres/plant/day up to 2 years are suggested. For adult plants after 2 years, 15 litres/plant/day are suggested. With good mulching at the base, this quantity of water should be adequate depending upon the climate, soil conditions and canopy growth (Rethinam, 2008).

2.6. Green manure crops

Growing green manure crops like sunhemp, daincha, indigo and green leaf manure crops like cassia, glyricidia, agathi (*Sesbania sp.*), subosavi, kubdool etc., can produce adequate biomass which can be recycled. Growing green manure crops in the planted area for a period of 2 to 3 months will provide shade as well as bio mass besides creating an ideal micro climate to the plants during summer. These crops also fix atmospheric nitrogen. If these are cut and spread at the base, it becomes mulching. Mulching will reduce evaporation losses, prevent weed growth and conserve moisture. Up on slow decomposition in the basin, the physicochemical and biological properties of soil improve and water holding capacity and the over all soil health increase. (Rethinam 2008).

2.7. Inter and mixed crops

Eventhough systematic studies on inter/mixed cropping have not been conducted, there is lot of scope for such crops to increase the unit income in unit time and also to provide biomass for organic recycling. Growing greengram, blackgram, cowpea, horse gram, groundnut, rain fed millets *etc.* during monsoon season utilizing the available moisture are suggested. In case if irrigation facilities can be extended to the intercrops, the interspaces can be used for growing vegetables, flowers, medicinal plants, fodder crops etc., which have good market potential in that area. Noni crop can be well fitted in coconut and arecanut plantation as mixed crop. In mango and cashew plantations also, noni can be planted in between the rows. However, crops like banana, sugarcane, papaya, rose and other crops which require more water should be avoided as they may promote the nematode problem (Rethinam, 2008).

3. Harvest and Post Harvest Processing

Noni bears fruits in about nine months to one year after planting. Harvesting can be done throughout the year although there are seasonal patterns in flowering and fruiting (Nelson, 2001 and 2003). However, the commercial harvest can be done from 20-24 months after planting and it is suggested to remove all flower buds up to one year and six months and then allow for flowering so that the plant can be studied. Depending on the postharvest technology programme adopted, the fruits may be harvested at different stages of development. After harvesting, the fruit ripens within a week at ambient temperature and also because of its short storage life, the fruits cannot be transported to the distant places even with in the country. To overcome this problem of harvesting, the fruits with pedicel help to maintain

better quality and market acceptability as the highest spoilage of fruits was observed in fruits harvested without pedicel (Fruits with pedicel performed well in terms of keeping quality, ascorbic acid and TSS). Among the accessions, SPG-2 recorded minimum loss of weight (2.90%) followed by Pbay-7 (3.74 %) in 9 days during storage (Singh *et al*., 2007e). Nonetheless, most of the processors buy the fruits harvested at the ''hard white'' stage for juice production, as the fruits become soft too quickly once this stage is reached (Nelson, 2001, 2003). The change from stage 4 to stage 5 occurs very quickly (a few hours) and the pulp practically liquefies and turns from green to white, as well as develops the characteristic butyric smell. The fruits are individually selected on the tree and harvested by hand. At the ''hard white'' stage, they are able to withstand transportation in baskets or containers, and exposure of the fruits to light or high temperatures immediately after harvest does not affect their overall quality. Before processing, fruits are ripened at room temperature for a day or more, depending on the end product (tea, juice, pulp, dietetic products, etc. (Nelson, 2003). Recently a protocol for packaging and shipment of noni fruits has been developed which needs further scaling up (Singh *et al*., 2008d).

4. Yield

Morinda citrifolia is a perennial bush and it is possible to find fruits at different stages of maturity on the same plant at the same time. Some producers choose not to harvest in the first year, and they prune in order to let the bush grow stronger. In Hawaii, Noni fruits are harvested throughout the year, although there are seasonal patterns in flowering and fruit bearing (meteorological factors, fumigation, and irrigation) (Nelson, 2001; 2003). In India, the plants are allowed to grow for two years without any side growth by periodical pruning so as to make the plant sturdy. It is reported that Noni plant is capable of giving yield up to 250-300 kg after 7-8 years of planting under better conditions of cultivation. However, in the initial stages, yield may range from 30-40 kg per plant and the well grown tree will produce an average of 90 – 100 kg. It is also reported that the productivity of the trees will be up to 40-50 years and the harvest can be done 6 - 7 times in a year (Nelson, 2001; 2003).

In Hawaii, Noni fruits are usually harvested two or three times per month, although fruit production is lower during winter. With a density of 638 plants per hectare with good soil fertility, drainage and irrigation and appropriate pest, disease and weed control, along with an appropriate fertilization plan, it is possible to obtain yield from 7 tonnes/ha/year in the second year after planting to approximately 70 tonnes/ha/year after the fifth year (Nelson,

2001; 2003). With a juice extraction rate of approximately 50% (w/w), one hectare can thus yield around 35 tonnes of juice. However, many factors may affect these yields and most producers do not obtain such good results because of diseases or poor agricultural practices. In Hawaii, an average annual yield of 50 tonnes/ha is generally attained (Nelson, 2001 and 2003).

The cost of cultivation under Andaman conditions is worked out to Rs.42,425 per ha (Singh *et al.*, 2007f). It was observed that five years old plantations in Bay Islands gave a gross income of Rs.4,68,750 /- with a net income of Rs.2,00,731/- (Subhash Chand *et al.*, 2007).

5. Storage

Ripened fruits can be stored up to 9 days and juice need to be extracted before 9 days of maturity (Peter, 2007). Singh *et al.* (2007e) studied the effect of storage of different quality parameters and reported that the physiological loss of weight was the highest (28.26%) and spoilage was higher in non pedicillate fruits. Fruits harvested with pedicel had maximum ascorbic acid and less spoilage.

6. Future research programmes

The overall scanning of available literature has shown that very little research work has been done on Noni on the production of crop including selection of varieties while bulk of the research have been done on the pharmaceuticals and health aspects of Noni and its products. Keeping in view of its immense potential for this under utilized crop, the needs are outlined for future research.

6.1. Widening genetic variability

Collection of indigenous and exotic germplasm

1. The existing variability of the crop in India including Andaman and Nicobar Islands have to be collected, conserved and catalogued at two places viz., at Andaman and Nicobar Islands and World Noni Research Foundation.

2. Germplasm has to be collected from South East Asian as well as Pacific Countries and conserved for their evaluation and breeding programme. (collections to be made from Thailand, Malaysia, Indonesia, Sri Lanka, Myanmar, Maldives, Tahiti, Marshall Islands, Kiribati, Samoa, Federated Micronesia, Brazil *etc.*).

3. The germplasms have to be evaluated for desirable/promising traits and catalogued for using in the breeding programmes.

6.2. Noni crop improvement

Breeding for high yielding varieties and hybrids through conventional breeding and biotech approaches

1. Evaluation through selection of varieties using the available local germplasm to start with and later with exotic germplasm
2. Selection of good plant types and multiplication of the planting material like Noni selections 1, 2, 3, NS-4 *etc*.
3. Developing hybrids using the germplasm based on their desirable traits.
4. Attempting inter specific hybridization.
5. Biotech approach to develop new varieties.

6.3. Standardization of nursery techniques

1. Standardisation of nursery techniques using seeds
2. Standardisation of vegetative propagation using stem cuttings (hard/ semi hard/tip cutting)
3. Propagation through airlayering and cuttings using growth regulators.
4. Micro propagation for mass multiplication

6.4. Development of sustainable production technology

1. Standardization of planting geometry and plant canopy architecture.
2. High density cropping system.
3. Standardization of appropriate dose of nutrients for *Morinda citrifolia L.* through organic sources.
4. Developing drip irrigation scheduling and moisture conservation techniques.
5. Developing DRIS norms for recommending fertilizer/manure application.
6. Root development studies at various stages of Noni crop growth right from seedling stage.
7. Studies on the nutrient deficiency symptoms for the major, secondary and micronutrients.
8. Assessment of uptake of nutrients and critical leaf nutrients of Noni.
9. Evaluation of different organic sources on the growth and yield which are cost effective for different agro climatic zones.
10. Evaluation of the role of micro nutrients.

6.5. Noni based cropping systems

Inter cropping with annual crops, medicinal and aromatic crops, vegetables.

6.6. Noni based mixed farming system –

Growing grasses and rearing animals and recycling biomass to the plantations.

6.7. Evaluation of *Morinda citrifolia,* L. in challenged environment - viz., marshy areas, waste land, drought situations, saline/alkaline soils, sloppy land etc., and developing suitable management practices

6.8. Development of integrated pest and disease management techniques

1. Identification of insect pests, pathogens and nematodes of *M. citrifolia* through filed survey
2. Evolving suitable biocontrol methods for economically important pests and diseases.
3. Evaluation of organic and botanical insecticides including bio pesticides
4. Standardization of IPM packages against major pests and diseases

6.9. Harvest and post harvest Studies

1. Development of pre and post harvest technologies including maturity indices.
2. Storage, packaging including cold storage, transport, juice extraction, product development, diversification and shelf life studies.
3. Possibilities of using the extract of various parts of Noni as Noni based plant products for management of pests and diseases as well as for growth promoting properties
4. Exploring the possibilities of using the by product after extraction of juice for cattle, poultry and fish feed
5. Enriching functional foods with Noni fruit juice for infant foods and food for convalescents.

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Genetic resources of Noni (*Morinda citrifolia L.*) - Conservation efforts at NBPGR

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Key words : *Morinda citrifolia* - Noni – Germplasm – collection - conservation.

Abstract : Noni (*Morinda citrifolia* L.), belonging to family Rubiaceae, is a well known Polynesian folk remedy that has been rediscovered as the 'Modern Day Sanjivini' because of its diverse medicinal properties. Visualizing the importance of this highly nutritive horticultural crop as a source of income and for elevating the socio-economic status of the farmers, NBPGR initiated collection and conservation of the seed germplasm of this crop. Germplasm characterization was done for various fruit and seed characters. Preliminary seed storage experiments have shown that seeds are orthodox in nature but have low viability.

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Introduction

Morinda citrifolia L., commonly called as Noni, belongs to family Rubiaceae comprising eighty species primarily originated in the old world tropical regions (Smith, 1998). It is found growing profoundly in rocky terrains, fertile lowlands and sandy areas in most of the island terrains of the South Pacific of Tahiti, Hawaii, Malaysia, Indonesia, Taiwan, Philippines, Vietnam, India, Africa and West Indies. In India, it is found in Western Ghats, Rajasthan, Gujarat, Andhra Pradesh, Assam and Bihar. It is probably native to Maritime forests of Andaman and Nicobar Islands (India).

Materials and Methods

Germplasm collection

Eight species are reported to occur in India of which two are synonyms (Table 1). A small tree with straight trunk having both vine and small tree type plants were observed throughout the coastal line of Kerala. Leaves are broadly elliptic, bright green and glabrous. Flowers are white with dense ovoid heads. Fresh fruits (syncarpous) are ovoid, weighing 25.86-33.35 g/ fruit and glossy. Around 50-80 seeds were present in a single fruit with 100 seed weight of 2.20-2.32 g. Seeds are compressed, elliptical in shape and winged on edges with a prominent air sac. In addition to Noni, collection of

other two species was also done. A comparative account of mature fruits and seeds was undertaken (Table 3).

Protein estimation

For SDS-PAGE of the total seed proteins, 0.1 g of the fine defatted seed powder was added to 0.3ml of tris-glycine buffer and left overnight at 10°C. Next day, the samples were centrifuged at 10,000 rpm for 15 minutes. Forty ml of the protein samples along with equal amount of working sample buffer with pyronin-G dye were hydrolyzed in boiling water for 2-5 minutes, cooled and loaded in wells with micropipettes. The gel was run initially at 30 amps with final running at 50 amps till the tracking dye reached bottom of the gel. The gel was removed and fixed in 15% TCA for 16 h, washed with distilled water and stained with 2% Coomassie brilliant blue for 4-5 h. The gel was scored after washing in distilled water.

Results and Discussion

During the exploration trip to the coastal areas of Kerala (Thrissur and Cochin) including Vadanapalli Beach, Nattaka Beach, Fort Cochin (old fort reserve areas) and Cherai beach, a total of seventeen accessions were collected and conserved at the National Gene Bank (Table 2). *Morinda tomentosa* was collected from the Satgutti forests, Belgaum (Karnataka) whereas *Morinda pubescans* was collected from the vetal forest area near Pune (Mahatrastra). The fresh fruits collected were air dried and then seeds were extracted. The moisture content and germination studies were done as per ISTA standards. The various treatments (acid scarification, hot water treatment and chipping the seed) were given to enhance the germination but only 10% success was observed. The seeds with 5-7% moisture content were sealed in specially designed aluminum foil pouches and finally conserved in National GeneBank at -20°C. At present the NGB has a total of thirty one accessions of Noni and of the earlier collected accessions, IC 524021 and IC 524022 were registered for salinity resistance and high nutrient value at NBPGR (Gupta *et al.,* 2007)

Table 1 : Genetic Diversity in Morinda species reported from India

Name of the Species	Distribution	Major usage
Morinda angustifolia Roxb.	Eastern Himalayas, Assam, Bihar, Orissa and Andhra Pradesh	Roots yield yellow dye used for coloring cotton yarn
Morinda citrifolia Linn.	Cultivated throughout India, occurs wild in West Coast and Andaman and Nicobar Islands	Used in various medicinal preparations
Morinda coreia Buch & Ham.syn *M. tinctoria* Roxb.	Dry forest area	Wood for making plates, dishes and toys
Morinda umbellata Linn.	Khasi hills (Meghalaya), Bihar and Deccan peninsula	Root bark for dyeing
Morinda bracteata Roxb. Syn *M. citrifolia* var. *bracteata*	Coastal forests of Bengal	Roots for dyeing
Morinda tomentosa Heyne ex Roth. Syn *M. tinctoria* var. *tomentosa*	Throughout Northern India and Deccan peninsula	Fruits are eaten; root bark yields a red dye and wood used for making plates, dishes and toys.

Seed protein profile

The seed protein analysis by SDS-PAGE is an effective method of revealing the difference and similarity among various accessions and can be of help in ascertaining the diversity / polymorphism in the germplasm conserved. The dendogram pattern of the total proteins studied revealed that all the accessions under present study showed 54% similarity. This shows that Noni germplasm thus collected has very little diversity. Therefore, there is need to collect more germplasm from other areas like Andaman-Nicobar Islands and coastal areas of Thrivananthapurum, Kerala.

Fig. 1 : SDS-PAGE protein profile of the 12 accessions of Noni

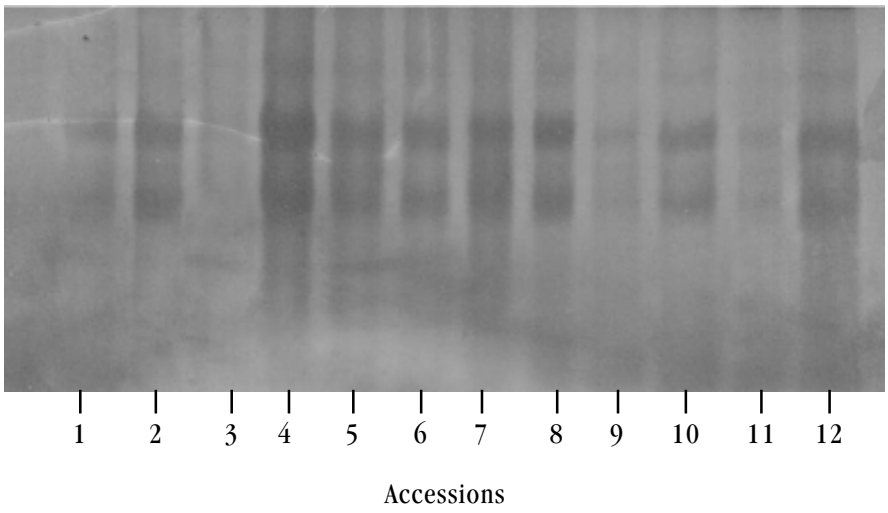


Fig. 2 : Dendogram showing similarity Index in 12 accessions of Noni

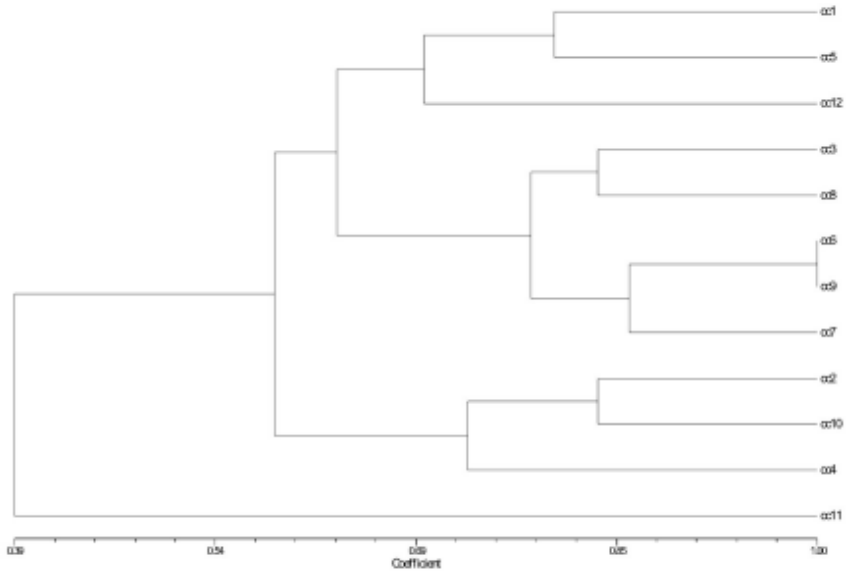
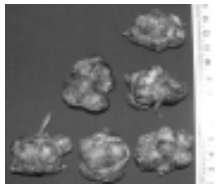


Table 2: Germplasm collections of Noni from Western Ghats

Collection No.	IC No.	Genus	Species	Collection Source	Village	District	State
V-LM/08-01	IC565450	*Morinda*	*citrifolia*	Disturbed wild	Thambakadavu beach	Thrissur	Kerala
V-LM/08-02	IC565451	*Morinda*	*citrifolia*	Disturbed wild	Thalikkulam	Thrissur	Kerala
V-LM/08-03	IC565452	*Morinda*	*citrifolia*	Disturbed wild	Thalikkulam	Thrissur	Kerala
V-LM/08-04	IC565453	*Morinda*	*citrifolia*	Disturbed wild	Thalikkulam	Thrissur	Kerala
V-LM/08-05	IC565454	*Morinda*	*citrifolia*	Disturbed wild	Thalikkulam	Thrissur	Kerala
V-LM/08-06	IC565455	*Morinda*	*citrifolia*	Disturbed wild	Thalikkulam	Thrissur	Kerala
V-LM/08-07	IC565456	*Morinda*	*citrifolia*	Disturbed wild	Thalikkulam	Thrissur	Kerala
V-LM/08-08	IC565457	*Morinda*	*citrifolia*	Disturbed wild	Thambakadavu beach	Thrissur	Kerala
V-LM/08-09	IC565458	*Morinda*	*citrifolia*	Disturbed wild	Natika Beach	Thrissur	Kerala
V-LM/08-10	IC565459	*Morinda*	*citrifolia*	Disturbed wild	Natika Beach	Thrissur	Kerala
V-LM/08-16	IC565465	*Morinda*	*citrifolia*	Natural wild	Methala	Ernakulam	Kerala
V-LM/08-20	IC565469	*Morinda*	*citrifolia*	Natural wild	Methala	Ernakulam	Kerala
V-LM/08-23	IC565472	*Morinda*	*citrifolia*	Disturbed wild	Cherai Beach	Ernakulam	Kerala
V-LM/08-24	IC565473	*Morinda*	*citrifolia*	Disturbed wild	Cherai Beach	Ernakulam	Kerala
V-LM/08-25	IC565474	*Morinda*	*citrifolia*	Disturbed wild	Cherai Beach	Ernakulam	Kerala
V-LM/08-26	IC565475	*Morinda*	*citrifolia*	Disturbed wild	Cherai Beach	Ernakulam	Kerala
VG/KD/07-21	IC559551	*Morinda*	*tomentosa*	Disturbed wild	Sutgatti forest	Belgaum	Karnataka

Table 3 : Seed and fruit characters of three species of *Morinda*

No.	Fruit characters	*Morinda citrifolia*	*Morinda tomentosa*	*Morinda pubescens*
1.	Mature fruits			
2.	Colour	Parrot green when immature, but yellowish white and glabrous at maturity.	Dark green till maturity and non-glabrous.	Dull green till maturity and non-glabrous.
3.	Surface texture	Hard when immature, but soft and fleshy when fully mature	Hard and turgid during all stages of development	Rough and hard when immature but soft at maturity
4.	Odour or smell	No odour when immature. Ripe fruit bears characteristic cheesy odour	Ripe and unripe fruits have spicy odour	No odour
5.	Dimension and shape	Strictly geometrical, about 4-6 cm long and 2-3 cm in diameter	Roughly round with smooth ends, 5 cm long and 3 cm in diameter	Highly irregular, 0.4 to 0.6 cm in dimension

No.	Seed characters	*Morinda citrifolia*	*Morinda tomentosa*	*Morinda pubescens*
1.	Mature seeds			
2.	Seed colour	Dark brown with prominent air sac and a tapering paddle	Dark brown without prominent air sac	Dark brown with no air sac
3.	Seed texture	Smooth plane	Rough with articulate ornamentation	Rough with articulate ornamentation
4.	Seed shape	Drop shaped	Roughly triangular	Triangular, elongated
5.	Seed size(mm)	3-7	5-9	6-8
6.	Number of seeds per fruit	Approximately 100	Approximately 60	Approximately 7-10
7.	100 seed weight (g)	2.20	1.90	8.28
8.	Initial seed moisture (%)	10-12	10-12	12-14
9.	Seat coat	Covered by a hydrophobic membrane	No hyaline covering present	No hyaline covering present

Future thrust areas

Since Noni is a magic ethanobotanical realm becoming highly important in modern medication, a systematic study involving the collection, conservation and characterization is the need of the hour. Collection of germplasm from different parts of the country as well as from abroad, development of *in situ/ ex situ* conservation strategies along with the protocols for seed germination under controlled conditions are required to be studied. The genetic diversity available in Indian coastal regions is required to be analyzed for morphological as well as molecular level for better management and utilization of the Noni germplasm in drug industry.

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Genetic diversity of *Morinda citrifolia* L. accessions across Andaman and Nicobar Islands using RAPD markers

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Keywords : *Morinda citrifolia* – genetic diversity – RAPD-DNA markers-PCR.

Abstract : *Morinda citrifolia* L. an important medicinal plant was collected from 14 localities of Andaman and Nicobar Islands through recurrent survey. All the fourteen accessions were fingerprinted using 48 random amplified polymorphic DNA (RAPD) markers. Among the 48 primers, 28 showed amplification and out of the total 811 loci generated, 335 loci were polymorphic. Unweighted pair-group method with arithmetic averages (UPGMA) analysis showed 41% variation in the collections. The RAPD technique proved that inspite of morphological identity, substantial polymorphism was observed among fourteen accessions studied.

Introduction

Andaman and Nicobar Islands is one of the 25 hot spots of world belonging to Indo - Burma region and are home to a large number of rare and endangered, even undocumented species of flora and fauna (biodiversityhotspots.com). A large number of plants of this region have medicinal values. *Morinda citrifolia* L. is one of them which belongs to *Rubiaceae* family. The *genus Morinda* originated in India and has been naturalized in many parts of Asia, South America, Caribbean and Polynesian Islands and presence of 80 different species is already reported (Singh *et al.*, 2007). Since time immemorial, this plant which is locally known as Lorang, Burmaphal, Noni, Surangi *etc.* are being used by the tribals and aboriginals in this remote region (Sherif and Rao, 2007) Noni is reported to have antibacterial, antiviral, antifungal, antitumor, antihelminthic, analgesic, hypotensive, anti-inflammatory and immune enhancing effects (Duke *et al.*, 2002; McClatchey, 2002; Wang and Su, 2001; Liu *et al.*, 2001). This plant has also been popular as a source of red, yellow and purple dyes (Gurib-Fakeem and Brendler, 2004; Wiart, 2002).

The 26 December 2004 Tsunami caused huge loss of the lives and the infrastructure in the Islands. A report from the state agriculture department showed that about 8000 hectares were damaged and become unsuitable for

agricultural, especially paddy cultivation (Kesavan and Swaminathan, 2007) under low lands due to inundation of sea water during high tide. Interestingly, it was found that *Morinda citrifolia* which is tolerant to saline soils (Norman Bezonal *et al.*, 1996) can have a good sustainability for cultivation in the affected lands. *Morinda citrifolia* fruit is used world wide for production of different products. More than 200 commercial entities sell Noni products, which are distributed across the globe, and it enjoys an enormous market share (Nina *et al.*, 2002).

Fig. 1 *Morinda citrifolia* on Tsunami affected land

The recent effort of introduction of contract farming for this fruit crop with Health India Laboratories, Chennai which is in the need of assured supply of raw Noni fruits for making health tonic, soap, biofertilizer, biopesticides, cosmetics etc. has opened new opportunity for Island farmers by using their waste land through cultivation of this plant.

This agreement has provided assured market with contract buy back guarantee (Singh *et a*l., 2005). Due to this development popularity of this plant has increased and has led to expansion in commercial cultivation to meet the growing demand for raw fruit material.

Until recently, genetic diversity among species or cultivars was determined with morphological or biochemical markers. However, these markers have their own limitations. In the last fifteen years, techniques based on DNA markers have been used to detect variation at DNA level and have proven to be very effective for distinguishing between closely related genotypes. DNA markers have several advantages over phenotype markers and are not affected by age, physiological conditions as well as environmental factors. The

RAPD technique (Williams *et al.*, 1990; Welsh *et al.*, 1991) provides a convenient and rapid assessment of the differences in the genetic composition of the related individuals and has been employed in a large number of plants including medicinal plants (Myburg *et al.*, 1997; Nebauer *et al.*, 1999; Padmesh *et al.*, 1999; Hosokawa *et al.*, 2000; Raina *et al.*, 2001; Neraj, 2003; Vieira *et al.*, 2003) In this paper, we report the results from the application of RAPD PCR profiling for the determination of differences among fourteen accessions of *Morinda citrifolia* collected from different locations of Andaman and Nicobar Island (Table 1).

Fig.2. Fruit of *Morinda citrifolia*

Materials and Methods

The genomic DNA was isolated from *Morinda citrifolia* leaves by CTAB method (Suman *et al.*, 1999) DNA quantification was estimated through UV-VIS spectrophotometer and agarose gel electrophoresis with known standard (Lambda DNA). DNA quality was checked under UV-light and the purity was calculated from O.D.260/O.D.280 ratio, and also by electrophoresis on 0.8% agarose gel.

Table 1: The collection sites (across Andaman and Nicobar Islands)

Garacharma	GAH-1
Sippighat -1	SPG-1
Sippighat -2	SPG-2
Memeo	MEM-1
Bambooflat-1	ABF-1
Bambooflat-2	ABF-2
Light House	LH-1

Bahai House	ABH-1
Hadoo	AHD-1
Jungli Ghat	JGH-1
CARI	GAH-2
Hut bay	TRA-1

The re-suspended DNA was then diluted in sterile distilled water to 25ng/µl concentration for use in amplification reactions. A set of 48 random decamer oligonucleotides (Bangalore Genei) were used as single primers for the amplification of RAPD fragments. All the 48 primers were tested at least twice for reproducibility of banding pattern and 28 exhibited amplification. Polymerase chain reactions (PCR) were carried out in a final volume of 25 µl containing 25 ng template DNA, 100 µM of each of the four deoxynucleotide triphosphate, 20 ng of decanucleotide primer, 1.5 mM $MgCl_{2,}$ 10X Taq buffer (10mM Tris HCl pH9.0, 50 mM KCl) and 0.5 U Taq DNA polymerase. All the chemicals were purchased from Bangalore Genei. PCR amplification was carried out on thermal cycler well blocks (M.J.Research Inc. USA) under the following conditions, initial denaturation of 4 minutes at 94ºC, followed by 40 cycles of denaturation at 94ºC for 1 minute, primer annealing for 1 minute at 32 ºC, extension for 1 minute at 72ºC followed by a final extension for 7 minutes at 72ºC. Amplification products were maintained at 4ºC until electrophoresis.

The reaction products were separated along side a molecular weight marker (1 kb ladder and 100bp ladder) by electrophoresis on 1.5% agarose gel using 1X TBE buffer at 8V/cm for 3 hours. The gel was visualized and photographed with gel documentation system (Vilber Loubmet, France, Cat.No. Bio ID++ver.99.04).

Table 2: Amplified primer code and sequences for RAPD Profiling

S.No	Primer code	Sequence 5'-3'
1.	OPH-1	AATCGGGCTG
2.	OPH-2	CAATCGCCGT
3.	OPH-3	TCTGTGCTGG
4.	OPH-4	GACCGCTTGT
5.	OPH-5	GTTGCGATCC
6.	OPH-6	TCGCCGCAAA
7.	OPH-7	AGCGTCACTC
8.	OPH-8	GTCCGTACTG
9.	OPH-9	GGTGCTCCGT
10.	OPH-10	GACCGACCCA

S.No	Primer code	Sequence 5'-3'
11.	OPH-11	CGGTTCCCCC
12.	OPH-13	GTCTGACGGT
13.	OPH-14	CAGCTCAAGT
14.	OPH-15	CGATCGAGGA
15.	OPH-17	AAGCAGCAAG
16.	OPH-19	AGCATTCGG
17.	OPH-20	CACCGTTCTG
18.	OPH-21	ACTCCGCAGT
19.	OPH-31	TAGACAGTCG
20.	OPH-33	AGGCCGTATC
21.	OPH-34	ATGAGTCCAC
22.	OPH-36	TCAAACTCGG
23.	OPH-39	TAGCCGTCAA
24.	OPH-41	ATTTGATCGC
25.	OPH-42	ACGCTGATCA
26.	OPH-43	AACCGACGGG
27.	OPH-44	TGCCCTGCCT
28.	OPH-45	CTTGCCTCCC

The image profiles of banding patterns were recorded and molecular weight of each band was determined by Molecular Analyst (version 1.5) software.

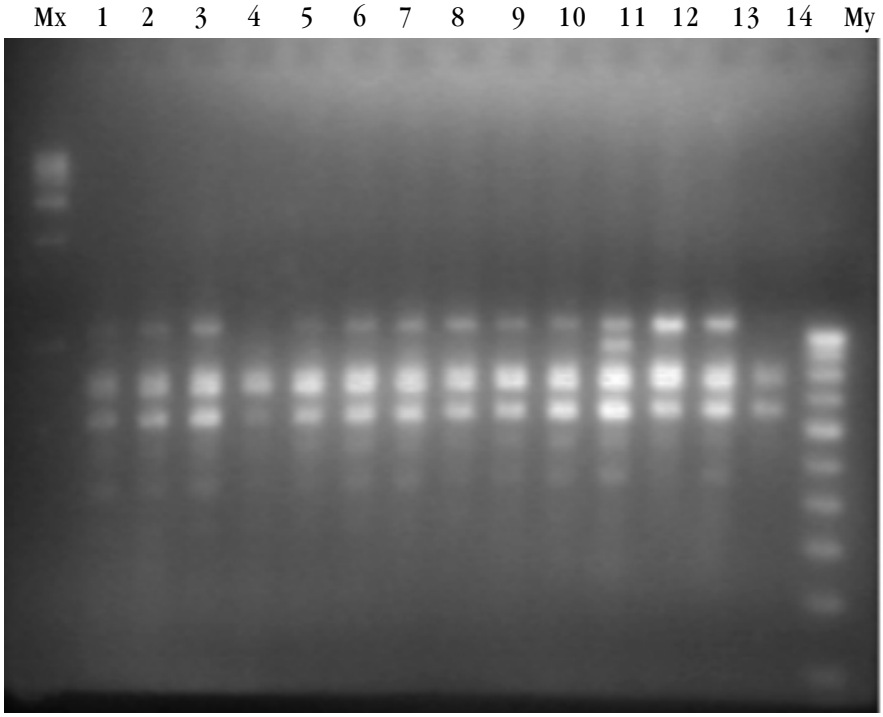

Fig. 3

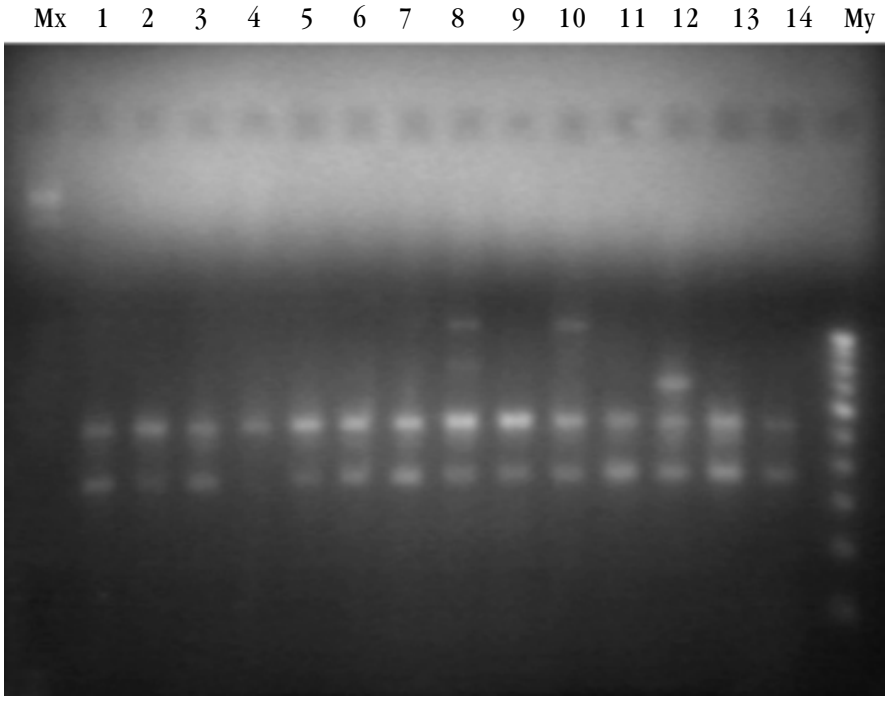

Fig. 4

Fig. 3 & 4 show the amplification of primer OPH 31& OPH-11 respectively.

RAPD Profile of 14 accessions of *Morinda citrifolia* collected from Andaman and Nicobar Islands.

Mx-1Kb ladder, My- 100 bp ladder 1- GAH-1, 2- SPG-1, 3- SPG-2, 4- MEM-1, 5- ABF-1, 6- ABF-2, 7- LH-1, 8- ABH-1, 9- AHD-1, 10- JGH-1, 11- GAH-2, 12- TRA-1, 13- TRA-2, 14- MAN-1

Data were recorded as presence (1) or absence (0) of band products from the examination of photographic negatives (William *et al.*, 1990) Using Dice coefficient, a similarity matrix involving 14 accessions was generated with NTSYS-pc (Numerical Taxonomy System, Applied Biostatistics, Inc., New York, USA, software version 2.02e). Dendrograms were created by UPGMA cluster analysis (Nei.and Li, 1979) (Fig. 5).

Results and Discussion

The accessions have been grouped into two major clusters A&B having 74% similarity The first major cluster A was grouped into two sub clusters A1 and A2 having 79% similarity, sub cluster A1 was further subdivided into two groups A1X and A1Y having 82% similarity. The A1Y had a solitary collection from AHD-1 while A1X was further subdivided into two subgroups $A1X^1$ and $A1X^2$. $A1X^1$ had two accessions JGH-1 and GAH-2 which are closely related and showed 88.5% similarity.

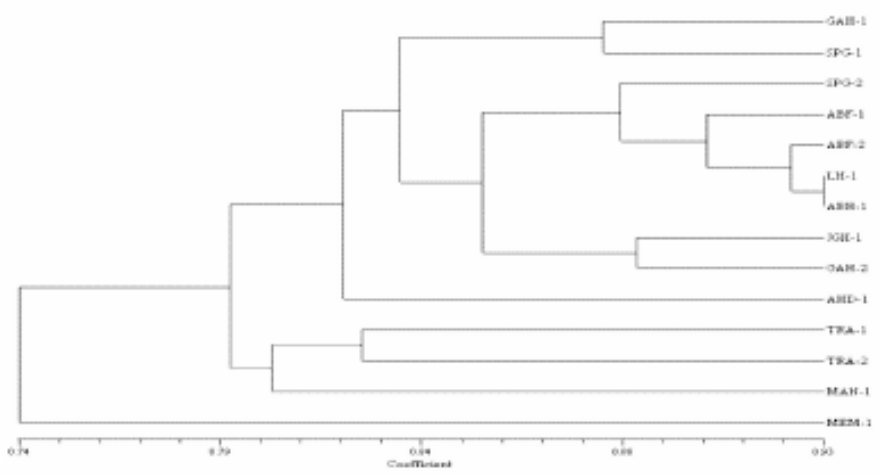

Fig. 5 : Dendrogram showing diversity of *Morinda citrifolia* accessions based on RAPD

A1X² grouped into A1X²A and A1X²B having 86% similarity.A1X²A comprises collection from SPG-2, ABF-1, ABF-2, LH-1 and ABH-1, in which accessions belonging to LH-1 and ABH-1 had 93% similarity while A1X²B had two accessions JGH-1 & GAH-2 having 88% similarity. The second sub cluster A2 was grouped into A2X and A2Y. A2Y represented by only accession MAN-1 while A2X had two accessions TRA-1 & TRA-2 having 83% similarities. The second major cluster B represented by only one accession MEM-1. Out of 811 bands, 335 bands were polymorphic. The polymorphism percentage was calculated as per the method suggested by Blair *et al.*, (1999). The present study had shown 41% polymorphism among the 14 accessions of *Morinda citrifolia.*

The result from the study indicates that inspite of their morphological identity, substantial polymorphism was observed among *Morinda citrifolia* accessions collected from different Islands. Even though there is no other molecular data to support the present classification, it is clear that identification based on morphology alone can not correctly designate different accessions of *Morinda citrifolia* as observed earlier in rice (Virk *et al.*, 1995) and costuss (Asit *et al.*, 2007).

Extensive use of RAPD-PCR technique and other molecular characterization methods of *Morinda citrifolia* germplasm are envisaged to identify markers of commercial traits and will also help in planning future germplasm collection and selection of parents for breeding programmes.

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Noni juice challenges the neuroglial tumor in rats - Preliminary biochemical assessment

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Keywords : Noni juice – glioma - neuroglial tumor

Abstract : Glioma, a neoplasm of neuroglial cells is a leading cause of central nervous system tumor related death even after conventional therapies necessitated search for new drug without side effects. Thus, the present study attempts to assess the impact of *Morinda citrifolia* (Noni) in the management of glioma. C6 cell lines were sterotactically injected into brain of group II rats where as medium alone in group I. Noni juice (NJ) was orally administered in Group III. Group IV rats were treated as in Group II and III. Implantation of the gliomal cell line was confirmed by the MRI. Brain tissues were removed and subjected to histopathological and biochemical analyses. Glioma induced rats showed increase in the activities of glial cell markers and antioxidant enzymes, whereas treatment with extract reduced the activities of markers, as well as enzymatic antioxidants. Histological architecture, lipids and protein bound carbohydrates were also altered in the treated groups on comparison with group II. These preliminary observations suggested that, *Morinda citrifolia* (NJ) might play a significant role against glial tumorigenesis which requires, further, detailed investigations.

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Introduction

A glioma is a type of primary central nervous system (CNS) tumor that arises from glial cells. The most common site of involvement of gliomas is the brain, but they can also affect the spinal cord or any other part of the CNS, such as the optic nerves. When a malignant brain tumor develops, its growth is regulated not only by intrinsic tumor cell kinetics, but also by the interaction of tumor cells with normal and reactive cells in the tumor environment (Rutka *et al.*, 1988). Glial tumors are typically angiogenic and infiltrate surrounding brain parenchyma. Even the most aggressive brain metastases tend to form Non-infiltrative masses with well-defined borders (MCrk *et al.*, 1984). Three experimental methods are commonly used to induce cerebral tumorigenesis, *viz.,* radiation, viruses and chemical carcinogens (Janiseh and Schreiber, 1977). Such methods do not easily offer an opportunity to create identical tumors in similar locations in all experimental animals (Auer *et al.*, 1981) and hence cell lines were reported as

better choice (Auer *et al.*, 1981 and Farrell *et al.*, 1987) and thus used in the current study for induction.

Astrocytoma C6 is a well established *in vitro* cell line initially induced in rats by N-nitroso-methylurea (Barde *et al.*, 1978), and extensively characterized thereafter (Bissell *et al.*, 1974, Parker *et al.*, 1980). Implantation of C6 glioma into the rat brain mimics many of the growth and pathological characteristics of human gliomas (Stewart *et al.*, 1985). In this study, the pathological and biochemical changes of rat brain bearing C6 glioma cells were investigated. *Morinda citrifolia* L (Noni juice (NJ)) was used in folk remedies by Polynesians for over 2000 years and was reported to have a broad range of therapeutic effects, including antibacterial, antiviral, antifungal, antitumor, antihelminthic, analgesic, hypotensive, anti-inflammatory and immune enhancing effects. Hence, the potent drug has been desired to be investigated for its anti-glioma property.

Materials and Methods

Cell culture

Rat C6 glioma cell line originally cloned from an N-nitrosomethylurea- induced glioma by Benda *et al.* (1968) was obtained from the NCCS, Pune and maintained in monolayers in 100-mm dishes at 37 ° C under humidified 5 % CO_2 - 95 % air. The cells were cultured in Eagle's minimum essential medium (MEM, Bio Corporals, Chennai.) supplemented with fetal bovine serum (FBS 10 % final concentration), penicillin-G (50 unit/ml) and streptomycin (50 µg/ml). Cells were harvested during the log phase of growth.

Animals and microinjection of tumor cells

Adult male Wistar rats weighing between 100-150 g were used. The animals were divided into four groups with six in each. All the animals were kept in polypropyelene cage provided with adequate water and pellet foods. They were anesthetized with Xylazine and Ketamine during surgery and injection. Each animal was securely placed in a stereotactic surgical frame. Hamilton Micro Syringe was inserted into the right caudate-putamen according to the rat brain atlas Figure 1.

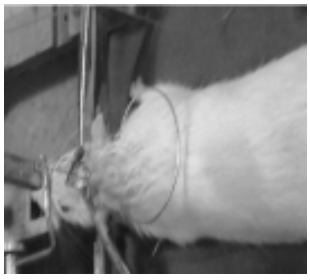

Fig. 1

Group I rats served as control and injected with 5 µ1 of MEM supplemented with 10 % FBS alone. Group II animals were injected with cell suspension of C6 glioma cells (5 µ1 of MEM supplemented with 10 % FBS containing 10^5 cells) under a controlled pressure. Group III animals served as drug control. NJ was administrated orally (4ml / kg of BW for 30 days). For Group IV animals, implantation of C6 glioma cells is same as that of Group II, followed by the treatment as in Group III. The craniotomy was sealed with bone wax and the overlying skin incision was closed.

Magnetic Resonance Imaging (MRI)

MR experiments were performed to confirm the induction and thus the model development. Anesthetized rats bearing C6 cells were subjected to MRI scanning, 1.5T (HDX T) (GE Medical System, USA). Images were acquired and interpreted by a radiologist.

Histopathology

The animals were anaesthetised and brains were gently removed and fixed in fixative containing formal saline. Sections were taken from right hemisphere of brain. Sections are mounted and stained with hematoxylin and eosin (H & E) staining. Imaging was done under canon light microscope with magnification of 300 X and bar scale 25 mm.

Biochemical parameters

Biochemical analyses of glial cell markers like glutathione–S-transferase, 5' nucleotidase (ND) and creatine kinase, followed by the study of lipid profile, enzymatic antioxidants and protein bound carbohydrates were carried out.

Statistical Analysis

Results were expressed as mean ± SD. Statistical analysis was performed using one way ANOVA.

Results and Discussion

Magnetic Resonance Imaging

All animals which were injected with C6 cells were investigated on MR after 30 days of injections. There were visible tumors in the animals scanned on MR. The rim of the tumors could be seen as images with contrast, the tumors were easily visualized as high signal intensity areas at the site of injection (Figure 2). No spread of tumor cells to other brain areas was seen. Necrosis was not observed, and no cystic parts could be detected within the tumors.

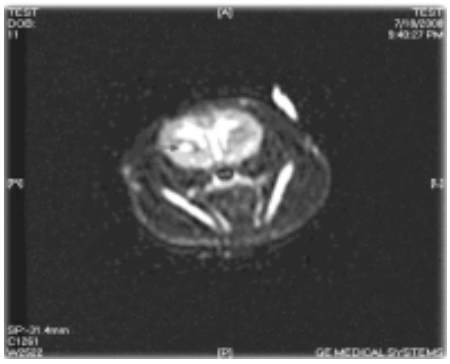

Fig. 2

The histopathological results are presented in Fig. 3 to Fig. 6. Figure 3 showed the normal architecture of Group I rats

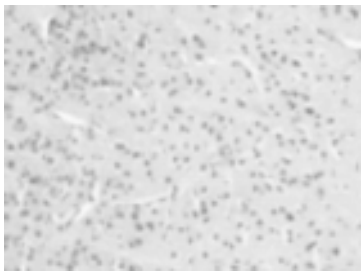

Fig. 3

In Figures 4 and 5, characteristic histology of group II rat brains were presented. The tumor has uniform, dense cellularity and is well vascularized with fibrillary background. The tumor-brain boundary is distinct, with little local infiltration of tumor cells.

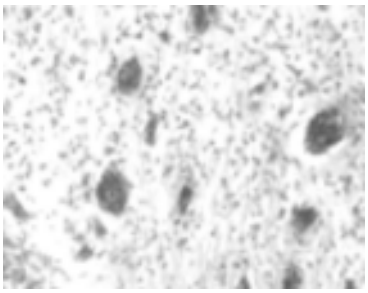

Fig. 4

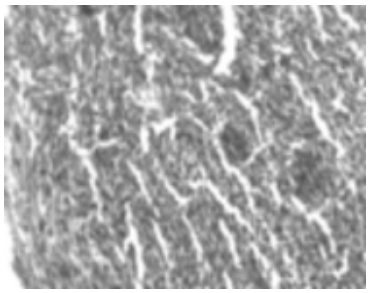

Fig. 5

Figure 7 depicts an architecture that resembles normal one with occasional tumor infiltration, while Figure 6 represents that from the drug control.

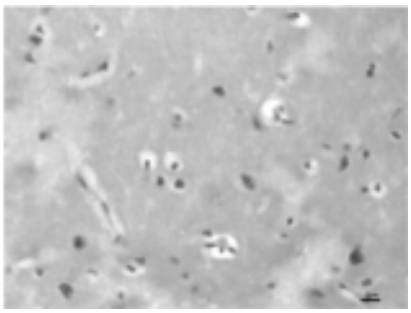

Fig. 6

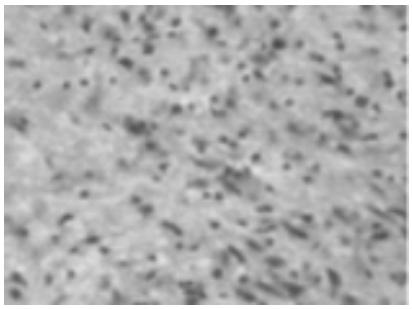

Fig. 7

Biochemical parameters

Estimation of protein

Table 1 : The level of protein in the brain of control and experimental groups

Particulars	Group I	Group II	Group III	Group IV
Protein	22.32±1.46	67.53±12.12*	35.54±1.15*	23.93±1.78NS

The values are expressed as mean ± SD (n=6); Proein mg/g of tissue; Statistical significance was represented as *p<0.05; Group II (glioma bearing rats) was compared with Group I;

Group III was compared with Group II ; NS = Non Significant. Group IV was compared with Group I

Glial cell markers

Table 2: The activities of markers like GST, 5' ND, CK in the brain of control and experimental groups

Particulars	Group I	Group II	Group III	Group IV
GST	17.44±1.53	35.59±1.43*	25.51±1.52*	17.04±1.73NS
5' ND	4.63±0.84	15.85±1.05*	9.66±0.75*	4.73 ±0.72NS
CK	152.21±12.40	213.54±31.25*	175.36±11.42*	156.67±18.25NS

The values are expressed as mean ± SD(n=6); GST- units/mg protein, 5'ND AND CK- nmoles of pi liberated / min/mg protein; Statistical significance was represented as *p<0.05

Group II (glioma bearing rats) was compared with Group I ; Group III was compared with Group II ; NS = Non Significant. Group IV was compared with Group I

Table 3: The activities of enzymatic antioxidants and catalase in the brain of control and experimental groups

Particulars	Group I	Group II	Group III	Group IV
SOD	11.78±0.85	20.45±1.20*	16.73±0.88*	11.99±1.20NS
Catalase	5.44±0.74	14.42±1.04*	10.23±1.43*	5.54±1.08NS

The values are expressed as mean ± SD(n=6); SOD - units/min/mg protein, Catalase-µmoles of H_2O_2 formed/min/mg protein ; Statistical significance was represented as *p<0.05

Group II (glioma bearing rats) was compared with Group I ; Group III was compared with Group II ; NS = Non Significant. Group IV was compared with Group I

Lipid profile

Table 4 : The levels of cholesterol, free cholesterol, ester cholesterol, triglycerides, free fatty acid and phospholipids in the brain of control and experimental groups.

Particulars	Group I	Group II	Group III	Group IV
Cholesterol	25.31±1.89	32.27±1.40*	22.04±1.25*	25.48±1.96NS
Free cholesterol,	20.73±1.27	16.43±1.06*	18.35±1.03*	20.94±1.83NS
Ester cholesterol	4.58±0.62	15.84±0.34*	3.69±0.22*	5.21±0.21NS
Triglycerides	84.28±0.87	122.45±1.60*	92.70±1.54*	79.84±5.13NS
Free fatty acid	33.73±1.17	62.82±1.81*	42.83±1.17*	34.52±1.31NS
Phospholipids	77.66±1.46	98.03±1.54*	85.71±2.30*	74.61±1.97NS

The values are expressed as mean ± SD(n=6); Lipid profile mg/g of tissue; Statistical significance was represented as *p<0.05; Group II (glioma bearing rats) was compared with Group I ; Group III was compared with Group II ; NS = Non Significant. Group IV was compared with Group I

Protein Bound Carbohydrates (PBC)

Table 5: The levels of hexose, hexosamine, sialic acid and fucose in the brain of control and experimental groups

Particulars	Group I	Group II	Group III	Group IV
Hexose	153.60±1.80	214.32±1.16*	174.45±1.86*	154.82±1.85NS
Hexosamine	143.93±2.08	196.68±3.29*	161.22±12.8*	145.97±1.52NS
Sialic acid	214.54±1.32	253.67±2.13*	242.00±1.20*	213.24±2,41NS
Fucose	97.47±1.67	155.65±1.30*	132.86±2.17*	114.02±1.43NS

The values are expressed as mean ± SD(n=6); PBC mg/g of tissue; Statistical significance was represented as *p<0.05; Group II (glioma bearing rats) was compared with Group I

Group III was compared with Group II; NS = Non Significant. Group IV was compared with Group I

The results (Table 1) showed the effect of drugs on the activity of total protein in the brain sample of control and experimental animals. There was a significant ($p<0.05$) increase in the levels of these protein in the brain tissue of the glioma induced Group II animals when compared with control and treated animals. On administration of drugs a significant ($p<0.05$) decrease in the levels of total proteins in the drug treated group (Group III) when compared with brain tissue of the glioma induced group II animals was observed. There was no significant difference between control and drug control groups.

Table 2 showed the effect of drugs on the activity of GST, 5'ND and CK in the brain sample of control and experimental animals. There was a significant ($p<0.05$) increase in the activities of these enzymes in the brain tissue of the glioma induced Group II animals when compared with control and treated animals. On administration of drugs, a significant ($p<0.05$) decrease in the activities of these enzymes in the drug treated group (Group III) when compared to brain tissue of the glioma induced group II animals was encountered. There was no significant difference between control and drug control groups.

The activity of GST was found increased in glioma. The expression of GST isoenzymes is changed in various pathological conditions, including neoplasia. The GST- pi expression was increased in glioma; change in GST isoenzymes expression can play an important role in the susceptibility of CNS to carcinogenesis (Usarek *et al.*, 2005). Expression was increased in glioma revealing malignant glioma such as astrocytoma and glioblastoma having

strong expression of the same. Hara *et al.* (1981) showed the existence of strong expression of GST in both astrocytoma and GBM. Similarly, CK activity was also seen in all the tissues but mostly in the brain and was found increased in many cancer conditions (Julie and Abbal, 1998) and it has an active role in the ATP regeneration in brain.

The 5' ND is an endonuclease which is expressed on normal and neoplastic glial plasma membranes (Ludwig *et al.*, 1999). The inhibition of 5' ND might result in a decrease in extracellular adenosine production with a consequent reduction in tumour progression (Wink *et al.,* 2003). In the present study, the expression of these markers which are related to inactivation of drug and chemoresistance was found to be comparatively higher in group II. The reverse picture was observed in drug treated groups. The inhibition of these enzymes might lead to the drug activation against the tumor cells and thus the observed reduction in the activity of these makers in the NJ treated groups might be interpreted as the influence of NJ on suppression of chemo resistance.

The results presented in Table 3 showed the effect of drug on the activities of enzymatic antioxidants SOD and catalase in the brain of control and experimental group. There was a significant ($p<0.05$) increase in the levels of these enzymes in the brain tissue of the glioma induced Group II animals when compared with control and treated animals. On administration of drugs, a significant ($p<0.05$) decrease in the levels of these enzymes in the drug treated group (Group III) when compared to brain tissue of the glioma induced Group II animals suggested the action of component of Noni on cell survival. There was no significant difference between control and drug control groups.

The levels of antioxidants were found increased in glioma (Pameeka *et al.*, 2007). The antioxidant enzymes are also known to be factors involved in radio resistance and chemo resistance (Grant and Ironside, 1995). Increased SOD activity in the meningioma and glial tumors was found (Tuna *et al.*, 2002). High expression of Cu / Zn SOD caused the suppression of both types of FAS induced cell death (Jayanthi *et al.*, 1999). The catalase activity was also found increased in the glioma (Popov *et al.*, 2003). In this study the activity of these enzymes was found decreased in drug treated group which might be due to the inhibition by the active components of Noni juice (NJ).

The results presented in Table 4 showed the effect of NJ on the levels of cholesterol, free cholesterol, ester cholesterol, triglycerides, free fatty acid and phospholipids in the brain tissue of control and experimental group. The levels of total cholesterol, ester cholesterol, triglycerides, free fatty acid and

phospholipids increased significantly ($p<0.05$) in the brain tissue of the glioma induced Group II animals whereas free cholesterol level decreased significantly ($p<0.05$) in the brain tissue of the glioma induced Group II animals when compared to control and treated animal.

The increased level of total cholesterol in the induced group showed that the process of carcinogenesis is known associated with alteration in the lipid metabolism affecting cellular function and growth. An altered lipid levels between primary tumors and metastasis were reported varying significantly (Chuanting Li, 2007). Increase in cholesterol reported in different grades of glioma correlates well with histological vascular proliferation (Tosi *et al.*, 2003). Changes in lipid composition may play a role in structural and functional membrane alteration in neoplastic cells (Ledwozyw and Lunicki, 1992). The data obtained suggested that the profound modification of lipids which are gradually, accompanied by a progressive increase in the malignancy of the tumor are also responsible for functional variation connected with neoplastic growth. The related lipid components were maintained to the levels very much comparable to that of normal on administration with NJ. This restoration of lipid levels could be due to strong hypolipidemic activity of the NJ. This suggests that the juice containing the active component that might exert effect on neoplastic induced perturbation of cell lipids.

The results presented in Table 5 showed the effect of drug on the levels of hexose, hexosamine, sialic acid and fucose in the brain of control and experimental group. There was a significant ($p<0.05$) increase in the levels of these PBC in the brain tissue of the glioma induced Group II animals when compared with control and treated animals. On administration of drugs, a significant ($p<0.05$) decrease in the levels of these PBC in the drug treated group (Group III) when compared to brain tissue of the glioma induced Group II animals was observed. There was no significant difference between control and drug control groups.

The level of PBC was altered in tumor condition. The abnormal level of these PBC is the major indicator of pathogenesis. The increased levels are associated with advanced stages of cancer (Baxi., 1993). In this study, the elevated level of PBC were found significantly decreased in NJ treated groups.

Conclusion

The MRI and histopathological experiments confirmed the implantation of C6 cell in the rat brain that leads to formation of experimental model of glioma. Glioma induced rats showed increase in the activities of glial cell markers and antioxidant enzymes, whereas treatment with Noni juice reduced the

activities of markers as well as enzymatic antioxidants. Histological architecture, lipids and protein bound carbohydrates were also altered in the treated groups on comparison with Group II. These preliminary observations suggested that Noni juice might play a positive role against glial tumorigenesis and on suppression of chemo-resistance which requires, further, detailed investigation for confirmation.

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Glycaemic and cholesterolaemic effect of nutritional supplement of Noni (*Morinda citrifolia*) on selected middle aged female Non Insulin Dependent Diabetic Mellitus (NIDDM) subjects

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Keywords : Noni juice – Glycaemic – cholesterolaemic - NIDDM

Abstract : Noni (*Morinda citrifolia*) juice is extracted from Indian Mullbery. This nutritional supplement contains high amount of calcium, potassium, phosphorus and magnesium. Because of its high nutritional content, the fruit is now used for extraction of juice which is used as a health drink throughout the world. Noni has sufficient amounts of the precursor of xeronine. This precursor is proxeronine which regulates the rigidity and shape of specific proteins. This action of Noni makes a person feel well. An attempt has thus been made to administer the Noni juice to selected middle aged female NIDDM subjects for a period of 60 days. Sixteen subjects were selected for the study and they were divided into two groups of eight subjects each, to one group there was no administration of Noni juice and they formed the control group and to the other group, Noni juice was administered (30ml / day in two equal doses) and they formed the experimental group. The blood was collected on the 0 day and on the 60th day of the study period and the biochemical parameters were analyzed. All the results obtained were statistically analyzed using Students 't' test. The study revealed that the subjects administered with Noni showed a marked decrease in blood sugar level, BMI and lipid profile when compared to the control group of the same age and condition of NIDDM who were not given the Noni juice. It is inferred that Noni juice could be a good hypoglycaemic and hypocholesterolaemic agent.

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Introduction

Thousands of people worldwide attributed improvements in their health due to the life saving benefits of Noni (*Morind citrifolia*) (Edwards, 1996). One such health challenge where Noni was found helpful was with Diabetes mellitus and hypercholesterolaemia. Noni has the ability to strengthen the immune system and help the body maintain strong insulin levels preventing Diabetes mellitus. Noni has powerful antioxidant properties that help to

neutralize free radicals role of oxidation of useful LDL cholesterol to harmful LDL cholesterol, thus preventing the condition of hypercholesterolaemia (Mathivanan, 2005). Both these conditions are emerging as one of the main threats to human health. India is reported as the capital of Diabetes mellitus and a co-capital of heart disease. About 90 per cent of Diabetes mellitus in India are NIDDM (Muralidharan, 1998; Murdiati, 2000).

Recent research on Indian traditional foods has opened up a new area of treatment through diet and diet regulation. Certain foods have known to have some functional properties with proven beneficial effects to bring a good control on blood sugar levels and blood cholesterol level (Ford, 2003). They should be made an integral part of the diet as their availability and acceptability in the Indian scenario is easier (Mala and Subramanian, 1993). Meal planning includes choosing healthy foods, eating the right amount of food and eating meals at the right time. The diabetic patient needs to be educated about change in life-style, diet, exercise and drugs (Ford, 2003).

Xeronine, the alkaloid of Noni in the presence of insulin activatea the peripheral cell membrane insulin receptors and helps in the normal absorption of glucose (Murdiati, 2000). Noni acts as a cell rejuvenator, healthy immune system promoter and has a very low glycaemic index and hence reduces the risk of complications of Diabetes Mellitus (Wang and Williams, 2002). The anti oxidants present in Noni prevent accumulation of cholesterol, a condition of severity known as hypercholesterolaemia (Ford, 2003).

Materials and Methods

Hundred middle aged female NIDDM subjects were randomly selected and their nutritional status was assessed by anthropometry, clinical examination and diet survey. Their biochemical parameters were recorded from the completed test results. Out of the 100 subjects interviewed, 16 subjects were selected for the study of which eight of the subjects formed the experimental group (those who were administered Noni juice) and eight of them formed the control group (those who were not administered Noni juice). The effect of Noni syrup on the anthropometric measurements and blood parameters were studied for a period of 60 days of supplementation.

The inclusion criteria used for the selection of study subjects were as follows:

They should be middle aged women (between 35-55 years).

Their fasting blood sugar level should be more than 130 mg/dl and a total cholesterol value more than 230 mg/dl.

They should be willing to participate for the entire period of study (60 days) and should be willing to give blood at the beginning and end of the study period.

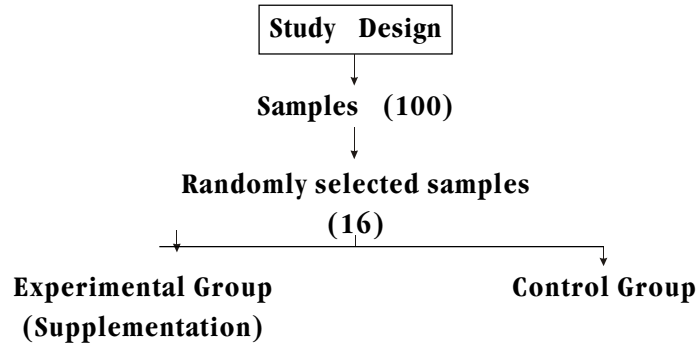
The objectives of the present study were :

To study the effect of nutritional supplement Noni on selected female NIDDM subjects for a period of 60 days on their-

1. Anthropometric indices of the experimental and control groups.

2. Biochemical parameters like haemoglobin levels, fasting and post prandial blood sugar and serum lipid profile of the experimental and control groups and

3. To compare the anthropometric indices and biochemical parameters of the experimental and control groups.



Note: Supplementation period : 60 days
Collection of blood: 0 day and 60th day

Study Design

The study was conducted from December 2007 to February 2008. Sixteen female middle aged NIDDM subjects were selected for the supplementation from those attending a polyclinic at Vellore. Each of the eight female middle aged NIDDM subjects from the experimental group was given 30 ml of Noni juice in two equal doses of 15ml each in the morning (before breakfast) and at bedtime (before dinner)for a period of 60 days (Peter, 2008). Periodic visit by the researcher was taken up to ensure that the subjects under study adhered to the dietary regimen and were taking Noni juice regularly. The anthropometric measurements like height and weight were measured and the blood sample was drawn from the selected subjects on day 0 and day 60 of the study period.

Results and Discussions

Effect of Nutritional supplement of Noni on anthropometric measurements

Anthropometric measurements are presented in Table 1.

Table 1: Effect of nutritional supplement of Noni (*Morinda citrifolia*) on anthropometric measurements

Anthropometric measurements	0 day	60 days	't' value
Weight	67.25±13.49	64.75± 13.69	11.832**
BMI	27.21 ± 5.75	26.20 ±5.85	11.769**

** Significant at 1% level (p< 0.01)

The mean initial weight of selected respondents on 0 day of the supplementation was 67.25 ± 13.49 which reduced to 64.75 ± 13.69 after 60 days of supplementation with Noni. There was a significant reduction in weight.

The Body Mass Index (BMI) of the experimental group on 0 day of supplementation was 27.21 ± 5.78 and on the 60th day the BMI came down to 2 6.20 ± 5.85 thus showing a significant reduction of BMI.

Effect of Nutritional supplement Noni on blood sugar

The results are presented in Table 2.

Table 2: Effect of nutritional supplement of Noni (*Morinda citrifolia)* on blood sugar

Blood Parameters	0 day	60 days	't' Value
Fasting blood sugar (mg/dl)	165.71±21.66	120.25±19.83	8.348**
Post prandial blood sugar (mg/dl)	269.25±28.01	171.13±21.81	22.294**
HbA_1C (per cent)	8.138± 0.859	7.175±0.649	7.773**

** Significant at 1% level (p<0.01)

The mean initial fasting blood sugar level on 0 day was 165.71 ± 21.66 which reduced to 120.25 ± 19.83 in 60 days of supplementation. The mean post prandial blood sugar level on 0 day was 269.25 ± 28.01 which showed

a gradual decrease to 171.13 ± 21.81 in 60 days of supplementation. The reduction was significant for both the parameters.

The mean initial HbA_1C value on 0 day of supplementation was 8.138 ±0.859. After 60 days of supplementation, there was decrease in the mean HbA_1C value to 7.175 ± 0.649. The significant reduction of HbA_1C was observed.

Effect of nutritional supplement of Noni (*Morinda citrifolia*) on serum lipid profile

The results of serum lipid profile are presented in Table 3.

Table 3 : Effect of nutritional supplement of Noni (*Morinda citrifolia*) on serum lipid profile

Blood Parameters	0 day	60 day	't' value
Serum total cholesterol (mg/dl)	248.25 ±17.98	195.50±15.84	9.254**
Serum triglyceride(mg/dl)	285.75±48.85	190.73 ±23.27	6.678**
HDL cholesterol (mg/dl)	47.13 ± 9.49	47.88±9.28	0.513NS
LDL cholesterol (mg/dl)	140.50± 20.51	109.50±18.72	3.870**
VLDL cholesterol(mg/dl)	56.88 ±9.98	38.13±4.55	6.180**

NS= Not Significant; ** Significant at 1 % level (p< 0.01)

The mean serum total cholesterol on 0 day was 248.25 ± 17.98 which got reduced to 195.50 ± 15.84 after 60 days of supplementation. The mean serum triglyceride level on 0 day of supplementation was 285.75 ± 48.85 which reduced to a mean value of 190.73 ± 23.27 after 60 days of supplementation.

The mean initial LDL cholesterol value was 140.50±20.51 which reduced to 109.50 ± 18.72 after 60 days of supplementation. The mean VLDL cholesterol values reduced from 56.88 ± 9.98 to 38.13 ± 4.55. The supplementation of Noni juice did not significantly change the HDL cholesterol level.

The mean BMI calculated using body weight and height of the selected respondents on the test days are presented Table 4.

Table 4: The mean Body Mass Index calculated using body weight and height of selected respondents

Anthropometric measurements	E 0 day	E 60 days	C 0 day	C 60 days	't' value	't'value value
Weight in Kg	67.25 ±13.49	64.75 ±13.69	74.13 ±10.64	74.8 ±11.48	1.132*	1.591*
BMI	27.21 ±5.78	26.20 ±5.85	30.69 ±5.01	30.97 ±5.27	1.288*	1.712*

NS = Not Significant; ** Significant at 1 % level (p< 0.01); E = Experimental; C = Control

The experimental group showed a marked decrease in weight and BMI due to supplementation of Noni.

Comparison of haemoglobin levels of experimental and control groups

The mean haemoglobin levels of he selected respondents of both the groups are presented in Table 5.

Table 5 : Comparison of haemoglobin levels of experimental and control groups

Blood parameters	E 0 day	E 60 day	C 0 day	C 60 day	't' value of E#	't' value of C#
Haemoglobin level mg/dl	13.24 ±0.51	13.46 ±0.31	13.38 ±0.82	13.25 ±0.93	0.402*	0.615*

E= Experimental; C = control; *NS = Not significant; ** Significant at 1 % level (p< 0.01)

The results showed that supplementation of Noni for 60 days did not increase the haemoglobin level significantly.

Comparison of blood sugar of experimental and control group

The results are presented in Table 6.

Table 6: Comparison of blood sugar of experimental and control groups

Blood parameters Mg/dl	E 0 day	E 60 day	C 0 day	C 60 day	't' value of E	't' value of C
Fasting blood sugar mg/dl	165.71 ±21.66	120.20 ±19.83	182.63 ±31.52	188.63 ±24.68	6.109**	1.251NS
Post prandial blood sugar mg/dl	269.25± 28.08	171.13± 21.81	270.63± 49.56	282.50± 31.33	8.252**	0.068NS
HbA1C	HbA1C (per cent)	8.14 ± 0.86	7.18± 0.65	8.44 ± 0.79	8.44 ± 0.71	3.707**

NS = Not Significant; ** Significant at 1 % level (p< 0.01); E = Experimental group; C = ontrol group

There was significant decrease in mean fasting blood sugar, mean post prandial blood sugar and mean HbA1C values in the experimental group after supplementation (from 165.71 to 120.25; from 269.25 to 171.13; from 8.14 to 7.18) as compared to the control group (from 182.63 to 188.63; from 270,63 to 282.50; from 8.44 to 8.44) respectively.

The results revealed that supplementation with Noni for a period of 60 days brought a significant reduction in fasting and post-prandial blood sugar and HbA1C values.

The results on serum lipid profile of experimental and control group are presented in Table 7.

Table 7: Comparison of Serum lipid profile of Experimental and Control group

Blood parameters mg/dl	E 0 day	E 60 day	C 0 day	C 60 day	't' value of E	't' value of C
Serum total cholesterol mg/dl	248.25 ±17.98	195.50 ±15.54	251.50 ±18.00	255.98 ±21.21	6.462**	0.361NS
Serum triglyceride mg/dl	248.75 ±48.85	190.73 ±23.27	298.25 ±53.58	300.33 ±44.27	6.198**	0.488NS
HDL cholesterol Mg/dl	47.13 ±9.49	77.88 ±9.28	33.42 ±9.66	33.81 ±7.26	3.370**	2.861**
LDL cholesterol mg/dl	140.50 ±20.51	109.50 ±18.72	158.38 ±20.26	166.63 ±57.58	2.669**	1.754NS
VLDL cholesterol mg/dl	50.88 ±9.98	38.13 ±4.55	59.75 ±10.67	60.00 ±8.72	6.292**	0.557NS

NS = Not Significant; **Significant at 1 % level (p< 0.01); E = Experimental group; C = Control group

The results showed that supplementation with Noni juice led to significant reduction in serum total cholersterol, serum triglycerides, LDL cholesterol and VLDL cholesterol while the HDL cholesterol signiciantly got increased.

Conclusion

The supplementation of Noni juice for 60 days resulted in desirable effect on glucose and cholesterol levels suggesting that Noni juice could be potential hypoglycemic and hypocholesterolemic agent.

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Callus and cell suspension studies of *Morinda citrifolia* L

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Keywords : *Morinda citrifolia* – cell suspension – callus – tissue culture

Abstract : Young leaves from 6-8 months old seedlings of Noni (*Morinda citrifolia* L.) were collected from Centre for Organic Indian Noni (COIN) at Chenglepat, Tamil Nadu. The leaves were constantly stirred with 1:3 sodium hypochlorite: sterile water for 30 minutes. Then the leaves were washed with sterile water for 3-4 times and treated with 0.1% mercuric chloride for 5 minutes. The sterilized leaves were transferred to sterile petriplates and cut into leaf discs of approximately 6mm diameter. The leaf discs were then inoculated to MS medium containing various concentration of 2,4-D. Cultures were incubated for callus initiation at 27 ± 2^{0}C in 16h photoperiod (1200-1500 lux). MS medium containing 2mg/l 2, 4-D produced proliferating golden yellow calli masses within 35-45 days. After several subcultures on semi solid medium, the healthy and fast growing calli of Noni were transferred to liquid MS medium containing 2,4-D for generation of cell suspension culture. The liquid cultures were kept on gyratory shaker at 120 rpm. The liquid cell culture was filtered through stainless steel mesh. 10ml of cells (250-300mg) were inoculated in 30ml of fresh MS medium containing 2mg/l 2,4-D. Thus pipettable cell suspension was obtained after 6-8 subcultures.

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Introduction

Noni, *Morinda citrifolia* L., an economically important plant belonging to the family Rubiaceae, has adapted to wide range of environments for its natural growth and development including flowering and fruit setting round the year. (Nelson, 2002). World Noni Research Foundation (WNRF) reported for the first time about Tissue culture (micropropagation) aspects of Noni (Subramani and Antony Selvaraj, 2006). In continuation of mass propagation of Noni *in vitro*, attempts were made to study the cell culture aspects. The callus and cell suspension culture of Noni were established in Tissue and Cell culture Laboratory at Chennai.

Materials and Methods

Young leaves from six to eight months old seedlings were collected from COIN at Chenglepat (Fig.1). These leaves were washed thoroughly in running tap water for ten minutes. After washing, the leaves were washed again with soap solution (Tween 20), then the explants were stirred with 1% sodium hypochlorite for 30 minutes. After washing with sterile water for 3-4 times, the leaf explants were treated with 0.1% mercuric chloride for 5 minutes and rinsed with sterile water before inoculation. Young leaves were cut to the size of 6mm diameter and inoculated into MS medium containing various concentrations of 2,4-D (2,4-Dichlorophenoxyacetic acid). After inoculation, the culture was stored in dark for 24 h and then exposed 16 h light at 1500 lux and and 8 h of dark. The culture room temperature was maintained at 27 ± 2 ^{0}C and humidity of 55 ± 10%.

Results and Discussion

Callus initiation and maintenance: Young leaves of Noni were cut to the size of 6mm diameter discs (Fig.2). These leaf discs were inoculated in MS basal media containing 2 mg/l 2,4-D. After two weeks of inoculation, initiation of calli all along the midrib and at the cut portions of the discs were observed (Fig. 3). After four weeks, the initiated calli were sub-cultured for further growth. After 3-4 sub-cultures, we could produce friable golden yellow calli masses (Fig. 4). These healthy and fast growing friable calli from the solid media were transferred to MS liquid media containing 2,4-D to initiate cell suspension culture.

Initiation and maintenance of cell suspension: MS liquid medium containing the friable calli masses were incubated in a gyratory shaker rotating at 120 rpm for generation of cell suspension. Liquid cultures were stored at 27 ± 2 °C and 55 ± 10% humidity. Photo period of 16 h light and 8 h dark was maintained. These cultures were sub-cultured at 4-5 weeks of intervals. After 5-6 subcultures, pipettable cell suspension was obtained (Fig. 5). These cell suspension cultures were used for cell plating and then for organogenesis. During the growth period, parameters like fresh weight, dry weight, packed cell volume and cell number of Noni were determined at 3-4 days of intervals with three replicates for each observation.

Cell plating and Organogenesis: From 7-8 months old batch suspension culture of Noni, 12-15 days old culture (*i.e.* expected in the rapidly dividing phase) was filtered successively through stainless steel mess of 320, 140 and 120 sizes. Filtrate of 120 mess size consisted of cells arranged in 3-5 celled linear filaments which were the plating units. They were centrifuged at 300G

for 10 min to remove the spent media and then suspended at a density of 2-3*10 cells /ml in fresh medium (Fig. 6). After 12-15 days of incubation, small calli clumps started appearing in the petriplates. After 30-35 days of incubation, these small calli clumps were sub-cultured for organogenesis.

MS basal medium containing 4 mg /l BAP and 0.5 mg /l kinetin were used for organogenesis. The tiny calli clumps after 25 days of incubation in the above medium started producing *Morinda* shoot (s) (Fig. 7).

Further these calli were sub-cultured to MS medium with 2 mg /l BAP, 1 mg /l kinetin and 0.1 mg /l IAA

Somatic cell genetical approach to produce resistant clone of *Morinda citrifolia* L.

The use of synthetic medium supporting the indefinite growth of plant tissues *in vitro* was first reported in 1939 (White, 1939). Cultured tissues are used as research tools in studying specific problems of plant cell physiology, bio-chemistry, genetics and molecular biology. The potentials for applying microbial selection technique to obtain mutant lines of higher plant cells have been well discussed in numerous reviews (Subramani, 1990; Selvapandiyan, 1998). Selection of cell lines with novel phenotypes is recognized as important in obtaining cultivars useful to agriculture, horticulture and in the elucidation of basic problems. Selection of trait at single cell level, somatic hybridization and transformation *in vitro* have given rise to the field of somatic cell genetics which is the extreme simplicity of experimental selection over conventional genetics. Here the unit of mutation, selection and hybridization is single cell grown *in vitro* in contrast to whole plant in conventional system.

This tremendous reduction in size of experimental system allows to handle numerous experimental units in given space; hitherto an advantage offered previously by organisms like yeast and bacteria. Besides this, other advantages offered by the system of somatic cell genetics are:

Purely homozygous plants even from self incompatible plants could be obtained in one year.

Plants resistant to fungal toxin or herbicide can be produced in shorter period using single cell plating technique as in tobacco (Chaleff and Ray, 1994; Selvapandiyan, 1998)

Selection can be done in highly controlled condition in the single cell plating technique. These results are reproducible.

his type of reproducibility is difficult to achieve in the conventional approach as the selection is done in the open environment which is variable.

Conclusion

Keeping the advantages offered by Somatic cell genetics, callus and cell suspension cultures were established at WNRF laboratory for the improvement of Noni.

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Fig.5 Suspension culture

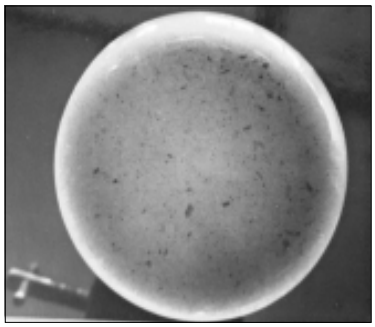
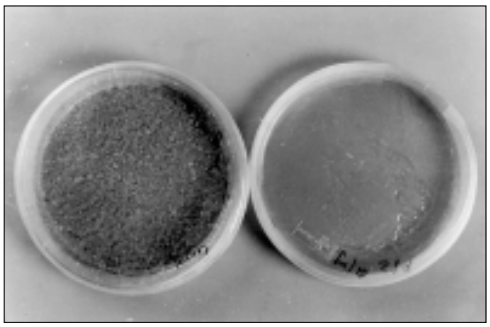


Fig. 6 Cell Plating



S. John Britto, SJ.

A review of the taxonomy of *Morinda L.* (Rubiaceae)

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Abstract : *Morinda* of *Rubiaceae* is a genus of warm tropical countries with 8 species occurring in the India. Recently it has regained its popular use in diverse applications. This taxonomic review of *Morinda* notes its systematic status, distributional patterns, species enumeration, and useful chemotaxonomical compounds. The species in general show a wide spectrum of phenotypic variations. There is an urgent need to assess the genetic status of these variations by adopting molecular tools.

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Introduction

Linnaeus (1753, 1754) circumscribed genus *Morinda* of *Rubiaceae*. The family is cosmopolitan, though the species are mostly confined to the warm tropics. It is one of the most speciose families, especially in the tropics, with about 10,000 species (Mabberley, 1990). Biologically and morphologically the family is diverse, with many different life forms and reproductive traits. The classification of the family and its principal genera are still debated and are likely to be much modified. Presently four subfamilies are recognised (Mabberley, 2008). They are *Chinchonoideae, Ixoroideae, Antirheoideae* and *Rubioideae. Morinda* is placed in sub family *Rubioideae* and in tribe *Morindeae*. The latter is known for syncarpic fruits and heterogeneous. Subfamily *Rubioideae* is characterised by the presence of raphides and heterostyly.

History of Classification

The earlier classifications of *Rubiaceae* follow the classical system of Hooker 1873, Verdcourt, 1958 and Bremekamp 1966. Takhtajan, (1980) in his general outline of the subfamilies proposed a system with five subfamilies. Some recent studies approached the problem of subfamilial classification of *Rubiaceae* using methods other than classical taxonomy. (Lee and Fairbrothers, 1978) adopted serological study. (Kisakurek, Leewenberg and Hesse, 1983) employed the occurrence of alkaloids for the *Cinchoneae-*

Cinchoninae complex. A numerical chemotaxonomical study was devised by (Kiranmai *et al*. 1985), which included only 19 species of *Rubiaceae*. Two other chemotaxonomic studies have used the occurrence of iridoid glycosides (Kooiman, 1969, Inouye *et al.*, 1988). Kooiman's study detected asperuloside and Galium glycosides which occur only in Bremekamp's subfamily *Rubioideae*. Inouye *et al.*'s study confirmed the family classification that has been proposed by (Robbrecht, 1988). Kirkbride, (1982) applied a cladistic interpretation to Verdcourt's, (1958) division into three subfamilies.

Recent systematic studies also support the above divisions which are monophyletic on the basis of molecular sequence data (Bremer *et al*., 1995, Bremer and Thulin 1998, Andersson and Rova 1999, Bremer *et al*., 1999, Bremer and Manen 2000, Fay 2000, Heywood *et al*., 2007). Separation between subfamilies *Cinchonoideae* and *Ixoroideae* from *Rubioideae* is relatively easy. The raphides are almost universally present in *Rubioideae* while in the *Cinchonoideae* they are almost entirely absent. Other than presence of raphides, morphological delimitatins of subfamilies is not as clear cut and the recognition of subfamilies currently depends on combinations of morphological characters rather than synapomorphies (Heywood *et al*., 2007).

Occurrence of *Morinda* in the tropics

Roxburgh (1814) in *Hort. Beng*. had cited the following species for India, namely *M. angustifolia* Roxb., *M. bracteata* Roxb., *M. exserta* Roxb., *M. multiflora* Roxb., *M. scandens* Roxb. and *M. tinctoria* Roxb.

Hooker, J.D. (1880) grouped seven species of *Morinda* from India in two groups. He placed *M. tinctoria, M. angustifolia, M. citrifolia* and *M. persicaefolia* in one group while *M. umbellata, M. rigida* and *M. villosa* were in the second group.

Gamble (1921) had listed five species for the Madras Presidency namely *M. citrifolia, M. angustifolia, M. tinctoria, M. umbellata* and *M. reticulata*.

W.J. Hooker for *Niger Flora* (Western Tropical Africa) enumerated *M. quadrangularis* G. Don, *M. lucida* Benth., *M. longiflora* G. Don, *M. geminata* DC., *M. palmetorum* DC. and *M. chrysorhiza* DC.

Kurz (1877) in Forest Flora of British Burma listed 9 species which are *M. exserta* Roxb., *M. leiantha* Kz., *M. tomentosa* Heyne, *M. citrifolia* L. *M. angustifolia* Roxb., *M. persicaefolia* Buch-Ham., *M. Wallichii* Kz., *M. umbellata* L. and *M. speciosa* Wall. (climber).

Species such as *M.angustifolia* Roxb. are in the Tropical Himalaya, wild and cultivated from Nepal eastwards, ascending to 6000 ft. in Sikkim and are also found in Assam. *M. persicaefolia* Buch.-Ham. is Distributed in Malaysia, Singapore, Burma, Bangladesh while *M. villosa* Hook.f. a climber is found on the Khasia mountains.

Bentham and Mueller (1866) in *Fl. Australiensis* described for Australia *M. citrifolia* L., *M. jasminoides* A. Cunn., *M. umbellata* L., and *M. reticulata* Benth. The latter is characterised by one large coloured leafy bract to each flower head.

Backer and van den Brink (1965) in *Fl. Java* enumarated *M. jackiana* Korth., *M. umbellata* L., M. *sarmantosa* Bl., *M. citrifolia* L. and *M. tomentosa* Roth.

Ridley (1923) in the *Fl. Malay Peninsula* accounted for *M. citrifolia,*L., *M. elliptica, M. scortechinii,* Ridl. *M. Ridleyi* Ridl., *M. rigida* Miq., *M. lacunosa* King and Gamble and *M. umbellata* L.

Merril (1923) in the '*An Enumeration of Philippine Flowering Plants* listed the following species namely: *M. bartlingii* Roxb., *M. bracteata* Roxb., *M. celebica* Miq., *M. citrifolia* L., *M. coriacea* Merr., *M. nitida* Merr., *M. parvifolia* Bartl., *M. philippinensis* Elm., *M. platyphylla* Merr., *M. tinctoria* Roxb., *M. umbellata* L. and *M. volubilis* (Blasco) Merr.

There are other species from adjacent Malay regions such as *M. beccariana* Baill., *M. cinnamomea* Craib., *M. longifolia* Craib., *M. nana* Craib., (Siam) and *M. pumila* Craib.

It is worthwhile noting common species from other tropical regions. The *N. Caledonia species are M. alyxioides Guillaumin, M. ilicifolia* Guillaumin and *M. moaensis* Alain (Cuba). In N. Guinea the following species occur: M. hirtella Merr. & Perrey, *M. morindoides* (Baker) Milne-Redhead, *M. oligocephala* Merr. & Perry, *M. reticulata* Valeton, *M. schultzei* Valeton and *M. gjellerupu* Valeton.

From China *M. brevipes* S.Y., *M. brevipes* Hu var. *stenophylla* Chun and How (Hainan) *M. bowiana* S.Y. Hu., *M. hypeheursis* S.Y. Hu., *M. hainnanensis* Merr., *M. officinalis* How and *M. shuanghuaensis* C.Y. Chen & M.S. Huang are recorded.

The Hawaiian Islands have the following species: *M. lanaiensis* H. St. John var. *glabrata* (Degener) H. St. John var. *horakae* H. St. John, *M. waikapuensis* H. St. John, Verdcourt (1976) in Fl. Trop. E. Africa had described *M. lucida* Benth., *M. asteroscepa* K. Schum., *M. titanophylla* Petit., *M. angolensis*

(Good) F. white and *M. titanophylla* E. Petit, (Congo, Uganda). Thus the worm tropics abound in Morinda species.

Description of Genus

MORINDA

L., Sp. Pl.: 176 (1753) & Gen. Pl., ed. 5: 81 (1754)

A genus of about 80 species distributed throughout the tropics and about 8 species occur in India.

Trees, shrubs or less often lianes, with mostly glabrous, less often hairy of tomentose stems. Stipules leafy, undivided, free or forming a sheath with the petioles. Leaves opposite or rarely in whorls of 3, sometimes only 1 at flowering nodes. Flowers often heterostylous, hermaphrodite or rarely unisexual, in tight capitula. The flowers usually joined, at least by the bases of the calyces, the capitula sometimes bearing single large coloured bracts or occasionally many smaller bracts; capitula 1-several at the nodes, frequently arranged in umbels, pedunculate or rarely sessile. Calyx-tube urceolate or hemispherical, the limb short, truncate or obscurely to distinctly toothed, persistent. Corolla ± coriaceous, funnel-shaped or salver-shaped; lobes (4-)5(-7), valvate; throat glabrous or pilose. Stamens (4-)5(-7), inserted in the throat; filaments short; anthers and style included or exserted. Disc swollen or annular. Ovary 2-4-locular, sometimes imperfectly so; style with 2 short to long linear branches; ovules solitary in the locules, attached to the septum below the middle or near the base, ascending, anatropous or amphitropous. Fruit syncarpous (very rarely scarcely so), succulent, containing several pyrenes; pyrenes cartilaginous or bony, 1-seeded or joined into a 2-4 locular woody structure. Seeds obovoid or reniform, with a membranous testa and fleshy endosperm.

Key to Species in India

1 Climbers	
2 Corolla tube glabrous within	***M. villosa***
2 Corolla tube hairy within	
3 Leaves with elongate acumen at apex. Endemic to Southern Western Ghats	***M. reticulata***
3 Leaves acute at apex. Distribution wider	***M. umbellata***
1 Shrubs or trees	
4 Stipules 2-lobed	***M. citrifolia***
4 Stipules unlobed	
5 Leaf margin undulate	***M. angustifolia***
5 Leaf margin entire	***M. pubescens***

Morinda angustifolia Roxb., Pl. Corom., 3(2): t. 237. 1815; Fl. India. 2: 201, 1824; Hook. f., Fl. Brit. India 3: 156. 1880; Kanjilal *et al*., Fl. Assam 3: 79 1939; Deb in Bull. Bot. Surv. India 3: 311. 1961.

A large shrub or a small tree, 4-10 m tall. Bark greyish, exfoliating in thin pieces; blaze whitish. Leaves coriaceous, large, elliptic or obovate-lanceolate, (6)15-13(22) × 2-10 cm, varying in size and shape, caudate-acuminate at base, margin undulate, shortly acuminate or acute at apex, glabrous; petiole 3.5 cm; stipules acute. Flowers white, fragrant usually 5-merous *ca*. 3 cm long, terminal, long on short peduncles, in globose heads, leaf-opposed, peducle elongating in fruit. Calyx nearly truncate or obscurely toothed. Corolla salver-shaped; tube up to 2.5 cm long. Syncarps 2 cm, turbinate, black.

Distribution: Eastern Himalayas, north-east India, Assam, Bihar, Oirssa and Andhra Pradesh up to 1800 m.

Fl. & Fr. : February – October

The root is used for dyeing.

Morinda citrifolia L., Sp. Pl. 176. 1753; DC., Prodr, 4: 466. 1832; Hook. f., Fl. Brit. India 3: 156. 1880; Gamble 2: 652 (459). 1921. Ridsdale in Manilal, Bot. Hist. Malab. 137. 1980; Manilal & Sivar., Fl. Calicut 144. 1982;

Cada-Pilava Rheede, Hort. Malab. 1: 97-98, t. 52. 1678.

Morinda bracteata Roxb., Fl. Ind. 2: 198. 1824; Thw., Enum. Pl. Zeyl. 144. 1859.

M. citrifolia L. var. *bracteata* (Roxb.) Hook.f. F. Br. Ind. 3: 156. 1880.

Shrub or small tree, 3-6 m tall; trunk not branched, smooth. Branches tetragonal, angles not sharp. Leaves 10-20 × 5-9 cm, elliptic, shining green, glabrous, membranous, 5-6 pairs of distinct lateral nerves, margin entire, apex obtuse or acute-acuminate, base attenuate ending in 1-2 cm long petiole, petiole sinuate; stipules 6-9 × 4-7 mm, rounded, oblong or somewhat semi-circular, glabrous, persistent, often 2-lobed. Inflorescence axillary, usually in every alternate pair of leaves, peduncle *ca*. 1 cm long. Head oblong, 1.5-2,5 × 1-1.5 cm, green, enlarged in fruit, lower flowers of the head open first. Calyx-tube cupular, *ca.* 2 mm in diameter, lobes truncate or obscure. Corolla white, tube *ca*. 12 mm long, cylindrical, glabrous, lobes oblong, 5-6 mm long, recurved, *throat hairy*, pubescent. Stamens yellowish, somewhat exserted. Heads of fruit ovoid, glossy, 4-6 × 3-4 cm, whitish or pale-yellow, pyreness compressed on one side, winged on edges, *ca*. 8 × 5 mm, with basifixed white hairs specially on edges and wing.

Cada-Pilava (*M.citrifolia*) finds a place in Hortus Malabaricus of van Rheede (1678). The details outlined by him, enshrine the indigenous pre-Ayurvedic knowledge of the hereditary medical practitioners of the Kerala state.

Distribution: Warmer parts of India, Sri Lanka, Bangladesh, Malaya, N. Australia and Pacific Islands, cultivated elsewhere. In Pakistan it is cultivated as an ornamental.

Fl. & Fr.: Almost throughout the year.

Its powers: The juice pressed out from the whole plant and cooked with oil from the leaves is good for parts affected by gout, for which also is helpful smearing of the root crushed and mixed with water. Cultivated widely in many places throughout India, found also as an escape, but not truly wild. The roots furnish a valuable red eye.

Morinda pubescens J.E. Smith in Rees. Cyclop. 24: n. 3. 1813.

Leaves narrowly linear-oblong var. **stenophylla**

Leaves broadly obovate-elliptic var. **pubescens**

var. **pubescens:** Verdc. in Kew Bull. 37: 543. 1983. *M. tinctoria* Roxb. Fl. Ind. 2: 197. 1824, non Noronha 1790; Hook.f., Fl. Brit. India 3: 156. 1880; Gamble 2: 652 (459). 1921. *Morinda coreia* Buch-Ham., Trans. Linn. Soc. London 13: 537. 1822; *M. tinctoria* Roxb. var. *tomentosa* (Heyne ex Roth) Hook. f. Fl. Brit. India 3: 156. 1880; *M. tomentosa* Heyne ex Roth, Nov. Pl. Sp. 147. 1821.

Small tree to 6(-10) m, bark thick, rough, fissured and somewhat corky. Branchlets often with a characteristic thin pallid to yellowish bark. Leaves (broadly-) elliptic, less frequently linear-lanceolate or obovate, 6-12(-20) × (1.5-) 3.5-6(-8) cm, above glabrous, below glabrous or tomentose, apex acute to acuminate, base to cuneate, frequently decurrent, lateral nerves 4-9 pairs, domatia in axils usually hairy, rarely glabrous. Stipules deltoid to narrowly triangular, 3-8 mm long, apex acute, sometimes slightly bifid. Petiole up to 3.5 cm. Inflorescence a solitary, axillary or rarely terminal, ovoid or globose, capitulum, often appearing leaf-opposed or subtended by a reduced leaf, pedicels up to 3.5 cm. Flowers 5(rarely 6)-merous. Calyx limb short, truncate. Corolla white, tube 10-15 mm, inside glabrous in the throat, lobes oblong, 6-8 mm long, recurved. Stamens exserted or partially included, anthers oblong, 4-5 mm. Style 10-18 mm, stigma 2-lobed. Syncarp 1.5 - 2.5 cm dia.

Distribution: Sri Lanka, India, Malay Archipelago.

Fl. & Fr.: *May – Nov.*

var. **stenophylla** (Spreng.) Kumari in Henry *et al*. Flora of Tamilnadu 2: 14. 1987. *M. stenophylla* Spreng. Syst. Veg. 1: 749. 1824. *M. tinctoria* Roxb. var. *stenophylla* (Spreng.) Gamble, Fl. Pres. Madras 652 (460). 1921.

A shrub, up to 3 m tall, stem pubescent. Leaves narrowly linear-lanceolate or oblong, 6-12.5 × 1.2-1.8 cm, cuneate at base, entire margin, acute-acuminate at apex, slightly pubescent; petiole very short, 0.6 cm; stipules very large, linear, 1.5 × 0.5 cm. Flowers in axillary cyme, white. Syncarp 0.5 × 0.4 cm.

Distribution: Andhra Pradesh and Tamil Nadu.

Fl. & Fr.: May – Nov.

Morinda reticulata Gamble, Gamble in Bull. Misc. Inform. 248. 1920 & in Fl. Pres. Madras 652 (460) 1921; A.N. Henry *et al*. in J. Bomaby Nat. Hist. Soc. 75(3): 690. 1978; A.N. Henry & Swamin. in Indian J. For. 3(2): 140. 1980; Vajravelu & P. Daniel in Jain & Sastry. Mat. Cat. Threat. Pl. India 24. 1983; Swamin. in A.N. Henry *et al*., Fl. Tamil Nadu Ser. I. Analy. 2: 14 . 1987.

Climbing shrubs, stem terete, slender. Leaves opposite, simple; lamina elliptic to oblanceolate, 2.5-17 × 0.5-5 cm, coriaceous, shining, cuneate to attenuate at base, entire, acuminate (acumen up to 2.5 cm long) at apex; nerves 5-12 pairs, midrib prominent, raised beneath, less prominent and sunken above; lateral nerves prominent beneath less above, curved upwards, joined and looped towards the margin, reticulation fine. stipules small, connate at base; petioles upto 12 mm long, grooved adaxially, rounded abaxially, turgid at base; Inflorescence terminal, compound umbels; peduncles 10-25 mm long, glabrescent; bracts small, cupular, glabrescent. Flowers sessile or subsessile, bisexual; calyx-tube saucer-shaped, *ca*. 1 mm long, glabrous, lobes 0, rim wavy, glabrous. Corolla tube short, up to 1 mm long; lobes 4, linear-oblong, *ca*. 3 × 1.5 mm, thick, cottony wooly within, glabrous without, entire, obtuse and mucronate at apex, incurved. Stamens 4, alternipetalous; filament short *ca.* 1 mm long, glabrous; anthers oblong glabrous; anthers oblong-linear, *ca*. 2 mm long, 2 loculed, introrse, dorsifixed. Ovary semi-inferior, glabrous, small, globose, *ca*. 0.5 × 0.5 mm. Fruits globose, 9-18 × 9-18 mm, syncarpous, pyrenes pyriform. Seeds oblong-obovoid, not winged.

Distribution: A very rare climber in evergreen forests. Southern Western Ghats. 200-850 m. Tamil Nadu (Kanniyakumari district) and Kerala (Thiruvananthapuram district) Endemic.

Fl. & *Fr.*: June- October.

Morinda umbellata L. Sp. Pl. 176. 1753; Hook. f., Fl. Brit Ind. 3: 157 1880; Gamble 2: 652 (460) 1921) Ridsdale in Manilal, Bot Hist. Hort Malab. 137. 1980. – *Morinda pada-vara* Juss. ex Schult. in Roem. & Schult., Syst. Veg. 5: 216. 1819. *Morinda tetrandra* Jack, Malayan Misc. 1(5): 13. 1820. nom. illeg. (incl. type of *M. pada-vara* Schult. 1819).

Pada vara Rheede, Hort. Malab. 7: 51-52, t. 27. 1688.

A climbing or straggling shrub, up to 3 m tall. Leaves coriaceous, shining, broadly obovate-elliptic or linear-lanceolate, 6-17.5 × 2-9 cm, cuneate-acuminate at base, margin entire, acute at apex, glabrous or pubescent above, tomentose below, nerves very distinct; petiole 3 cm; stipule connate, acuminate. Flowers in terminal umbellate heads, white, peduncle pubescent. Calyx *ca*. 1 mm long, truncate. Corolla tube *ca.* 1 mm long. villous within, lobes 4, recurved. Stamens 4. Syncarp 1.2 cm wide, occasionally separate, irregularly lobed 0.5 × 4 cm, green; pyrenes pyriform. Seeds oblong.

Distribution : Sri Lanka, India, through to China, Japan, N. Australia

Fl. & Fr. : January to September.

The name Padavara (Malayalam script on t. 27) is still used, meaning of which is not clear. It is also called Kudalchurukki; kudal means intestine and churukki means to shrink, referring to its use as medicine for intestinal disorders. It occurs in sacred groves through out Malabar.

Morinda villosa Hook. f., Fl. Brit. India 3: 158. 1880; Kanjilal *et al*., Fl. Assam 3: 80 1939.

A Climbing shrub; branches more or less hirsute, longitudinally ribbed. Leaves 2-10 × 2.5-3.5 cm, elliptic to elliptic-lanceolate, pubescent above, hirsute beneath, pale greenish when dry; lateral nerves prominent, 10-15 on either half; base narrowed into the petiole. Flowers usually 4-5-merous in terminal, long pedunculate umbels. Corolla white, tube short. Syncarp deep orange, 1.5 cm across.

Distribution: Khasi Hills Cachar, Kameng, Tirap; 500-800 m, in primary forests.

Fl. & Fr.: April - Oct.

Phytochemical studies

These studies are being extensively undertaking in order to exploit their usefulness. The data also enable the taxonomic delimitation of the species.

M. angustifolia has revealed the presence of Rhein, aloe-emodin and morindone isolated from heartwood; ursolic acid and rutin isolated from leaves (*Indian J. Pharm. Sci* 1978, 40. 169). *M. citrifolia* is characterised by the following: Acacetin -7 O-ß-D glucopyranoside and 5,7 –di-O-methylapigenin-4'-O-ß-D-galactopyranoside (I) isolated from flowers (J. Indian Chem. Soc. 1976. 53, 424); glycoside (II) isolated from flowers charaterised as 6,8 dimethoxy-3 methylanthraquinone-1-O-ß-rhamnosyl (1) glucopyranoside (*J. Indian chem. Soc*. 1977 54 429) asperuloside, glucose, caprylic acids identified in ripe fruits *Planta Med.* 1970) 36. 186). Anthraquinones – nordamnacanthal (1.7), morindone (0.5%), rubiadin, rubiadin-1-methyl ether – and a pigment, mp. 295°, from roots (*Aust. J. Chem*. 1962, 15. 332). Ursolic acid and ß-sitosterol isolated from leaves (*J. Chem. Soc*. Pakistan 1980, 2, 71; *Chem, Abstr*. 1981, 94 27438b). Seed oil contained ricinoleic acid (6.8%) *J. Oil Techno. Assoc. India* 1989, 21 26: *Chem. Abstr. 1990, 113,* 148862 e); two new anthraquinones – moreone1 and morenone 2- isolated from roots and their structures elucidated (*Proc. Natl. Acad. Sci India* 1992, 62A 11: Chem. abstr. 1994 121 175126 q); another anthraquinoe from heartwood and its characterisation as physcion-8-O [{. a-L-arabinopyranosyl (1 3)} {ß-D-galactopyranosyl (1 6) }-ß-D- galactopyranoside] (Int. J. Pharmacog. 1993, 31. 182. *Chem Abstr.* 1994 120 265759 g).

M. pubescens is noted for the presence of Primeveroside of morindone isolated from root bark (*Indian J. Chem*. 1977, 15B. 497).
Morindone, damnacanthal and nordamnacanthal (1,3-dihydroxy-2-formylanthraquinone) from heartwood (J. Sci. Ind. Res. 1959, 18B. 367); morindone diglucooside (morindomin), mp. 255°, isolated from root bark and characterised (*JSci. Ind. Res.* 1960. 19B, 433) In addition to morindone, damnacanthal and nordamnacanthal, a new anthraquinone ester – tinctomorone – isolated from heart wood and its structure established established (*Indian J. Chem.* 1979, 17B. 650); morindone synthesised (*Phytochemistry* 1980, 19, 2493); morindone, damnacanthal, nordamnacanthal and ß-sitosterol isolated from roots (*J. Inst. Chemists, Calcutta* 1982, 54, 22; *Chem. Abstr*. 1982, 96 159377 g); ursolic acid isolated from leves; alizarin-1- methyl ether, rubiadin and mannitol from stem bark and anthragallor-2-3-dimethyl ether, soranjidiol, ibericin and morindone-6- primeveroside from root bark (*J. Indian Chem. Soc*. 1983, 60, 585); fatty acids (C16-22_ campesterol, stigmasterol and ß-sitosterol isolated from oil (*Plant. Med. Phytother*. 1980 14 29 *Chem. Abstr*. 1980 93, 191886 w).

M. reticulata, a less known species shows the following: n-Triacontano, a-amyrubm ß-sitosterol glucoside, alanine, arginine, glutamic acid and glycine isolated from leaves (*J.Sci. Res. Plants Med*. 1981, 2, 107; Chem. Abstr. 1982, 97, 178768 x).

M. umbellata has revealed compounds such as 2-Hydroxyanthraquinone, alizarin, alizarin 1-Me ether, rubiadin, rubiadin 1-Me ether, xanthopurpurin, alizarin 2-Me ether, 1-hydroxy-2-methylanthraquinone, 2-methylanthraquinone, munjistin and lucifin from roots and stems (*Phytochemistry)* 1968 7. 1421)

Conclusion : This review has outlined the taxonomy of six commonly known Indian species. Among them *M. pubescens* and *M. citrifolia* are much in demand for their economical usefulness. However their taxonomy poses big challenge owing to wide spectrum of variations indicating their phenotypic and genetic traits. Their taxonomic problem can be solved to a large extent by adopting molecular tools.

Rubiaceae

Plate 1. ***Morinda angustifolia*** Roxb. : habit.

Plate 2. *Morinda citrifolia* L.: 1 &10. habit; 2. inflorescence; 3. flower; 4 & 5 corolla, split open; 6. stamen; 7. pistil; 8 & 9. ovary, t.s. & l.s.; 11. syncarp; 12. seed.

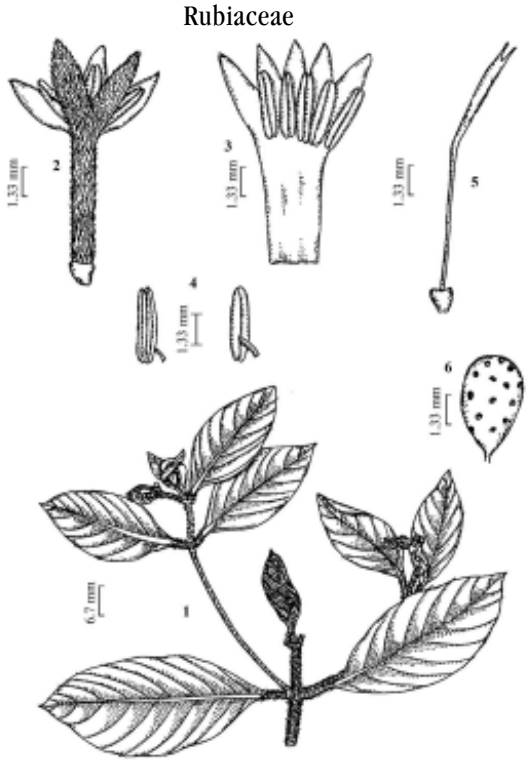

Plate 3. *Morinda pubescens* J. E. Smith. var. *pubescens*: 1. habit; 2. flower; 3. corolla split open; 4. stamen; 5. pistil; 6.syncarp.

Rubiaceae

Plate 4. *Morinda* reticulata Gamble: 1. habit; 2. flower; 3 & 4. calyx; 5. corolla split open; 6. stamen.

Rubiaceae

Plate 5. ***Morinda umbellata*** L.: **1**. habit; **2** & **3** flower (**5** & **4** corolla-lobes); **4**. corolla, split open; **5**-**7** stamen; **8**, **9** & **12** pistil: entire, l.s. & t.s.; **10**, **11** syncarp: **13**, **14** seeds.

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