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Research Review

Current Horticulture **6**(1): 3–14, January–June 2018 ISSN: 2347-7377; RNI No.: UPENG03865/24/1/2013-TC

Management of genetic resources of perennial horticultural crops: a review

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ABSTRACT

India has a rich and varied heritage of biodiversity, encompassing a wide spectrum of habitats from tropical rain forests to alpine vegetation and from temperate forests to coastal wetlands. Out of 18 biodiversity hot spots identified in the world, four hotspots, i.e. The Western Ghats, Eastern Himalaya, Western Himalaya, and Nicobar islands are in India. Besides, India has 26 recognized endemic centres which are home to one-third of all the flowering plants identified and described so far. There are 8.7 million species of the world's biota. Of them, only 1.7 million have been described to date, and their distribution is highly uneven. About 7% of the world's total land area is home to half of the world's species, with the tropics alone, accounting for 5 million. India contributes significantly to the biodiversity of the world, accounting 7.31 % of the global plant diversity from 2.4% of the world's area.

Key Words: Genetic resources, Perennial horticultural crops, Biodiversity, Habitats, Tropical rain forests, Alpine vegetation, Temperature forests, Coastal watlands

India has two major realms called the Palaearctic and the Indo-Malayan, and three biomass namely, tropical humid forests, tropical dry/deciduous forests, and the warm desert/semi-deserts. The endemism of Indian biodiversity is high. About 33% of the country's recorded flora are endemic to the country and are concentrated mainly in the North-East, Western Ghats, North-West Himalaya and the Andaman and Nicobar islands. Of the 49,219 plant species, 5,150 are endemic and distributed in 141 genera under 47 families, corresponding to about 30% of the world's recorded flora, which means 30% of the world's recorded flora is endemic to India. Of these endemic species, 3,500 are found in the Himalayas and adjoining regions and 1,600 in the Western Ghats alone. India is a centre of crop diversity — the homeland of 167 cultivated species and 320 wild relatives of crop plants. India's record in agro-biodiversity is equally impressive that it has 167 crop species and wild relatives. It is considered to be the centre of origin of 30,000-50,000 varieties, comprising rice, pigeonpea, mango, turmeric, ginger, sugarcane, gooseberries *etc.* and ranks seventh in terms of contribution to world agriculture. India is one of the 17 mega diverse countries of the world, holding approximately 8% of global biodiversity with about 45,000 plant species in 16 agroclimatic zones.

A large number of crops such as cucumber, brinjal, melon, mango, banana, coconut, black pepper, ginger, turmeric, cardamom *etc.* are important horticultural crops native to India (Table 1). Historically, the Kings and rulers used to conserve many of this diversity in then gardens around their palaces or in the vicinity of temples. Documented evidences indicate that the orchard in Lakhbagh in Darbangha established by Mughal Emperor Akbar consisted of one lakh mango trees, proving eloquently the interest the Mughal emperors had in making selections for quality. The Mughal gardens in Kashmir, Punjab, Delhi and Uttar Pradesh are testimony to their contributions to floriculture. The conservation of diversity and plants of different species in Botanic Gardens, on the pattern of Royal Botanic Garden at Kew, was replicated by Britishers. Other introductions are pineapple through Philippines, papaya, guava, cashew, chili and tomatoes were the introductions. Grapes were an introduction by Mughals in 1300. Grape was also introduced in southern part of the country into Salem and Madurai districts of Tamil Nadu by the Christian missionaries around 1832.

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Table 1. Diversity of world plant species

Approximate number of plant species	13-14,000,000
Number of described plant species	1,750,000
Number of higher plant species	300,000 to 500,000
Approximate number of edible plant species	75,000
Number of plant species used for food	7,000
Commercially important plant species	150
Plant species producing 90% of calories in human diet	30
Crop species producing 60% of global food requirement (rice, wheat, maize)	3

Sources: Wilson (1992); Dhillon and Saxena (2003); Engels and Visser (2006)

The father of modern conservation of Plant Genetic Resources in India is Prof. Harbhajan Singh who laid emphasis on introduction of germplasm which was later acclimatized and directly released for commercial cultivation as primary introductions or after selections or utilized as donor for specific traits in breeding varieties. His leadership during late-forties to mid-fifties resulted in selection of 60 varieties in 27 vegetable crops. Some of them, Golden Acre (cabbage), California Wonder (capsicum), Nantes (carrot), Arkel (garden pea), Japanese White (radish), Sugar Baby (watermelon) and Contender (French bean) are still popular in many parts of our country due to their farmer-and consumer-friendly characteristics. The Division then graduated to become a separate world famous institute called National Bureau of Plant Genetic Resources.

Table 2. India-centre of diversity of horticultural crops

Fruits	**Primary centre:** Mango, citrus, jackfruit, bael, aonla, ber, khejri, jamun, tamarind, phalsa, lasora, karonda, wood apple, pilu, bilimbi, Garcinia
	Secondary centre: Banana, pomegranate, mulberry, *Malus, Pyrus, Prunus, Rubus*
Vegetables	**Primary centre:** Brinjal, smooth guard, ridge guard, cucumber, parwal, *Amaranthus, Basella,* sword bean, winged bean, kundru, Dolichos bean, Indian lettuce, drumstick.
	Secondary centre: Cowpea, okra, chilli, pumpkin and Brassicas
Ornamentals	**Flowers:** orchids, rhododendrons, musk rose, begonia, balsam, globe amaranth, glory lily, foxtail lily, primula, blue poppy, lotus, water lily, clematis, tulip
	Trees: Kachnar, amaltas, pink cassia, *Butea,* India coral tree, Pride of India, Scarlet cordial, yellow silk cotton tree, karanj, tecomella, tulip tree, chalta, sita ashok, arjun, milchelia, kadamba, maulsari.
	Shrubs and climbers: *Jasmine, Ixora, Hamiltonia, Clerodendron, Crossandra,Plumbago, Tabernamontana, Trachlospermum, Passiflora, Clitoria, Porana, Gloriosa, Clematis*
Plantation crops, spices and condiments	Pepper, greater galangal, Bengal cardamom, Anethum, sowa, ajowain, cinnamon, cumin, curcuma spp., curry leaf, long pepper, betelvine, long pepper, ginger, Indian cinnamon, Indian tamarind, kokum and tamarind.
Tuber crops	Greater yam, lesser yam, potato yam, elephant-foot yam, yam bean, winged bean, alocasia, giant taro, colocasia.
Medicinal and aromatic plants	Muskdana, belladonna, jamalgota, Malabar grass, rosha grass, citronella, grass, lemon grass, datura, puskarmul, jasmine, saya, isabgol, patchouli, sarpagandha, sandal wood, costus, nuxvomica, Indian almond, vetiver, kutaki, bank-kakri, asparagus, atees, vatsnabh, Indian ginseng, ashoka, arjuna, bijayasal, kurchi, neem, guruchi, lodhara

Source: Singh *et al.* 2009

Today India holds the world's largest germplasm in many crops, notable among them being coconut, areca nut, black pepper, mango, cardamom, cashew and many vegetable crops.

The National Active Germplasm Sites (NAGSs) is a unique feature in our system and a large number of germplasm are being maintained in these sites. Different field gene banks maintain a total of about 73,000 genetic resources having 9,240 accessions of fruits, 25,400 of vegetables and tuber crops, 25,800 of plantation crops and spices, 6,250 of medicinal and aromatic plants, 5,300 of ornamental plants, and 984 of mushrooms. There is International Gene Bank operating in crop like coconut which is hugely difficult to manage since they occupy a large area. There are about 129 horticultural crops on which NAGS works and the genetic resources are being maintained by various ICAR institutes and SAUs (Table 2).

Horticultural genetic resources (HGR) are a subset of agro-biodiversity that is related to horticultural plant species or their wild gene pool, having genetic material of actual or potential value. Horticultural plants comprise groups of important crop commodities which include fruits, vegetables, spices and condiments, ornamental plants, and aromatic and medicinal plants. These groups of crops, besides improving biological productivity and nutritional standards also have enormous export potential (Table 3).

Table 3. Genetic resources of horticultural crops

Crop	Total accessions
Fruits and nuts	7,084
Vegetables (including onion and garlic)	20,053
Ornamentals (including orchids)	3,499
Spices (including seed spices)	8,785
Plantation crops (including oil palm and cashew	2,709
Medicinal and aromatic plants+ RET species	2,570
Tuber crops and potato	10,094
Mushrooms	2,692
Total	57,486

Source : Ganeshan, 2015

GENETIC RESOURCES OF FRUIT CROPS

MANGO

Mango (*Mangifera indica* L.) is most important fruit crop in India having socio-economic significance. Mango originated as alloploid and its native home was recommended as Eastern India, Assam to Burma or possibly further in the Malay region. Introduction of superior types into Malay region from India is also an evidence of its origin in India. Based on detailed study of the history, phyto-geographical distribution of allied species, fossil records, and evidence of numerous wild and cultivated varieties in India, researchers considered origin of genus *Mangifera* probably in Burma, Siam, Indo-China and the Malay peninsula, but the birth of common mango is in Assam-Burma region and not in the Malay.

India is one of the world's richest germplasm centres where mango has been a predominant fruit crop for thousands of years. Genus *Mangifera* belongs to the family Anacardiaceae and almost all the commercial cultivars of mango are included in a single species *Mangifera indica*. However, a few commercial cultivars of South-East Asia belong to other edible species such as *M. altissima, M. caesia, M. cochinshinensis, M. foetid, M. griffithii, M. lagenifera, M. londipes, M. macrocarpa, M. odorata, M. pajang, M. pentandra, M. sylvatica* and *M. zeylanica.* There are 41 species in genus *Mangifera* with varied reports about number of species in this genus. Out of these, five species namely, *M. andamanica, M. indica, M. khasiana, M. sylvatica* and *M. comtosperma* have been reported from India.

A lot of genetic erosion in mango germplasm has taken place due to urbanization, industrialization and resultant felling of trees. On the other hand, most of the genepools have remained unexplored with regard to the study of the extent of variability in order to identity, collect and conserve the valuable germplasm *in-situ* or *ex-situ* for direct use as cultivars or in breeding programme to impart desirable traits to the commercial cultivars. The species of *Mangifera* occur mainly as complex biotic community in tropical humid forests, subtropical rain forests and tropical dry forests of Indo-Malayan biogeographic realm. For *in-situ* conservation, the region in hills of east Orissa, forests bordering Burma in Manipur valley which have rich genetic variability in wild forms of *M. indica* have been identified. There is urgent necessity to take measures for *in-situ* conservation of 15 species belonging to endangered, vulnerable and rare categories.

Substantial diversity of cultivated *M. indica* is being conserved in field gene banks at several centres in India, although very less variability of wild forms or other *Mangifera* species is represented in these collections like *M. Cambodiania, M. cochinchinensis, M. odorata* and *M zeylanica* also exist in few collections but the endemic India species like *M. sylvatica, M. andamanica, M. khasiana* need to be collected. Exploratory surveys were carried out at the Andaman and Nicobar islands to locate the genetic diversity and distribution of wild and indigenous mango species within the Islands. The indigenous mango species like *Mangifera andamanica, Mangifera griffithi* and *Mangifera camptosperma* were

found distributed in specific locations. The National Collection Centres have now been identified at Central Institute of Subtropical Horticulture, Lucknow, and Indian Institute of Horticultural Research, Bengaluru, to maintain the germplasm of mango. Besides this, many agricultural/horticultural universities are also maintaining the germplasm of mango.

In-vitro gene bank for mango is still not feasible since attempts to standardize tissue culture technique for mango are faced with problems of browning of both tissue and medium within few days of inoculation due to leaching of phenolic compounds from vegetative tissue into the culture media. Use of pollen storage and exchange of mango germplasm through pollen have a lot of advantages. This may be useful in hybridization programme designed to transfer genes for fruit colour to local varieties and the time lag can be shortened by the use of pollen rather than introduced bud wood.

BANANA

India is having vast diversity for banana and plantains, but only a few varieties/landraces are cultivated commercially and banana trade is dominated by only one or two cultivars especially Cavendish type. Genetic diversity of genus *Musa* comprising seeded wild species to seedless cultivars with various levels of ploidy, *viz.*, 2x, 3x, 4x *etc.* and different genomic compositions like AA, AB, AB, ABB, BB, ABBB, *etc.* In India, wild Musa species are largely distributed in the North-Eastern states, the Western and Eastern Ghats and Andaman and Nicobar Islands. The genus *Musa* has been classified into four major sections, namely *Eumusa, Rhodochlamys, Callimusa* and *Australiamusa,* but majority of the cultivated bananas originated from *Eumusa.* This is the biggest section in the genus and the most geographically widespread, with species being found throughout South East Asia from India to the Pacific Islands (Horry *et al.*, 1997). In India, more than 11 species have been reported including *M. acuminata* ssp. *Burmannicca, M. acuminata* ssp. *Burmannicoides, M. sikkimensis, M. balbisiana, M. nensium, M. thomsonii, M. itinerns, M. ochracea, M. flaviflora etc.*, which are widely distributed in the North-Eastern India.

Table 4. Distribution pattern of wild *Musa* species, Rhodochlamys and allied genus Ensete in India

Species	Distribution
M. ac.ssp.burmannicca	Western Ghats
M.ac.ssp.burmanicoides	Western Ghats and North-eastern India
M. balbisiana	Western Ghats, Andaman Nicobar Island and North-eastern India
M. itinerans	Arunachal Pradesh
M.cheesmanii	Arunachal Pradesh
M.ochracea	Tripura and Manipur
M.flaviflora	Arunachal Pradesh, Assam, Tripura, Mizoram, Meghalaya and Manipur
M.sikkimensis	Arunachal Pradesh, Nagaland, Manipur, Tripura and Meghalaya
M.nagensium	Nagaland and Mizoram
M. thomsonii	Nagaland and Mizoram
M. swarnaphalya	Arunachal Pradesh
M.saddlensis	Arunachal Pradesh
M.kuppiana	Arunachal Pradesh
M.velutinasubsp.markkuana	Arunachal Pradesh
M.velutinavar.variegata	Arunachal Pradesh
M. sabuana	Arunachal Pradesh
M.nagalandiana	Nagaland and Manipur
M.balbisianavar.andamanica	Andaman and Nicobar Island
Musa arunachalensis	Arunachal Pradesh
M. laterita	Western Ghats and Assam
M. aurantiaca	Arunachal Pradesh
M.velutina	Assam and Arunachal Pradesh
M. ornata	Tamil Nadu, Andhra Pradesh, Mizoram
M. rsaceae	Arunachal Pradesh
Ensete superubum	Western Ghats
Ensete glaucum	North-eastern India

Though there are many species in the section Eumusa, only *M. accuminata* and *M. balbisiana* are believed to have contributed to the evolution of resent day cultivated bananas which are sterile and parthenocarpic. The National Collection Centre for Banana is at National Research Centre for Banana, Trichy. Besides this, many agricultural / horticultural universities are also maintaining the germplasm of banana (Table 4).

GUAVA

Guava (*Psidium guajava* L.) is indigenous to tropical America, where it occurs in wild as well as in cultivated forms. It was common in many parts of the West Indies and that the improved forms were planted by the local people (Purseglove, 1981). It then spread throughout the tropics and has been naturalized in many countries. It has become a troublesome weed in Fiji. Burton, who visited in the early seventeenth century, mentioned the presence of guava trees in India in his memoirs. At present, it is grown throughout the length and breadth of the country from sea level to 1,300 m altitude and is so much acclimatized that it appears to be native of India.

The most important guava growing States are Uttar Pradesh, Bihar, Madhya Pradesh and Maharashtra. The genus Psidium of Myrtaceae family comprises about 150 species of small trees and shrubs (Hayes, 1970). About 20 species have edible fruits of which the most commonly cultivated is the common guava, *i.e. Psidium guajava* L. The value of the wild *Psidium* species mainly lies in their utilization as rootstocks for regulation of vigour, bearing, fruit quality and resistance to pests and diseases. *P. cujavillis, P. mole, P. cattlianum* and *P. guineense* can be used as rootstock (Mitra and Bose, 1990).

About Total 137 guava accessions are maintaining in field gene bank excluding Psidium species. Central Institute of Subtropical Horticulture, Lucknow and Indian Institute of Horticultural Research, Bangalore are NAGS for guava and maintaining guava germplasm. Apart from these, several universities also maintain germplasm of guava for conservation and utilization.

CITRUS

Citrus occupy an important position in India's fruit production. Citrus fruits, which include mandarin, sweet oranges, lemon, lime, pummelo *etc.*, are primarily consumed as fresh fruits and also processed, mainly to prepare squash, juice, marmalade and pickles. The indigenous Citrus species of India are *Citrus limon, Citrus karna, Citrus indica, Citrus nobilis, Citrus sinensis, Citrus assamennsis, Ciltrus limonica, Citrus megaloxycarpa, Citrus ichangensis, Citlrus latipes, Citrus macroptera, Citrus madurensis, Ciltrus limettiodes, Citrus rugulosa, Citrus pennivesiculata, Citrus medersapatana and Citrus nakoor*. During evolution, a remarkable diversity in citrus has developed due to natural hybridization and cultivation since ancient times.

There was a long period of progressive evolution of the genus citrus. It has become difficult to ascertain the centres of origin of most of the citrus cultivars because of natural hybridization. Both inter-specific and inter-generic hybrids have made the identification of citrus species more difficult. The germplasm has not been collected thoroughly for want of proper survey and utilization goals.

The indigenous germplasm and potential varieties with respect to physiographic conditions of growing area on a global basis needs to be conserved in situ, and collected for the expansion of gene banks for utilization in the future. There is a huge diversity in citrus in the north-eastern region of India and is also considered as one of the major centers of citrus diversity. The region holds an important position with respect of citrus wealth. Favorable climatic conditions aiding in easy hybridization amongst different species and genera, has brought about numerous forms of citrus growing in wild and semi-wild condition.

Yet very little attention has been given for the characterization and evaluation of citrus germplasm of this region. The region has remained isolated for a long time. Even today, the accessibility is rather poor in many parts of this region. Central Institute of Citrus Research, Nagpur is NAGS centre for Citrus and maintaining larger collections of citrus species. Apart from these the regional stations in different regions such as Chettalli, Ludhiana, Jorhat Akola, Parbhani, Tirupati, Periyakulam and some universities are also maintains the regional diversity of their regions.

GRAPE

Grape (*Vitis vinifera* L.) belongs to order vitals and family Vitaceae. Most of the commercial cultivars belong to Vitis family. Species of *Vitis* family persist with diploid chromosome number 38 except *Vitis rotundifolia* (muscadine grape) which contains 2n=40 chromosomes. Some of *rotundifolia* species have classified under separate genus, *Muscadinia*. Commercially grown varieties belong to *Vitis vinifera* and *Vitis labrusca*, whereas species *V. riparia, V.berlandieri, V. rupestris, V. champinii* and their hybrids are used as rootstock.

Major grape-growing countries are China, Italy, United States of America, Spain, France, Turkey, Chile, Argentina, India and Iran. In India, consumption and production of table grapes are comparatively on higher side than the wine grapes. In India, National Research Centre for Grapes, Pune, is the National Active Germplasm Site for grapes. It comprised 437 grape

accessions which includes; 110 indigenous, 305 exotic, 22 rootstocks. The available genetic resources of grape have been utilized in investigating the existing genetic variability.

POMEGRANATE

Pomegranate (*Punica granatum* L.) is widely cultivated in arid and semi-arid regions of the world. It is known to be dry and hardy fruit crop. It belongs to Lythraceae family consisting of two species *viz.*, *Punica granatum* L. and *Punica rotopunica* Balf. During the last decade, the area and production under pomegranate cultivation was rapidly increased because of concerted research efforts, value addition, outreach to farmers and marketing advisories. The National Research Centre on Pomegranate, Solapur, (Maharashtra), is national repository has 304 germplasm in the Field Gene Banks (FGBs). This includes 210 indigenous collections covering indigenous wild collections of North Eastern states and western Himalayas, cultivated and local types besides 94 exotic collections of USA, Turkey, Russia, Iran, Japan, Italy and Afghanistan. Recently, 120 USDA accessions have been collected from California, USA, by ICAR-IIHR, Bengaluru, through NBPGR, New Delhi.

Pomegranate cultivated types belongs to *Punica granatum* L. with two sub-species, *viz.* chlorocarpa and porphyrocrpa. Apart from edible types, ornamental types are also exists. The ornamental types are with double red flowers, largely sterile and no grown for edible fruit. Besides, there is a dwarf statured pomegranate which grows only up to 2-2.5 ft. with profuse, miniature flowers and small sized fruits called "nanna". Wild pomegranates are highly acidic types, popular as souring agent (anardana). "Bhagwa"is the most popular variety which occupies nearly 80% pomegranate area in India, due to its attractive red rind and red arils with low acidity (%). Super Bhagwa, Ganesh, Arakta, Mridula, Ruby, Dholka, Kandhari, Jyoti, G-137, Jallore Seedless, Jodhpur Red are the other important commercial varieties grown in different states of India. The available genetic resources of pomegranate have been utilized in investigating the existing genetic variability for different morphological, biochemical and molecular characters.

SAPOTA

Sapota was introduced in to India from Central and South America, specifically from the peninsula of Mexico. Its commercial cultivation was first taken up in Maharashtra during 1898. Since it is an introduced crop the natural seedling diversity is observed in pocket like, Gujarat, North Karnataka including Goa, Maharashtra. Important states of India where sapota is cultivated in commercial scale are Karnataka, Gujarat, Tamil Nadu, Maharashtra, Andhra Pradesh and West Bengal. The IIHR is National Active Germplasm Site (NAGS), and fifty accessions are maintained in field gene bank.

CUSTARD APPLE

Custard apple (*Annona squamosa* L.) was introduced in India from tropical America and found in wild form in many parts of the country. Custard apple growing regions in India includes Assam, Bihar, Madhya Pradesh, Maharshtra, Odisha, Rajasthan and Uttar Pradesh, Andhra Pradesh, Telangana and Tamil Nadu. Approximately 22,000 hectares area is under custard apple cultivation in India. Among the 166 species of Annona, six species such as *Annona squamosa, Annona reticulata, Annona atemoya, Annona cherimola, Annona glabra* and *Annona muricata* are commercially important and edible. At present, 10 varieties of custard apple and six species of Annona are conserved under in situ condition for its utilization in genetic improvement of custard apple at IIHR, Bangalore.

TEMPERATE FRUITS AND NUT CROPS

Temperate fruits comprising of pome (apple, pear and quince) and stone (cherry, apricot, peach, plum, nectarine) along with nuts (walnut and almond) contribute significantly to national economy. These crops adapted to the Himalayan ecosystem have high degree of biodiversity. Their cultivation is restricted to temperate region of India like Jammu and Kashmir, Himachal Pradesh, Uttarkhand, Arunachal Pradesh *etc.* Biodiversity in temperate fruit crops lies with the availability of alternate crops like Cape Gooseberry, Rose Hops, Minor nuts (Hazel Nut, Pecan Nut, Pistachio Nut) *etc* having chilling requirements which are met under temperate conditions. Thus adoptability of these crops will help in combating the challenges of climate change and thus act as alternate crops. Different biodiversity conservation sites in the form national repository is being planned by NBPGR and CITH for maintaining and conserving the biodiversity of temperate fruits and nuts at different places to ensure the security of germplasm.

ARID ZONE FRUITS

To harness genetic variability for varietal improvement of arid fruit crops such as ber, pomegranate, bael, aonla, custard apple, date palm, phalsa, lasoda, wood apple, tamarind, cactus pear, karonda, jamun, salvadora and fig, it is imperative to build a rich germlasms bank. The ICAR-Central Institute for Arid Horlticulture, Bikaner, Rajasthan ever since its inception is striding forward in this direction and has one of the richest germplasm pool of underutilized arid fruits. Besides, it has also been recognized as National Active Germplasm Site (NAGS) for arid fruits (Table 5).

Table 5. Status of germplasm of arid fruits at NAGS at CIAH.

Name	Scientific name	No. of Accessions
Ber	*Ziziphus mauritiana*	373
Bordi	*Z. roltundifolia*	22
Pomegranate	*Punica granatum*	195
Custard apple	*Annona squamosa*	09
Aonla	*Emblica officinalis*	24
Date palm	*Phoenix dactylifera*	64
Bael	*Aegle marmelos*	57
Jamun	*Syzigium cumini*	52
Tamarind	*Tamarindicus indica*	25
Cactus pear	*Opuntia ficusndica*	20
Phalsa	*Grewia subinaequalis*	8
Fig	*Ficus carica*	8
Mulberry	*Morus spp.*	15
Marula nut	*Sclerocarya birrea*	01
Mahua	*Madhuca latifolia*	50
Chironji	*Buchanania lanzen*	30
Khirni	*Manilkara hexandra*	30
Karonda	*Carissa carandus*	48
Lasora	*Cordia myxa*	65
Pilu	*Salvadora spp.*	02
Ker	*Capparis decidua*	06
Manila tamarind	*Pithocelobium dulce*	03
Wood apple	*Feronia limonia*	12

Source : CIAH, Bikaner

TROPICAL UNDERUTILIZED FRUITS

There are several fruits originated and naturized in the tropical humid region of India. These are either wild or cultivated in very limited area but these have potential to be a major fruit due to their nutritional value or other attributes. Among these mangosteen, durian, rambutan, avocado, kokum, Malabar tamarind, yellow mangosteen, kronda, Maayan apple, rose apple, langsat, egg fruit, carambola, dragon fruit, velvet apple, longan, macadamia nut, pummello, sour sop, pulasan, bilimbi, hog plum are important ones. A large number of accessions of these fruits have been collected and maintained in the field gene bank at IIHR and its regional stations for conservation and utilization.

CASHEW NUT

Cashew (*Anacardium occidentale* L.) belongs to the family Anacardiaceae and is a native of Brazil. The family comprises of bout 60 genera and 400 species of trees and shrubs with resinous bark and grows most abundantly in the tropics in both eastern and western hemisphere (Ohler, 1979). The Anacardium genus comprises of 20 species and the cultivated species *A. occidentale* L. is andromonoecious, with male and hermaphrodite flowers in the same inflorescence. Within the species *A. occidentale*, there is a wide variation in colour, size and shape of the apple, as well as in size and shape o the nuts. The time of flushing, flowering varies among different types. There are also differences in leaf size and leaf shape and numerous other characters. Cashew was introduced to India by Portuguese during 16th century. Molecular studies have

Table 6. Status of cashew germplasm in India

State	At NCFGB	At AICRP centres	Total
Andaman and Nicobar Islands	10	--	10
Andhra Pradesh	103	48	151
Arunachala Pradesh	2	--	2
Assam	3	--	3
Chhattisgarh	5	61	66
Goa	45	--	45
Karnataka	135	128	263
Kerala	72	181	253
Maharashta	45	297	342
Manipur	1	--	1
Meghalaya	11	--	11
Mizoram	1	--	1
Orissa	21	97	118
Tamil Nadu	46	200	246
Tripura	3	--	3
West Bengal	14	92	106
Exotic	22	--	22
Total	539	1104	1,643

Source: DCR, Puttur

shown the possibility of its introduction repeatedly over a period of time but at a single location, *i.e.* West coast (Archak *et al.*, 2009).

Presently, the cashew plants in wild state as well as in well managed orchards are seen in Maharashtra, Goa, Karnataka and Kerala along the west coast, Tamil Nadu, Andhra Pradesh, Orissa and West Bengal on the east coast. To a limited extent, the crop is also seen growing in Chhattisgarh, Gujarat, Assam, Arunachal Pradesh, Meghalaya, Tripura, Manipur, Nagaland and Andaman and Nicobar Islands. Seedling origin plants are in plenty in forests and plantations managed by state cashew development corporations throughout the country. This diversity is considerably captured in germplasm surveys and so far 539 accessions have been collected and conserved by the National Cashew Field Gene Bank (NCFGB) in the Directorate of Cashew Research, Puttur.

Similarly, Regional Cashew Gene Banks (RCGBs) have been established at All India Coordinated Research Project (AICRP) centres which are maintaining a total of 1104 accessions (Table 5). Three wild species namely, *Anacardium pumilum, A. othonianum* and *A. microcarpum* are also conserved. The collection also has seedling accessions of 23 exotic collections of which nine were collected from Brazil, Nairobi, Mtwara, Lindi, Nacala, Mozambique, Ex Tanganyka, Singapore and Australia and 14 from Republic of Panama (Table 6).

MANAGEMENT OF GENETIC RESOURCES

Exploration and Collection

India has rich biodiversity with respect to mango, Citrus spp, jackfruit and related species, Bael, *Garcinia, Prunus* spp, *Musa* spp. *Syzygium, Zizyphus, Terminalia, Punica* sp. *etc.* The culture effected are being made to collect the available diversity in fruit, vegetables, flowers, medicinal and aromatic crops, spices, plantation crops and mushroom. National Bureau of Plant Genetic Resources, conducted targeted exploration in the biodiversity hot spots in collaboration with other Horticultural Research Institutes, State Agricultural Universities and other concerned department. Apart from these, the crop specific institutes also conducted surveys and collect germplasm of their mandated crops. The IC numbers of all these collected accessions is collected from NBPGR after receiving the passport data and samples.

Germplasm Introduction

A large number of valuable germplasm of horticulture crops have been introduced in India. In recent years, the focus is on introduction of germplasm with resistance to abiotic and biotic stresses. Large collection of Papaya *(Carica papaya)* and its wild relatives, Cactus spp., Date palm, pomegranate, pear apple, peach, almond, plum, grape, oil palm and its wild relatives has been introduced from different countries.

Germplasm Characterization

Characterization is the description of plant germplasm which determines the expression of highly heritable characters ranging from morphological or agronomical features to seed proteins or molecular markers.

Morphological characterization : Characterization of germplasm is essential to provide information on the traits of accessions assuring maximum utilization of germplasm collection to final users. The recording and compilation of data on important characteristics which distinguish accessions within a species, enables an easy and quick discrimination among phenotypes. It facilitates a check on true-to-type of homogeneous samples, allowing detection of misidentifications or duplicates and indicating possible errors made during other gene bank operations. It can be carried out at any stage of the conservation process, as long as there are sufficient numbers of seeds or plant materials to sample. It should be done as soon as possible to add value to any collection. It is, however, very time consuming and expensive and therefore, often delayed or done during regeneration in many gene banks to reduce costs.

Most genetic resources collections are made up of population or landraces which are genetically variable. It may therefore, be necessary to collect data at the plant level, rather than at the plot level, because knowledge of the average value of a descriptor for an accession as a whole is not always sufficient. In order to facilitate standardization of information obtained during characterization, Bioversity International has been coordinating the development, publication and updates of various plant descriptor lists in close cooperation with crop experts and gene bank curators. The crop descriptors are available for major horticultural crops. Characterization is also increasingly done using complementary characterization of methods to capture the full information. Characterization may include morphological descriptors, herbarium samples, digital pictures, nutritional traits etc. A set of morphological descriptors can be used to describe the phenotype. The descriptive traits used will vary with the species.

Molecular characterization : New methods have made molecular analysis and genotyping useful techniques for studying diversity. A variety of molecular techniques are used, including cytological markers, biochemical markers and molecular genetic markers such as SSR, EST-SSR, AFLP, RAPD. Their choice depends on the research into molecular methodologies for the crop,

facilities and expertise available in each gene bank. Characterization of various fruit plants can be carried out with the help of different markers. Markers are those particular plant features which can be documented with confidence, comparative affluence and ease. However, two basic types of markers have been reported i.e., non-morphological makers (molecular markers) and naked eye polymorphism or morphological makers. DNA fingerprinting, genetic diversity analysis and marker assisted selection using the frontline DNA technologies are used for molecular characterization of germplasm.

Germplasm Evaluation

Preliminary evaluation of germplasm consists of recording a limited number of additional agronomic traits thought desirable by users of the particular crop. Characterization of physiological characters by curators can be of considerable help to the breeders through providing baseline data, such as vernalisation requirement, times of flowering and maturity, which would help to narrow the selection of potential breeding stock. Further, characterization consists of recording potential agronomic characters which will determine the usefulness of an accession for a specific purpose in specific circumstances. Typically, these include stress tolerance, disease and pest resistance and quality characters. Evaluation for many of these traits is outside the ability of most curators.

In the widest sense, the detailed evaluation of large collections requires multidisciplinary approach, specific testing conditions involving disciplines of cytogenetics and evolution, physiology, pathology, entomology, biochemistry and agronomy. They all contribute information that bears on the choice and utilization of genetic resources by the breeders. Cytogenetic information is essential for the use of many of crops. The genetics of host-parasite interaction is equally essential for the choice of resistant genotypes of any status. In horticultural crops, the evaluation of the germplasm is done by the respective crops institute.

Germplasm Conservation

The aim of conservation is to support sustainable development by protecting the using biological resources in such a way that do not diminish the diversity available in genus and species or destroy important habitats and ecosystems. Biodiversity can be conserved either *in-situ* or *ex-situ*.

In-situ conservation : *In-situ* conservation is on-site conservation or the conservation of genetic resources in natural populations or in the area where it grows naturally. It includes establishment of national park, biosphere reserve or gene sanctuary. In India, government has established 18 biosphere reserves for conservation of flora and fauna under Ministry of Environment, Forest and Climate change based on the UNESCO Man and the Biosphere (MAB) programme. In *in situ* conservation, plant species are promoted to grow in their natural habitats where evolutionary processes continue to operate, making it a dynamic system. Genetic variability is generated through mutation, pollen and seed dispersal, and recombination within and among populations.

Selection operates on this variability leading to the development of new plant types with improved adaptability. *In situ* conservation, in addition to natural habitats in protected areas and national reserves also needs to be carried on-farm in the areas where landraces and locally adapted farmers varieties are cultivated. This requires active farmers participation to conserve landraces and traditional farmers varieties. The novel genetic resources may be conserved even in home gardens. On-farm conservation is of particular importance in countries like India, to conserve local genetic diversity and to provide diverse food and other products for household needs and local markets, where livelihood depends on traditional subsistence farming systems. Some on-farm conservation models have been developed to define priorities for what and where to conserve based on biological and socio-economic criteria.

Ex-situ conservation : Conservation of plant genetic resources outside their natural habitat is known as ex-situ conservation. It facilitates conservation in controlled conditions and makes possible reintroduction of species into wild. It can be achieved in the following ways (1) Seed gene banks, (2) Botanical garden (3) Field gene banks (4) in-vitro banks (5) Cryopreservation banks and (6) DNA banks.

Seed conservation : Seed conservation is aimed at maintenance of high seed quality in terms of viability and vigour for various periods. Two types of collections are maintained in gene bank (i) active collections under medium term condition (4°C) from which samples are drawn for evaluation and distribution and ii) base collections are maintained at (-20°c) for long term storage. Most of fruits crops are vegetatively multiplied and their seeds are not used for multiplication. But most of the rootstocks are multiplied through seeds. These seeds can be stored for short term storage for next season. The seeds of some rootstocks alike Rangpur lime etc. loose their viability quickly these can be stored at low temperature. The seed propagated fruits like papaya seeds can be stored at low temperature for short term and midterm conservation.

Botanical garden : Botanical gardens are used to conserve those species which are loses their viability

during seed gene bank storage. There are many field gene banks/botanical gardens maintained by government and non-governmental organizations in India. National Biodiversity Authority (NBA), an autonomous and statutory body of the Ministry of Environment and Forests, Government of India listed existence of 109 botanical gardens across 18 states in India. The role of most botanical gardens in conserving intra species diversity is limited because these conserves only few accessions per species or taxon. However, this plays a greater role in public awareness and education. Botanical gardens are mainly used to display a great number of different exotic species. There is a possibility that a few well managed gardens lay emphasis on conservation of certain group of species as living collections.

Field gene banks : Field gene banks are important for conservation of germplasm of perennial crops. All the horticultural research institutes and research stations are maintaining field genes of the important crops of their respective regions. National Bureau of Plant Genetic Resources has designated National Active Germplasm Sites for different horticultural crops (Table 7). These designated sites have the responsibility of overall germplasm management including conservation of the allotted crop(s) and work in association with the NBPGR.

Pollen cryobank conserves nuclear genetic diversity (NGD) of important horticultural crops. Long term conserved pollen in the form of nuclear genetic diversity of citrus, papaya, grape, mango, tomato, eggplant, onion, capsicum, rose, gladiolus, gerbera, carnation and RET species of medicinal plants are continued to be cryopreserved in liquid nitrogen. The pollen cryobank was maintained and managed by periodic replenishment of the cryogen, for maintaining a constant cryogenic temperature throughout the storage duration. Cryopreservation involves storage of ant material at low temperature in liquid nitrogen or nitrogen vapour (-154°C to -196°C). At this temperature the cell division and metabolic processes stop and hence the plant material can be stored for larger period without alteration.

Cryopreservation of those species tht can easily be regenerated into whole plant from the stored propagules is a promising option for safe, long-term storage of germplasm. Status of cryopreserved germplasm of horticultural crops at the cryobank NBPGR, New Delhi include 1071 accessions of fruits and nuts (*Aegle marmelos, Citrus* sp., *Capparis decidua, Juglans regia, Prunus* sp., *Zizyphus* sp.) covering 111 species. Cryopreservation requires limited space, involves very little maintenance and is considered to be a cost effective option. Engelmann (1997) has reviewed cryopreservation protocols developed using different techniques. Once these techniques are further refined, large-scale adoption should be possible.

Pollen storage : Pollen storage was mainly developed as a tool for controlled pollination of asynchronous flowering genotypes especially fruit tree species. The potential advantage of this method in conserving germplasm can be readily appreciated; the relatively small quantity of the specimen required for a single accession and exchange of germplasm through pollen

Table 7. Designated National Active Germplasm Sites (NAGS) for horticultural crops

Crop	Designated NAGS
Arid fruits	Central Institute of Arid Horticulture, Bikaner
Banana	NRC Banana, Tiruchirapalli
Cashew	Directorate of Cashew Research, Puttur
Citrus species	Central Institute of Citrus Research, Nagpur
Grapes	NRC for Grapes, Pune
Aonla, Bael& Litchi	NRC Litchi , Muzaffarpur
Jackfruit	Indian Institute of Horticultural Research, Bangalore
Mango	Central Institute for Sub-Tropical Horticulture, Lucknow Indian Institute of Horticultural Research, Bangalore
Subtropical fruits	Central Institute for Sub-Tropical Horticulture, Lucknow Indian Institute of Horticultural Research, Bangalore
Mulberry	Central Silk and Mulberry Genetic Resources Centre, Hosur
Oil Palm	Indian Institute of Oil Palm Research, Pedavegi
Plantation Crops	Central Plantation Crops Research Institute, Kasargod
Temperate horticulture Crops	Central Institute of Temperate Horticulture, Srinagar NBPGRRS, Shimla
Tropical fruits	Indian Institute of Horticultural Research, Bangalore

poses fewer quarantine problems compared with seed of other propagules. In recent years, cryopreservation techniques have been developed for pollen of an increasing number of species and cryobanks of pollen have been established for fruit tree species in several countries (Alexander and Ganeshan, 1993). Pollen collections of mango cvs. 'Totapuri', 'Alphonso', Langra and a dwarf variety were cryopreserved. *In vitro* germination of pollen cultured in sucrose medium using the cellophane procedure was found to be ideal. Pollen collections from different species, cultivars and hybrid lines were made from Vitis, Poincirus, Citrus and Musa species and preserved at -20°C and -196°C.

Pollen of *Citrus limon Burm*, cvs. 'Sevelle' 'Eureka' and 'Italian' retained the viability under storage conditions of -18oC and germinated moderately after 100 days of storage without appreciable loss of viability. It was observed that the percentage germination decreased to 25-30 per cent in 'Seville' after 150 days and 25 per cent in 'Eureka' and 'Italian' of fresh pollen after 100 days. Long-term pollen preservation of Citrus aurantifolia and 4 cultivars of Citrus limon Burn 'Seville', 'Hill Lemon', 'Italian' and 'Nepali Obong' was initiated at Cryo-genic temperature with pre-treatment like freeze-drying and pre-freezing in liquid nitrogen fumes.

All cultivars and flower types retained their capacity to germinate *in-vitro* after 3.5 years of cryogenic storage. Papaya cvs. Washington and Coorg Honey Dew and *Carica cauliflora* L. pollen were preserved at -3°C, -18°C and -196°C. Pollen preserved in liquid nitrogen (cv. Washington and *Carica cauliflora*) continued to maintain high viability, profiled *in-vitro*, after a period of one year and 8 months. Pollen samples have been retained in liquid nitrogen for prolonged storage durations, since gemination capacity was not affected even after 7 year of storage.

The investigations on pollen preservation in grape varieties viz, Ánab-e-Shahi, Bangalore Blue, Bangalore Purple, Black Champa and Queen of Vineyards revealed that pollen collected from these varieties were assessed for their capacity to germinate *in vitro* and stored at -196°C in liquid nitrogen. It was successfully cryopreserved for 5 years without any loss of viability. However, pollen storage alone cannot conserve the cytoplasmic genetic diversity of a species. There is a need to assess the potential drawbacks of excluding maternal genes and their feasibility of ovule storage and in vitro fertilization techniques. In addition, effective sample techniques to cover a population or gene pool are needed.

DNA storage : Storage of DNA is another approach to conservation. Genetic engineering has broken down the crossability barriers and transgenic plants incorporating genes from virus, bacteria, fungi and even mice have become reality. Such efforts have lead to realization of storage of total genomic information in the form of DNA libraries. However, strategies and procedures have to be developed on how to use the material stored in the form of DNA Therefore, the role and value of this method for PGR conservation is not completely clear as yet.

In vitro conservation : Tissue culture techniques are of great interest for collecting, multiplication and storage of plant germplasm. Tissue culture systems allow propagation of plant material with high multiplication rates in an aseptic condition. Virus free plants can be obtained through meristem culture in combination with thermotherapy, thus ensuring disease free plants and simplifying quarantine procedures. Some crop species such as banana and plantain (*Musa* spp.) do not produce seeds or produce recalcitrant seeds such as coconut, cacao, and many tree and shrub species. Crops such as potato, yam, cassava and sweet potato have either sterile genotypes or produce orthodox seeds which are highly heterozygous, therefore, making seed storage of limited interest for the conservation of particular gene combinations.

These species are mainly propagated vegetative to maintain clonal genotypes. The miniaturizations of explants allow reduction in space requirements and reduce labour costs. Protocols have been optimized for 23 horticultural crops, which are being conserved under normal and reduced culture conditions. *In-vitro* plants of Jackfruit accessions have been successfully conserved for 4 years under standard culture conditions prior to first subculture. While more jackfruit accessions are accessed *in vitro*, 4 citrus accessions are maintained *in vitro* and conservation attempts has resulted in maintaining *in vitro* plants under reduced culture conditions for 6 months.

Registration of germplasm : Unlike the developers of released cultivars, scientists associated with the development of improved germplasm and genetic stocks (new sources of resistance, male sterility, varied types of mutants, cytogenetic stocks etc.) have no mechanism for recognition. Lack of formal recognition of such useful materials and role of scientist in development of these materials, discourages them from sharing valuable materials with other workers. Consequently, most of such valuable material remains underutilized or get lost. With the recent developments concerning IPR and other related issues, due recognition of these materials has become all the more important. Keeping these considerations in view, the Indian Council of Agricultural Research has identified National Bureau of Plant Genetic Resources (NBPGR) as the nodal agency for implementation of plant germplasm registration.

These are maintained at National Gene Bank, NBPGR, New Delhi or different National Active Germplasm Sites (NAGS). Further to promote on farm conservation of elite lines and germplasm and encourage farmers, Protection of Plant Varieties and Farmers' Right Authority (PPV & FRA) is registering farmers varieties and awarding custodian farmers.

FUTURE THRUSTS

- Use of GIS for geo-referencing/gap analysis and prediction and distribution of species using environment variables to plan future explorations.
- Use of GIS for mapping of trait-specific germplasm with respect to bioactive compounds.
- Use of biotechnological tools like *in-vitro* storage/cryopreservation including pollen preservation to strengthen the conservation of germplasm.
- Use of molecular marker tools like SSR/SNP/GWAS) improve the understanding of extent, nature and distribution of diversity and develop the varieties with high yield and quality for sustainable production.
- Priority for collection of wild relatives and under exploited genetic resources.
- Introduction of targeted germplasm for crop improvement
- Evaluation of germplasm for yield, quality, shelf-life, and resistance to biotic and abiotic stresses.
- Registration of germplasm, breeding lines and parental lines
- Awareness generation related to patenting, farmers right and benefit sharing.

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Analysis of root mycorrhizal colonization and soil GRSP of *Osmanthus fragrans*

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ABSTRACT

The experiment was conducted to analyze the status of root colonization by arbuscular mycorrhizal (AM) fungi and soil glomalin-related soil protein (GRSP) in plant of *Osmanthus fragrans* (YinGui) in the field. Three treatments, 1.0 m ($S_{1.0m}$), 1.4 m ($S_{1.4m}$) and 1.8 m ($S_{1.8m}$) away from the trunk of plants of *O. fragrans* were designed. The results showed that plants were AM plants, as observed the AM structure. The AM colonization varied from 10.2% to 21.2% in field and greater mycorrhizal colonization was found in $S_{1.4m}$. Easily extractable GRSP (EE-GRSP) ranged from 0.457 to 0.565 mg/g soil and was higher in $S_{1.4m}$ than in $S_{1.0m}$ and $S_{1.8m}$. No any significant difference was observed in difficultly extractable GRSP (DE-GRSP) and total GRSP. A greatly significantly positive correlation was found between AM colonization and EE-GRSP.

Key Words: Arbuscular mycorrhiza, EE-GRSP, Glomalin, Mycorrhizal colonization, Osmanthus fragrans.

Arbuscular mycorrhizal fungi (AMF), a kind of beneficial soil microorganisms, can form mycorrhizal association with more than 80% of land's plants (Bainard 2011; Ortas *et al.,* 2015; Barman *et al.,* 2016). The AMF promotes plant growth, stimulates nutrient acquisition, and enhances the plant tolerance to several abiotic stresses, such as salinity, drought, high temperature, heavy metals and so on (Ortas *et al.,* 2015; Tuo *et al.,* 2015; Barman *et al.,* 2016). Further, mycorrhizal hyphae can secrete a special glycoprotein, glomalin, named as glomalin-related soil protein (GRSP) in soil (Wright *et al.,* 1996; Wright and Upadhyaya, 1998; Du *et al.,* 2005). Glomalin is derived from extraradical mycelium and spores of AMF, showing important functioning in terrestrial ecosystems. For the instance, GRSP could improve soil structure and promote root growth (Wu *et al.,* 2014). The GRSP is related to soil aggregate stability and also influences soil carbon (C) storage indirectly by stabilizing soil aggregates and soil stability. Although some studies had made the progress about GRSP, few details about the molecular properties of GRSP was known. Nowadays, GRSP is believed to be an N-linked glycoprotein composed of 36-59% C, 4 -6% hydrogen, 33-49% oxygen, 0.03-0.1% phosphorus and other components (Zhang *et al.,* 2017).

The *Osmanthus fragrans*, is famous flowering plant in China, occurring in eastern China. It is widely grown (24-33°N) and used chiefly as ornamental plants (Tang et al., 2013), and traditional medicinal plants (Liu *et al.,* 2008). Until now, it is not known whether native AMF can colonize roots of *O. fragrans*. The GRSP levels of *O. fragrans* are not clear. Therefore, we observed root mycorrhizal colonization and soil GRSP levels in *O. fragrans.*

MATERIALS AND METHODS

The experiment was conducted at Yangtze University, China (30°36'N, 112°14'E). The location has the North subtropical humid monsoon climate, with four distinct seasons, plenty of rain, suitable light, and a long frost-free period. The annual total radiation is 4367-4576 MJ/m, with annual sunshine hour 1823-1987, average annual temperature 16.2-16.6°C, and annual precipitation 1100-1300 mm. The 29-year-old plants of *Osmanthus fragrans* at Yangtze University campus were selected as the plant material. Three treatments, 1.0 m ($S_{1.0m}$), 1.4 m ($S_{1.4m}$) and 1.8 m ($S_{1.8m}$) away from the trunk of plants were designed. The samples (roots and soils) were collected randomly at 10 cm depth from

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four trees on 14 October, 2016. Soil and root samples from all the four trees per block were well mixed as a composite sample.

Parameters determined : The root samples collected were cleared with 10% KOH solution, stained with 0.05% trypan blue in lactophenol, and root colonization was observed using microscope and number of entry points in infected root. The AMF infected percentage was calculated by following formula: AMF colonization (%) = 100 × root length infected / root length observed (Wu *et al.*, 2016).

Rhizospheric soil was collected, mixed, air-dried, and sieved (2 mm) for further analyses. Briefly, for easily extractable glomalin-related soil protein (EE-GRSP), 1.0 g substrate sample was incubated with 8 mL 20 mM citrate (pH 7.0), autoclaved at 121 °C and 0.11 MPa for 30 min, and then centrifuged at 10,000×g for 3 min. After collection of EE-GRSP from the supernatants, difficultly extractable glomalin-related soil protein (DE-GRSP) was subsequently extracted from remaining residue with 8 mL 50 mM citrate (pH 8.0) for 60 min and centrifuged at 10,000×g for 3 min. The supernatants of EE-GRSP and DE-GRSP were separately assayed with bovine serum albumin as standard according to Bradford (1976). Total GRSP was the sum of the EE-GRSP and DE-GRSP.

Root soluble sugar was determined by anthrone method (Wu *et al.*, 2006) using the sucrose as the standard.

Statistical analysis : Statistical analyses of data (means ± SD, n = 4) were performed using SAS software (v 8.1). The Pearson's correlation coefficients between variables were performed using the Proc Corr's procedure of SAS.

RESULTS AND DISCUSSION

Root AMF colonization : confirmed that AMF promoted plant growth, increased the absorption of water and nutrient, maintained quality and yield of crop plants, helped the host plants to enhance the resistance against pathogenic organisms and disease, as well as formation and stabilization of soil water-stable aggregates, contributing toward soil health resilience Wu *et al.*, (2013; and Clark *et al.*, 1999). Glomalin is a protein secreted by hyphae and spores of AMF (Wright and Upadhyaya 1996). The concentration of GRSP is closely related to AM colonization. We confirmed that *O. fragrans* belonged to AM plants, because of the presence of AM structures (Fig. 1). Root AM colonization was 13.8%, 21.2% and 10.2%, at $S_{1.4m}$, $S_{1.0m}$ and $S_{1.8m}$. treatments, respectively (Table 1). Compared with $S_{1.4m}$, root AMF colonization was significantly decreased in treatments of $S_{1.0m}$ and $S_{1.8m}$.

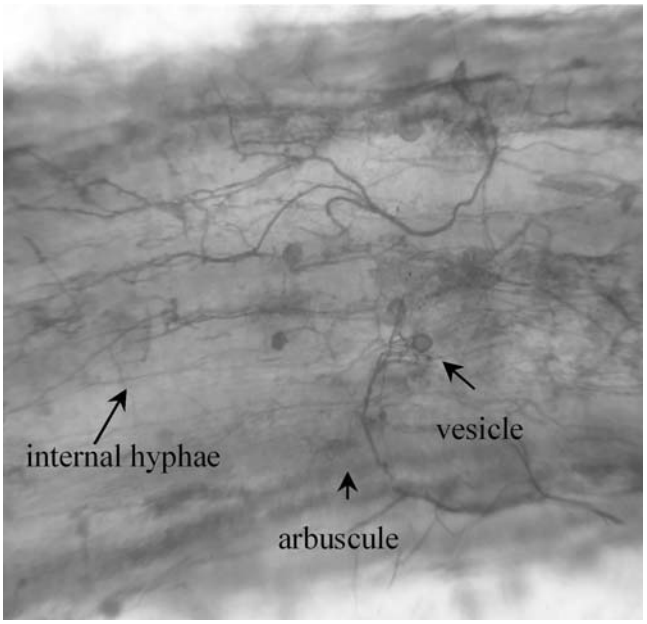

Fig. 1. Root mycorrhizal colonization of *Osmanthus fragrans* (YinGui) plants in the field

Table 1. Root mycorrhizal colonization and root soluble sugar in *Osmanthus fragrans* (YinGui) plants in field

Sample	Root mycorrhizal status		Root soluble sugar (mg/g)
	root colonization (%)	entry point (#/cm)	
$S_{1.0\ m}$	13.8±1.4b	13.4±1.1a	47.65±3.93a
$S_{1.4\ m}$	21.2±1.7a	5.2±0.5c	43.91±3.70a
$S_{1.8\ m}$	10.2±0.7c	5.9±0.4b	46.73±3.39a

NOTE: $S_{1.0m}$: sample was collected from 1.0 m away from the trunk of *O. fragrans.*

$S_{1.4m}$: sample was collected from 1.4 m away from the trunk of *O. Fragrans.*

$S_{1.8m}$: sample was collected from 1.8 m away from the trunk of *O. Fragrans.*

Data (means ± SD, n = 4) followed by different letters in same column are significantly different at $P < 0.05$. The same below.

On the other hand, significantly higher entry points number in root was recorded as $S_{1.0m} > S_{1.8m} > S_{1.4m}$ (Table 1). Interestingly, most number of entry points in roots was $S_{1.0m}$, but root AMF colonization in $S_{1.4m}$ was the most. Moreover, mycorrhizal status increased with decrease in trunk distance.

Soluble sugar is an important photosynthate and metabolic energy of plants (He *et al.*, 2006). The AMF obtains up to 20% of photosynthetic carbohydrates from host plant (Parniske, 2008). The AMF could help the host plants to improve photosynthesis, and synthesize more photosynthetic carbohydrates for host and mycorrhizal symbiosis. The AM symbiosis could alter the allocation of carbohydrate to roots and appear to intensely compete for root-allocated carbon, resulting in an enhanced allocation of carbohydrates to roots for AM growth and development (Wu *et al.*, 2007). However, soluble sugar in root had non-significant difference among $S_{1.0m}$, $S_{1.4m}$ and $S_{1.8m}$ (Table 1), and there was non-significant correlation between root AM colonization and root soluble sugar (Fig. 2). May be, these soluble sugars are further utilizated and stored by AMs, although these fungal characteristic components were not determined in the present study.

The concentration of EE-GRSP in $S_{1.4m}$ was dramatically higher than $S_{1.0m}$ and $S_{1.8m}$ (Table 2). Possibly, roots of *O. fragrans* plants focused on the 1.4 m range. The concentration of DE-GRSP was gradually decreasing as increase distance from tree trunk, but concentration of total GRSP in $S_{1.4m}$ was highest among three treatments. However, there was no notably difference of the concentration of DE-GRSP and total GRSP among $S_{1.0m}$, $S_{1.4m}$ and $S_{1.8m}$. Interestingly, there was a greatly significant correlation between root AM colonization and EE-GRSP in rhizosphere soil (Fig. 3). This result was similar to Wu *et al.* (2014) in citrus, since EE-GRSP is new producted glomalin originated from AMF (Wu *et al.* 2015).

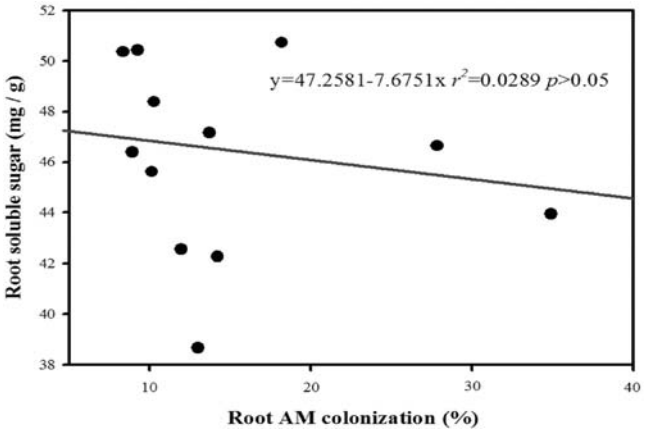

Fig. 2. The relationship of root AM colonization and root soluble sugar in root of *Osmanthus Fragrans* (YinGui) plants in the field

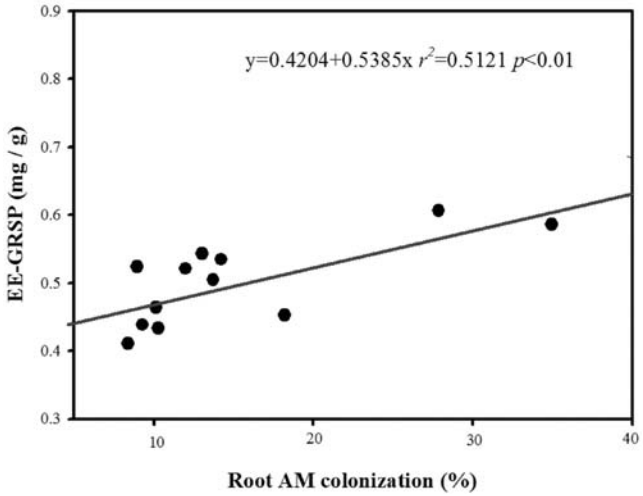

Fig. 3. The relationship of root AM colonization and EE-GRSP in rhizosphere soil of *Osmanthus Fragrans* (YinGui) plants in the field

Thus, the plants of *O. fragrans* were AM plants, as observed AM structure. The AM colonization varied from 10.2% to 21.2% in field and greater mycorrhizal colonization and EE-GRSP was found in 1.4 m of trunk distance. There was a greatly significant correlation between root AMF colonization and EE-GRSP in rhizosphere soil.

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Table 2. Concentrations of GRSP in soil of *Osmanthus fragrans* (YinGui) plants in field

Sample	GRSP (mg/g)		
	EE-GRSP	DE-GRSP	Total GRSP
$S_{1.0\ m}$	0.483±0.045b	0.940±0.091a	1.422±0.073a
$S_{1.4\ m}$	0.565±0.038a	0.932±0.085a	1.497±0.082a
$S_{1.8\ m}$	0.457±0.048b	0.925±0.062a	1.383±0.046a

Outstanding Young, Hubei Provincial Department of Education (T201604).

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Effect of fertigation schedule on production potential, quality and nutrient uptake of elephant-foot yam *(Amorphophallus paeoniifolius)*

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ABSTRACT

The field experiment was conducted to determine the effect of fertigation schedule on productivity and quality of elephant-foot yam (*Amorphophallus paeoniifolius* Dennst. Nicolson) at Bhubaneswar Regional Centre of CTCRI, Thiauvananthapuram, during 2013 and 2014. The experiment was laid out in split plot design with different fertigation intervals (T_1:2, T_2:3 and T_3:4 days intervals) in the main plots. The recommended dose of fertilizers (soluble fertilizer N, P_2O_5, K_2O @ 120-60-120 kg/ha) were applied in splits (S_1:30, S_2:40 and S_3:50) through drip irrigation in sub-plots. A check [surface irrigation with P_2O_5 60 kg/ha as basal and N, K_2O @ 120-120 kg/ha during first (40%), second (30%) and third (30%) month after planting (MAP) applied to soil] and the control (surface irrigation without fertilizer) treatments were also included. All the treatments had three replications. The plant height, canopy spread and pseudostem (leaf petiole) girth at third and fifth MAP were maximum in treatments which received maximum nutrients. The treatments T_3S_2 (fertigation in 40 split doses at 4 days interval) and T_2S_3 (fertigation in 50 split doses at 3 days interval) resulted in more corm yield, dry matter and starch content, nutrient (N, P and K) uptake and use efficiency (agronomic efficiency, recovery efficiency and partial factor productivity) but difference between the two treatments were not statistically significant. The control (surface irrigation without fertilizer) resulted in lower calcium oxalate content in corm. Dilution effect of calcium oxalate content was recorded in treatments that resulted in maximum corm yield.

Key Words: Elephant-foot yam, Fertigation, Nutrient uptake, Quality, Yield, Canopy, Pseudostem, Dry matter

Elephant-foot yam (*Amorphophallus paeoniifolius* Dennst. Nicolson) is a member of Araceae family grown for underground tuberous vegetable botanically known as corm. In India, it is cultivated in Andhra Pradesh, West Bengal, Bihar, Uttar Pradesh, Tamil Nadu, Kerala, Maharashtra, Odisha and Karnataka (Nedunchezhiyan and Byju, 2005). Recently, interest has been increasing as a commercial cash crop in India due to its high productivity and profit (Nedunchezhiyan *et al.,* 2010). The pseudostem and corm are consumed after chopping and boiling (Nedunchezhiyan *et al.,* 2006). The corms are also eaten after baking and frying. The corms are rich in minerals (calcium and phosphorus) and vitamins (vitamin A and C) (Nedunchezhiyan *et al.,* 2008). It is also used as medicine in many preparations (Mondal *et al.* 2012; Nedunchezhiyan *et al.,* 2016a).

Fertigation is an innovative cultural method by which fertilizers are applied along with irrigation water. Fertigation through drip system enables adequate supply of water and nutrients with precise timing and uniform distribution to meet the crop demand so as to get maximum yield (Fanish *et al.,* 2011). Phene *et al.* (1979) reported 25-50% reduction in fertilizer requirement under drip fertigation compared to surface broadcasting without yield reduction. Drip fertigation not only improves the yield but also saves water and nutrients (Behera *et al.,* 2013). Elephant-foot yam is a long duration (8-10 months) crop with continues nutrient uptake up to 7th MAP (Kabeerathumma *et al.,* 1987). In potato, fertigation of nutrients with very great dilution in each irrigation increased the fertilizer-use

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efficiency far beyond the previously possible level (Menzel and Obe 1990). In elephant-foot yam, drip fertigation schedule has not yet been reported so far. Since commercial cultivation of elephant-foot yam is constrained in water limited areas, in experiment was conducted to determine the effect of fertigation schedule on productivity, quality and nutrient-uptake of elephant-foot yam.

MATERIALS AND METHODS

The field experiment was conducted for two seasons during 2013 and 2014 at the Regional Centre of ICAR-Central Tuber Crops Research Institute (20°14'53.25" N and 85°47'25.85" E and 33 m above mean sea-level), Bhubaneswar, Odisha, India. The soil at the experimental site had 26.8% water-holding capacity, 1.54 g/cc bulk density, 6.4 pH, 0.36% organic carbon, 184 kg available N/ha, 21.2 kg available P/ha and 243 kg available K/ha. The recommended dose of fertilizer was N, P_2O_5, K_2O 120-60-120 kg/ha (Nedunchezhiyan *et al.*, 2016b).

The experiment was laid out in split plot design with fertigation intervals in main plots, *viz*. T_1-2 days, T_2-3 days and T_3-4 days and the recommended dose of water soluble fertilizers in 30 splits (S_1) (N-P_2O_5-K_2O 4-2-4 kg/ha/dose), 40 splits (S_2) (N-P_2O_5-K_2O 3-1.5-3 kg/ha/dose) and 50 splits (S_3) (N-P_2O_5-K_2O 2.4-1.2-2.4 kg/ha/dose) in sub plots. A check [surface irrigation with P_2O_5 60 kg/ha applied as basal and N-K_2O @ 120-120 kg/ha at first (40%), second (30%) and third (30%) MAP applied in soil] and the control (surface irrigation without fertilizer application) treatments were also included. The treatments were replicated thrice. The fertigation was given through drip irrigation. Water-soluble N, P and K fertilizers (urea, urea phosphate and potassium sulphate) were used in drip fertigation treatments. Urea, single superphosphate and muriate of potash were used as source of N, P and K respectively for soil application.

The field was ploughed once and tilled twice and ridges were made at 90 cm spacing. On the top of ridges whole seed corms weighing 400 g were planted at 90 cm spacing. Thus, a plant spacing of 90 cm × 90 cm was followed. The seed corms were placed 5-7 cm below the soil. The fertigation treatments were imposed 10 days after planting because the seed corm takes 10-15 days to initiate root and shoot primordia. The crop was drip irrigated at 80% CPE as recommended by Nedunchezhiyan *et al.* (2016b). Weeding followed by earthing-up was done at first, second and third MAP. The irrigation was withheld 15 days before harvesting. During the first season, the crop was planted on 17 May 2013 and harvested on 16 January 2014, while during the second season crop was planted on 12 May 2014 and harvested on 11 January 2015. The crop duration was 8 months.

The climate of the experimental site is characterized by hot and humid summer, and cold and dry winter. The rainfall received during the crop growth period was 1762.1 mm in 67 rainy days during 2013 and 1504.8 mm in 72 rainy days during 2014. During 2013, average maximum temperature varied between 28.8 and 38.7°C, whereas average minimum temperature varied between 14.4 and 27.0°C. During 2014, average maximum temperature varied between 27.7 and 39.2°C, whereas average minimum temperature varied between 14.2 and 26.3°C. The average mean humidity during the crop growth period was 77 and 72% during 2013 and 2014 respectively. During pre-and post-monsoon as well as dry spell of crop growth period 175.4 and 209.7 mm of water was applied through drip irrigation (at 80% CPE) during 2013 and 2014 respectively.

Elephant-foot yam produces crown-shaped crop canopy on pseudostem (Nedunchezhiyan, 2014a). During growth period, it produces on average 2-3 pseudostems (leaves) per plant or hill when whole corms are planted. Usually second leaf emerges at third MAP. The canopy expands continuously up to fifth MAPs and then slows down. The canopy growth between 5-6 months was negligible and then decline as senescence starts. Observations on plant height, canopy spread and pseudostem girth at collar region were recorded from the first leaf at third MAP and the second leaf at fifth MAP (Nedunchezhiyan, 2014b). The plants withered/dried at eighth MAP, no growth observations were recorded. Yield attributes and yield were recorded at harvesting.

The dry matter, starch and calcium oxalate were analysed (AOAC, 1980) at harvesting. The N, P and K content in shoots, corms and roots were estimated (Jackson, 1973) at third, fifth and eighth MAP (at harvesting). Nutrient uptake was calculated by multiplying nutrient content with dry matter production.

Nutrient (NPK)-use efficiency was estimated using the differences between fertilized treatment and unfertilized treatment (Cassman *et al.*, 1998). The indices calculated are Agronomic efficiency (AE; kg yield increase per kg NPK applied), recovery efficiency (RE; kg increase in NPK uptake per kg NPK applied), physiological or internal efficiency (PE; kg yield increase per kg increase in NPK uptake) and partial factor productivity (PFP; kg yield per kg NPK applied):

Agronomic efficiency (AE) = $(Y_t - Y_0)/F$

Recovery efficiency (RE) = $(U_t - U_0)/F$

Physiological efficiency (PE) = $(Y_t - Y_0)/(U_t - U_0)$

Partial factor productivity (PFP) = Y_t/F

where, Y_t is corm yield in test plot (t/ha); Y_0 is corm

yield in control plot (t/ha); U_t is total NPK uptake in test plot (kg/ha); U_0 is total NPK uptake in control plot (kg/ha); F is NPK applied in test plot (kg/ha).

The data collected were subjected to analysis of variance (ANOVA) using SAS statistical software (SAS Institute Inc. 2002). Treatment means were compared for significance at 0.05 level of probability using the critical differences (CD) as suggested by Gomez and Gomez (1984).

RESULTS AND DISCUSSIONS

At third MAP, fertigation in 30-40 split doses at 2-3 days interval (T_1S_2 and T_2S_1) resulted in maximum plant height, canopy spread and pseudostem girth. It indicated that treatments which received maximum amount of fertilizers within 3 months resulted in taller plants with more canopy spread and pseudostem girth (Table 1). Similarly, at fifth MAP also the treatment [fertigation in 50 split doses at 3 days interval (T_2S_3)] which received maximum amount of fertilizer within 5 months resulted in taller plants with more canopy spread and pseudostem girth (Table 1). However, between third MAP and fifth MAP, plant height increase was 20.6% only (Table 1). Ravi *et al.* (2015) reported that full development of first leaf occurs during 2-3 MAP. Between third and fifth MAP, canopy spread increased by 31.4%.

Thus, increase in canopy spread was 10.8% more than plant height. Between third and fifth MAP, pseudostem girth increased by 24.2% and increase in pseudostem girth was 3.6% more than plant height. This indicated that more nutrients are to be applied before third month for taller plants and before fifth month for enhancing canopy spread and pseudostem girth. Sahoo *et al.* (2014a, 2015) also noticed maximum growth attributes at higher level of nutrient application. Adequate supply of N has direct impact on plant vegetative growth (Lockhart and Wiseman, 1988). However, nutrient application beyond fifth month did not influence shoot growth in elephant foot yam. The growth attains a plateau between fifth and seventh month and then senescences (Ravi *et al.*, 2011). Significantly lower plant height, canopy spread and pseudostem girth was recorded in plants that were not fertilized under surface irrigation (the control) (Table 1).

The fertigation in 40 split doses at 4 days interval (T_3S_2) and 50 split doses at 3 days interval (T_2S_3) resulted in higher corm diameter, corm length and corm yield per plant (Fig. 1). The higher yield attributes in these treatments was due to higher growth attributes (plant height, canopy spread and pseudostem girth) (Table 1) and source activity, which increased corm bulking in these treatments. Lower corm diameter and length, and corm yield per plant were recorded in check (surface irrigation with the application of fertilizers in soil) and control (surface irrigation without fertilizer) (Fig. 1) due to lower growth attributes (Table 1).

The fertigation in 40 split doses at 4 days interval (T_3S_2) and 50 split doses at 3 days interval (T_2S_3) resulted in more corm yield (Fig. 2). This was due to more growth and yield parameters in these treatments (Table 1 and Fig. 2). In these treatments, fertilizer was effectively utilized for dry matter production and partitioning. It also implies that if fertigation was given in 40 splits then frequency of application was 4 days interval or if fertigation was given in 50 splits then the frequency of application was 3 days interval.

Table 1. Effect of fertigation schedule on plant height, canopy spread and pseudostem girth

Treatment	Plant height (cm)				Canopy spread				Pseudo stem girth (cm)			
	3 MAP		5 MAP		3 MAP		5 MAP		3 MAP		5 MAP	
	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014
T_1S_1	77	78	91	89	92	88	117	118	15.2	15.1	18.1	18.3
T_1S_2	83	81	91	90	96	92	121	119	15.4	15.3	18.5	18.4
T_1S_3	80	79	95	92	90	89	124	120	15.3	15.1	18.7	18.5
T_2S_1	82	82	90	88	93	93	120	118	15.3	15.3	18.3	18.4
T_2S_2	77	78	93	91	92	90	122	123	15.2	15.2	18.6	18.7
T_2S_3	75	74	98	96	90	88	128	124	15.1	15.0	19.0	18.8
T_3S_1	75	78	92	91	90	91	126	122	15.1	14.9	18.8	18.6
T_3S_2	73	74	93	92	87	85	122	123	14.8	14.6	18.8	18.9
T_3S_3	71	71	92	91	82	83	122	122	14.5	14.4	18.7	18.6
Standard check	72	72	93	90	84	82	123	116	14.6	14.8	18.6	18.0
Control	64	64	84	80	80	78	98	98	14.0	13.8	17.4	17.0
CD (P=0.05)	5	5	8	9	3	3	5	4	0.9	1.1	1.2	1.3

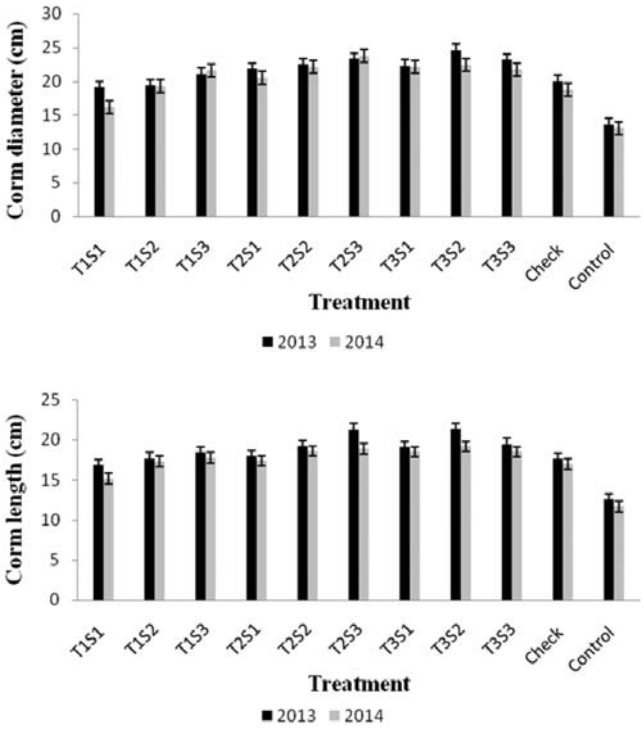

Fig. 1. Effect of fertigation schedule on corm diameter, length and yield / plant (means ± SE)

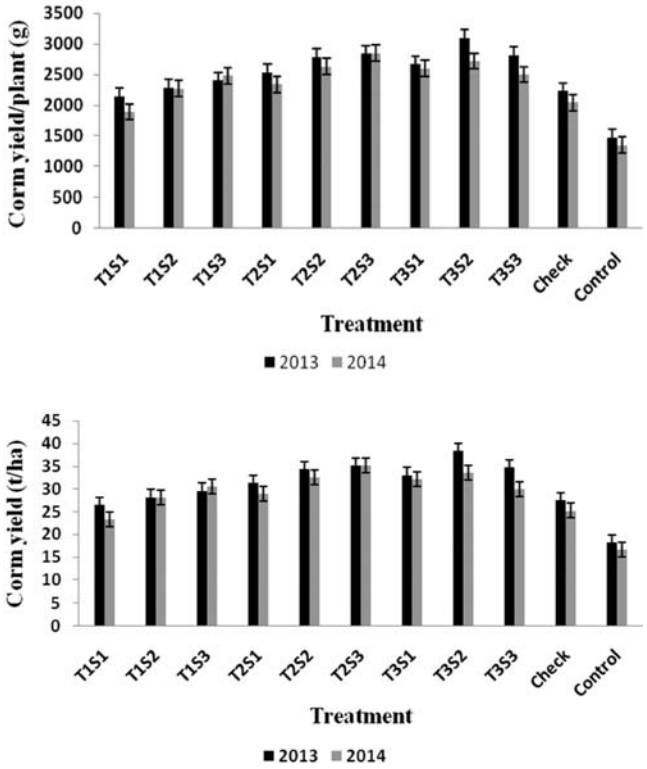

Fig. 2. Effect of fertigation schedule on corm yield (means ± SE; CD (P=0.05): 1.7 for both years)

Application of fertilizer in more than 40 split doses at 4 days interval or in lesser than 50 split doses at 3 days interval reduced corm yield drastically. In former case, crop was unable to utilize applied fertilizer, whereas in latter case crop was unable to get required nutrients throughout crop growth period due to various kinds of losses.

Drip fertigation of recommended dose at 2 days interval was inadequate as it is a longer crop duration. Tumbare and Bhoite (2002) reported that weekly fertigation through drip irrigation in 14 equal splits starting from the first week of transplanting was beneficial for green chilli grown in a sandy clay loam soil. The corm yield of check (surface irrigation with the application of fertilizers in soil) treatment was lower and at par with T_1S_1 (fertigation in 30 split doses at 2 days interval) (Fig. 2). This indicated that drip fertigation in 30 splits at 2 days interval was as equal as soil application of recommended dose of fertilizers. Nutrients applied in soil are subjected to various kinds of losses and fixation.

Compared to soil application, nutrients applied through drip irrigation are placed directly into root zone of plants, which reduces the loss of nutrients. However even in fertigation, there may be nutrient loss, if not used proper dose at proper interval. Plants need optimum dose at optimum interval for efficient utilization of applied fertilizers / nutrients. Nedunchezhiyan *et al.* (2010) and Jata *et al.* (2015) reported more corm yield with fertigation than soil application of fertilizers. Elephant-foot yam being a high-yielding crop mines high amount of nutrients from the soil. Hence, fertilizers should be applied to supplement the nutrients taken by the plants. When no fertilizer was applied, significantly lowest corm yield was observed in control (surface irrigation without fertilizer) (Fig. 2).

Elephant-foot yam is a starchy vegetable. High dry matter and starch give good consistency after cooking. All the fertigation treatments resulted in more dry-matter content in corms compared to the check except the treatment T_1S_1 (fertigation in 30 split doses at 2 days interval) (Table 2). The fertigation in 40 split doses at 4 days interval (T_3S_2) and 50 split doses at 3 days interval (T_2S_3) resulted in more corm dry matter. This may be due to higher amount of essential nutrients (NPK) available for plants uptake and utilization when applied spatially for a longer crop growth period.

All the fertigation treatments resulted in more starch content in corms compared to the check (surface irrigation with the application of fertilizers in soil) and the control (surface irrigation without fertilizer) except the treatment T_1S_1 (fertigation in 30 split doses at 2 days interval) (Table 2). The treatments T_3S_2 (fertigation in

Table 2. Effect of fertigation schedule on dry matter, starch and calcium oxalate content

Treatment	Dry-matter content (%)		Starch content (%)		Calcium oxalate (mg/100 g)	
	2013	2014	2013	2014	2013	2014
T_1S_1	19.2	18.8	15.6	15.2	81.4	81.0
T_1S_2	19.5	19.3	15.7	15.7	83.4	83.0
T_1S_3	19.8	20.0	16.1	16.5	83.6	84.0
T_2S_1	20.0	19.6	16.3	16.1	83.8	83.6
T_2S_2	20.3	20.1	16.5	16.4	84.5	84.3
T_2S_3	20.6	20.8	16.8	16.6	84.0	83.8
T_3S_1	20.2	20.0	16.7	16.5	84.6	84.4
T_3S_2	20.8	20.6	16.9	16.4	83.4	84.2
T_3S_3	20.5	20.1	16.4	16.3	84.0	84.4
Standard check	19.4	19.2	15.6	15.2	81.5	81.3
Control	19.0	18.8	15.2	14.6	80.5	80.3
CD (P=0.05)	0.8	1.1	0.4	0.6	3.8	3.6

40 split doses at 4 days interval) and T_2S_3 (fertigation in 50 split doses at 3 days interval) resulted in more starch content in corms. Potassium (K) is essential for translocation of sucrose from source (shoot) to sink (corm). In treatments T_3S_2 (fertigation in 40 split doses at 4 days interval) and T_2S_3 (fertigation in 50 split doses at 3 days interval) the uptake of K along with NP was more which helped plants to translocate sucrose from shoots to corms, and increased the starch content in corms. At higher level of N and K application, higher starch content was reported in elephant foot yam (Mukhopadhyay and Sen, 1986). In present study, starch content decreased in those treatments which received fertilizers at sixth MAP. It indicated that crop was not utilizing the nutrients when applied at sixth MAP. In standard check (surface irrigation with the application of fertilizers in soil) and control (surface irrigation without fertilizer) treatments, uptake of K was low, accordingly the starch content in corm was also lower.

Elephant-foot yam corm contains calcium oxalate (raphite) which is an anti-nutrition factor. Presence of calcium oxalate causes irritation in throat when corms are eaten. All the fertigation treatments increased the calcium oxalate content in corms compared to the check (surface irrigation with the application of fertilizers in soil) except the treatment T_1S_1 (fertigation in 30 split doses at 2 days interval). Lowest calcium oxalate content was observed in the control (surface irrigation without fertilizer). This indicated that more amount of nutrients (NPK) application to plants during active corm bulking period (4 MAP) increased the calcium oxalate content in corms. However, treatments T_3S_2 (fertigation in 40 split doses at 4 days interval) and T_2S_3 (fertigation in 50 split doses at 3 days interval) resulted in lower calcium oxalate content. This may be due to dilution effect as these treatments resulted in maximum corm yield. A decrease in calcium oxalate content at higher corm yield has been reported by Suja *et al.* (2012).

Nutrient uptake is the function of biomass production and nutrient accumulation. The fertigation in 40 split doses at 4 days interval and 50 split doses at 3 days interval resulted in greater NPK uptake (Table 3). Higher uptake of NPK can be related to greater shoot growth and corm yield, and higher dry-matter content. Byju *et al.* (2016b) reported linear relationship between elephant-foot yam yield and NPK uptake. The increase in uptake of nutrients by crop was due to continuous supply of essential plant nutrients to plants throughout crop-growth period. The treatment T_3S_2 (fertigation in 40 split doses at 4 days interval) had 45.7 and 37.6% greater NPK uptake than the check (surface irrigation with the application of fertilizers in soil) plants during 2013 and 2014, respectively (Table 3), whereas treatment T_2S_3 (fertigation in 50 split doses at 3 days interval) had 32.2 and 47.7% greater NPK uptake than the control (surface irrigation with the application of fertilizers in soil) plants during 2013 and 2014 respectively.

Presumably, application of less quantity of NPK in more number of doses resulted in increased availability of NPK throughout the active crop growth period. The NPK loss and fixation is minimal when applied in more number of splits. The fertigation interval (weekly or bi-weekly or monthly) was the best to maximize the nutrient uptake by the crop depending on the soil type (Hochmuth and Smajstrla, 2000). In control treatment (surface irrigation without fertilizer), lesser NPK available to plants in the rhizosphere led to significantly lowest NPK uptake, lower growth attributes and corm yield.

Table 3. Effect of fertigation schedule on NPK uptake, RE, AE, PE and PFP.

Treatment	NPK uptake (kg/ha)		AE		PE		RE		PFP	
	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014
T_1S_1	274.9	241.7	27.7	22.0	85.5	96.8	32.4	25.8	88.3	77.7
T_1S_2	294.4	291.0	33.3	38.0	85.8	97.8	38.9	42.3	94.0	93.7
T_1S_3	304.2	322.8	38.0	46.3	90.2	87.6	42.1	52.9	98.7	102.0
T_2S_1	320.1	292.5	43.7	40.7	92.0	95.1	47.4	42.8	104.3	96.3
T_2S_2	354.3	324.5	53.7	52.7	91.2	98.6	58.8	53.4	114.3	108.3
T_2S_3	360.2	370.2	56.3	61.7	92.7	89.8	60.8	68.7	117.0	117.3
T_3S_1	346.3	328.1	49.3	51.3	87.8	94.0	54.6	54.6	110.0	107.0
T_3S_2	396.6	345.0	67.0	56.3	92.3	93.5	72.6	60.3	127.7	112.0
T_3S_3	370.2	315.6	55.3	44.3	86.3	87.8	64.1	50.5	116.0	100.0
Std. check	272.5	250.7	31.0	28.3	98.2	98.3	31.6	28.8	91.7	84.0
Control	177.8	164.2	-	-	-	-	-	-	-	-
CD (P=0.05)	37.4	30.2	3.1	2.8	8.7	9.1	4.3	3.9	9.6	9.2

The AE is a useful measure of nutrient-use efficiency, as it provides an integrative index that quantifies total economic output relative to the utilization of all nutrient resources. The fertigation in 40 split doses at 4 days interval (T_3S_2) and 50 split doses at 3 days interval (T_2S_3) resulted in more AE and RE (Table 3). The treatments T_3S_2 (fertigation in 40 split doses at 4 days interval) and T_2S_3 (fertigation in 50 split doses at 3 days interval) resulted in 116.1 and 118.0% more AE and 129.7 and 138.5% greater RE over the control during 2013 and 2014, respectively.

The partial factor productivity (PFP) for applied nutrient is a useful measure of nutrient-use efficiency because it provides an integrative index that quantifies total economic output relative to utilization of nutrient in the system, including the indigenous soil nutrient supply and applied nutrient (Cassman *et al.* 1993). During 2013, the treatment T_3S_2 (fertigation in 40 split doses at 4 days interval) resulted in significantly greater PFP (39.3%) over the control (surface irrigation with the application of fertilizers in soil). During 2014, treatment T_2S_3 (fertigation in 50 split doses at 3 days interval) resulted in greater PFP (39.6%) over the control (surface irrigation with the application of fertilizers in soil) (Table 3). Sahoo *et al.* (2014b) reported that better utilization of N was observed at higher dose of nutrient application.

In the present study, AE, RE and PFP increased with fertigation duration and attained maximum when fertigation was given up to 170 days after planting. This implies that more number of doses of fertigation is essential for maintaining crop growth and greater AE. This also indicated that synchronizing split NPK application with crop demand enhanced AE, RE and PFP of NPK. Maximum fertilizer nutrient recovery was attained when more nutrients were available to plants (Cassman *et al.,* 2002). Application of recommended dose of N in 3-split doses resulted in more AE and RE than 2-split doses in wheat (Ratanoo *et al.* 2016).

The P spreads greater soil volume when applied as orthophosphoric acid through a drip irrigation system than triple super phosphate applied through the soil (O'Neill *et al.,* 1979). When P was applied as water soluble urea phosphate it moved in a calcareous loam soil to a depth of 30 cm, thereby increasing the availability to plants. The K application had more effect on yield when applied through drip irrigation (Dangler and Locascio 1990). The control (surface irrigation with application of fertilizers in soil) treatment resulted in the minimum AE, RE and PFP (Table 3). Adoption of inefficient nutrient management practices (application of fertilizer in the soil in 3 splits doses of N and K) resulted in minimum AE, RE and PFP. The minimum AE, RE and PFP of NPK in check (surface irrigation with the application of fertilizers in soil) plants may be attributed to the non-matching of NPK applied with the demand for a long duration. In cassava, minimum AE and RE were reported by Howeler (2014) and Byju *et al.* (2016a) when nutrients were applied directly in soil.

Since plant growth and nutrient uptake are closely interrelated, it is difficult to determine whether the poor AEN/P/K, REN/P/K and PFPN/P/K in the control treatment is due to inability of plants to recover nutrient or inability of plants to utilize that nutrient for growth and yield production (Craswell and Godwin 1984), and more than 50% of applied nutrients are lost in soil application compared to 10% loss in fertigation (Solaimalai *et al.,* 2005). Fertigation minimises loss of NK when precisely required quantity of nutrients are

supplied directly to the root zone in available forms at the right time (Jata *et al.*, 2013).

The PE is the fraction of applied nutrient utilized for yield production and RE is the fraction of applied nutrient that is absorbed by a crop. The PE was greater in plants under the check treatment (Table 3) and fertigation in 30-40 split doses at 2-3 days interval (T_1S_1, T_1S_2, T_2S_1 and T_2S_2). In these treatments, NPK uptake was less but utilized by the crop more efficiently for yield production. Paramasivan *et al.* (2014) also observed greater PE in medium fertilized plot than heavily fertilized plot. Kaur *et al.* (2010) also reported a 6% decrease in PE when they applied N in 3-split doses compared to 2-splits in wheat.

Thus, it can be concluded that fertigation of water-soluble fertilizers, *viz*. N, P_2O_5, K_2O 120-60-120 kg / ha in 40 split doses at 4 days interval (N, P_2O_5, K_2O: 3-1.5-3 kg / ha / dose) or 50 split doses at 3 days interval (N, P_2O_5, K_2O: 2.4-1.2-2.4 kg / ha / dose) can be recommended for more productivity, quality and nutrient-uptake use efficiency of elephant-foot yam.

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Effect of nitrogen levels on growth, yield, seed quality and economics of French bean *(Phaseolus vulgaris)* varieties

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ABSTRACT

An experiment was conducted to find out the effect of nitrogen in growth, yield, seed and economics of French bean (*Phaseolus vulgaris* L.) at Bahadari farm, College of Horticulture, Mandsaur, Madhya Pradesh, during *rabi* season of 2009-10. Fifteen treatment combinations comprising three varieties, *viz.* V_1, Swaran Priya; V_2, Arka Komal and V_3, Contender and five nitrogen levels, *viz.* N_1, 25 kg/ha N; N_2, 50 kg/ha N; N_3, 75 kg/ha N; N_4, 100 kg/ha N and N_5, 125 kg/ha N were tested in factorial randomized block design with three replications. The varieties differed significantly for growth, yield and quality parameters, and yield. Maximum plant height, leaf area index and dry weight of shoots/plant were recorden in Swaran Priya. Earliest 50 per cent flowering was commenced in Contender, followed by Swaran Priya with no remarkable difference. Maximum length of pod, seeds/pod, seed yield/plant, seed yield/ha, shelling percentage, test weight as well as germination percentage were recorded with Swaran Priya. Crude protein content in seed was highest in Arka Komal. Application of nitrogen levels indicated significant effect on growth, yield and seed quality. Maximum plant height, leaf area index and dry weight of shoots/plant were observed with application of N_4 (100 kg/ha N). Earliest 50 per cent flowering was observed under N_1 (25 kg/ha N) application. Maximum pod length (cm), seed yield (g)/plant, seed yield (q)/ha and shelling percentage were recorded with N_4 (100 kg/ha N). Maximum seeds/pod, test weight, crude protein content in seed and germination percentage were determined with application of N_5 (125 kg/ha N). Highest gross return, net return as well as net return per rupees investment were realized with Swaran Priya under N_4 (100 kg/ha N) application.

Key Words: French bean, Nitrogen levels, Varieties, Growth, Yield, Seed quality, Economics

French bean (*Phaseolus vulgaris* L.) grown in hilly areas of Himachal Pradesh, Jammu and Kashmir and north-eastern states in summer and winter and autumn season in parts of Uttar Pradesh, Maharashtra, Karnataka and Andhra Pradesh. In northern plains, it is cultivated on a limited scale as autumn or spring crop, because of susceptibility to low as well as high temperatures (Chadha, 2001). The yield is mostly dependent on genetic constitution of variety, environmental factors, soil, crop management practices and nutrition. The improved varieties in general gave higher yields, if supplied with optimum quantity of nutrition and grown under favourable conditions (Farkade and Pawar, 2002). This crop is especially characterized by lack of nodulation owing to absence of NOD gene regulator even though it is a leguminous crop. French bean is highly sensitive and responds well to nitrogen (Ahlawat and Sharma, 1989; Sushant *et al.*, 1999). Nitrogen is necessary for growth and chlorophyll synthesis. In plains, french bean seldom forms nodules and due to this the crop responds sharply to high doses of nitrogen (Ghosal *et al.*, 2000). It improves the quality of fruit, vegetable and grain crops. Nitrogen is essential constituent of amino acid and helps in protein synthesis. As compared to other leguminous crops, nodule formation in roots of french bean is very less or even absent plains. Since it is high nitrogen demanding crop, standardization of nitrogen dose and identification of suitable variety may help in popularization and enhancing the profitability of cultivation of french bean.

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MATERIALS AND METHODS

The experiment was conducted in *rabi* season of 2009-10 at Bahadari farm, College of Horticulture, Mandsaur, Madhya Pradesh. Mandsaur lies in western part of Madhya Pradesh, between latitude of 23° 45' - 24°13' North, longitude of 74° 44' - 75° 18' East and at an altitude of 435.20 m above mean sea-level. This region is under Malwa Plateau agroclimatic zone. The soil of experimental field was medium black (vertisols), clay in texture with 46% sand, 34% silt and 20% clay, 7.3 pH, 0.34 dS m EC and uniform plain topography. Available NPK in soil were 180, 18 and 360 kg / ha, respectively. Fifteen treatment combinations comprising three varieties. *viz.* V_1, Swaran Priya; V_2, Arka Komal and V_3, Contender and five nitrogen levels, *viz.* N_1, 25 kg / ha N; N_2, 50 kg / ha N; N_3, 75 kg / ha N; N_4, 100 kg / ha N and N_5, 125 kg / ha N were tested in factorial randomized block design with three replication. Pure and healthy seeds were sown in furrows at a spacing of 45 cm × 15 cm at a depth of 3-4 cm on 15 October. Nitrogen, phosphorus and potassium were provided through urea, DAP, SSP and muriate of potash. Sulphur was supplied through elemental sulphur. According to the treatment, full quantity of phosphorus, potash and sulphur, and half of nitrogen were applied as basal dose at the time of sowing. While the remaining half quantity of nitrogen was applied 30 days after sowing. Observations were recorded on plant height, leaf area index, dry weight of shoot (g) / plant, pod length, number of seeds / pod, seed yield / plant, seed yield / ha, shelling percentage, test weight, germination percentage and crude protein content (%). Economics of different treatments was calculated on the basis of prevailing market prices of inputs and output at the time of experiment. Data were analysed as per the standard procedure described by Panse and Sukhatme (1984).

RESULTS AND DISCUSSIONS

Growth parameters were recorded 30, 45, 60 days after sowing (DAS) and at harvesting stage. The results showed significant effect of varieties and nitrogen levels on various growth parameters, *viz.* plant height (cm), leaf area index and dry weight (g) of shoots / plant. There was linear increase in plant height with advancement of crop period which was reduced slightly at harvesting stage as compared to 45 and 60 DAS. It was due to drying and dropping of upper part of plant.

Plant height was observed highest with variety Swaran Priya, followed by Arka Komal and least under Contender. Arya and Rana (1999) and Rahman *et al.* (2007) also reported significant effect of varieties on plant height. Maximum leaf area index as well as dry weight (g) of shoots / plant was recorded with variety Swaran Priya. Higher plant height and leaf area index might have resulted in more photosynthesis and accumulation of food material consequently higher dry matter content in plant. These findings are in line with these of Singh *et al.* (2009).

Application of nitrogen exerted significant effect on growth parameters. Maximum plant height, leaf area index and dry weight (g) of shoots / plant were recorded under application of nitrogen at the rate of

Table 1. Growth parameters in French bean as influenced by varieties and nitrogen levels

Treatment	Plant height (cm)				Leaf area index			Dry weight of shoots / plant (g)			
	30 DAS	45 DAS	60 DAS	At harvest	30 DAS	45 DAS	60 DAS	30 DAS	45 DAS	60 DAS	At harvest
Varieties (V)											
V_1 (Swaran Priya)	28.44	52.94	56.44	45.25	0.339	0.370	0.595	2.19	5.75	7.84	14.56
V_2 (Arka Komal)	23.04	44.29	50.81	37.40	0.283	0.392	0.551	1.84	4.89	6.68	13.58
V_3 (Contender)	22.52	35.68	39.04	31.02	0.324	0.355	0.482	1.53	4.19	6.91	13.36
SEm±	0.92	1.44	1.21	0.70	0.007	0.008	0.009	0.05	0.09	0.15	0.21
CD at (5%)	2.67	4.17	3.50	2.03	0.020	0.025	0.026	0.15	0.27	0.45	0.60
Nitrogen levels (N)											
N_1 (25 kg / ha N)	21.77	39.80	43.84	34.29	0.266	0.321	0.427	1.56	4.32	6.38	12.17
N_2 (50 kg / ha N)	23.06	42.26	46.82	35.81	0.297	0.337	0.481	1.73	4.74	6.86	13.25
N_3 (75 kg / ha N)	24.63	44.74	48.27	38.43	0.310	0.359	0.523	1.89	5.06	7.08	13.79
N_4 (100 kg / ha N)	26.48	46.88	53.20	41.70	0.363	0.438	0.590	2.24	5.70	7.91	15.78
N_5 (125 kg / ha N)	27.39	47.81	51.69	39.22	0.342	0.407	0.692	1.86	4.91	7.50	14.17
SEm±	1.19	1.86	1.56	0.90	0.009	0.011	0.011	0.07	0.12	0.20	0.27
CD (5%)	3.45	5.38	4.52	2.62	0.026	0.032	0.033	0.19	0.34	0.58	0.77

Table 2. Effect of varieties and nitrogen levels on yield attribute, yield and quality of seed in French bean

Treatment	Length of pod (cm)	Number of seeds/ pod	Seed yield/ plant (g)	Seed yield (q/ha)	Shelling (%)	Test weight of seed (g)	Protein content in seed (%)	Germination (%)
Varieties (V)								
V_1 (Swaran Priya)	13.68	4.77	37.17	22.85	75.83	58.64	21.51	87.53
V_2 (Arka Komal)	13.16	3.89	31.58	20.77	72.06	37.33	21.73	86.13
V_3 (Contender)	12.91	3.48	26.02	13.94	65.30	50.89	21.36	85.40
SEm±	0.15	0.09	0.31	0.46	0.27	0.42	0.19	0.31
CD (5%)	0.42	0.25	0.91	1.34	0.77	1.21	NS	0.89
Nitrogen levels (N)								
N_1 (25 kg/ha N)	12.42	3.43	28.29	14.90	70.23	47.04	20.19	79.78
N_2 (50 kg/ha N)	12.83	3.79	30.55	17.26	71.18	47.49	20.68	82.67
N_3 (75 kg/ha N)	13.09	3.86	32.06	20.16	71.77	48.72	21.27	87.78
N_4 (100 kg/ha N)	14.09	4.18	35.47	22.87	72.26	50.66	22.54	90.22
N_5 (125 kg/ha N)	13.81	4.97	31.56	20.75	69.88	50.85	23.00	91.33
SEm±	0.19	0.11	0.40	0.60	0.34	0.54	0.24	0.40
CD (5%)	0.54	0.32	1.17	1.73	0.99	1.56	0.70	1.15

100 kg/ha, followed by 125 kg/ha >75 kg/ha > 50kg/ha > 25 kg/ha in descending order at all growth stages. Similar findings have been reported by Farkade and Pawar (2002) and Singh and Verma (2002) in french bean.

Yield parameters showed significant effect of varieties and nitrogen levels. Variety Swaran Priya recorded maximum length of pods (cm), number of seeds/pod, seed yield (g)/plant, seed yield (q)/ha and shelling percentage. It was followed by variety Arka Komal and Contender. Higher photosynthetic area, more dry-matter accumulation might have resulted in highest yield parameters and yield in variety Swaran Priya. Farkade and Pawar (2002) also reported effect of varieties on number of seeds/pod as well as seed yield (q)/ha. Kumar and Puri (2002) found significant effect of French bean varieties on number of pods/plant, number of seeds/plant as well as seed yield/ha.

Nitrogen application showed significant effect on yield parameters. There was a linear increase in yield parameters with increasing levels of nitrogen. Maximum length (cm) of pod, number of seeds/pod, seed yield (g)/plant, seed yield (q)/ha and shelling percentage were found with application of nitrogen at the rate of 100 kg/ha nitrogen, followed by 125 kg/ha >75 kg/ha > 50kg/ha > 25 kg/ha in descending order. Optimum availability of nitrogen with 100kg/ha N application might have resulted in higher growth parameters, yield parameters and yield which was declined with further increase in N application at the rate of 125kg/ha. Srinivas and Naik (1988), Farkade and Pawar (2002), Singh and Verma (2002) and Lal (2004) also reported higher growth parameters and yield with higher doses of nitrogen.

Seed quality was studied with respect to test weight, crude protein content and germination percentage in seed. The results revealed that there was no remarkable difference between varieties for crude protein content. Test weight and germination percentage in seed were significantly influenced with varieties as well as nitrogen levels.

Maximum test weight was recorded with variety Swaran Priya followed by Contender and Arka Komal. Highest crude protein content was recorded with variety Arka Komal, followed by Swaran Priya and Contender. Germination percentage in seed showed significant difference between varieties. Maximum germination percentage in seed was determined for variety Swaran Priya followed by Arka Komal and Contender. Arya *et al.* (1999) and Singh and Verma (2002) also reported increase in 100-seed weight with higher dose of nitrogen.

Nitrogen levels exerted significant effect on quality parameters, *viz*. test weight, crude protein content and germination percentage in seed. There was significant increase in test weight, crude protein content as well as germination percentage in seed with increasing levels of nitrogen. Maximum test weight, crude protein content and germination percentage in seed were found with application of N_5 (125 kg/ha N) which was at par to N_4 (100 kg/ha N) nitrogen level. Both of these treatments were significantly superior over other nitrogen levels. Similar findings were also reported by Amaral *et al.* (1980) and Chavan *et al.* (2000).

Table 3. Economic evaluation of different treatments for seed production in French bean

Treatment	Common expenditure (Rs / ha)	Expenditure on fertilizer (₹ / ha)	Total cost of cultivation (₹ / ha)	Seed yield (q / ha)	Gross return (₹ / ha)	Net profit (₹ / ha)	Cost : benefit ratio
V_1N_1	25175.5	3163.03	28338.53	17.42	78390	50051.47	1:2.76
V_1N_2	25175.5	4207.26	29382.76	19.71	88695	59312.24	1:3.01
V_1N_3	25175.5	4955.37	30130.87	24.85	111825	81694.13	1:3.71
V_1N_4	25175.5	5428.77	30604.27	27.18	122310	91705.73	1:3.99
V_1N_5	25175.5	5834.43	31009.93	25.11	112995	81985.07	1:3.64
V_2N_1	25175.5	3163.03	28338.53	17.26	77670	49331.47	1:2.74
V_2N_2	25175.5	4207.26	29382.76	19.45	87525	58142.24	1:2.97
V_2N_3	25175.5	4955.37	30130.87	21.29	95805	65674.13	1:3.17
V_2N_4	25175.5	5428.77	30604.27	24.31	109395	78790.73	1:3.57
V_2N_5	25175.5	5834.43	31009.93	21.57	97065	66055.07	1:3.13
V_3N_1	25175.5	3163.03	28338.53	10.03	45135	16796.47	1:1.59
V_3N_2	25175.5	4207.26	29382.76	12.61	56745	27362.24	1:1.93
V_3N_3	25175.5	4955.37	30130.87	14.33	64485	34354.13	1:2.14
V_3N_4	25175.5	5428.77	30604.27	17.12	77040	46435.73	1:2.51
V_3N_5	25175.5	5834.43	31009.93	15.57	70065	39055.07	1:2.25

Economic of seed production revealed highest gross return under V_1N_4 (Swaran Priya with 100 kg / ha N), followed by V_1N_5 (Swaran Priya with 125 kg / ha N), V_1N_3 (Swaran Priya with 75 kg / ha N), V_2N_4 (Arka Komal with 100 kg / ha N), V_2N_5 (Arka Komal with 125 kg / ha N) and V_2N_3 (Arka Komal with 75 kg / ha N). Minimum gross return was obtained with Contender under N_1 (25 kg / ha N) application. More availability of nitrogen under (100 kg / ha N) and better absorption and utilization by variety Swaran Priya might have resulted in superior performance of the combination V_1N_4 (Swaran Priya with nitrogen 100 kg / ha). Highest net return was realized with Swaran Priya under N_4 (100 kg / ha N) application, followed by V_2N_4 (Arka Komal with 100 kg / ha N), V_3N_4 (Contender with100 kg / ha N) application. Minimum net return was obtained under V_3N_1 (Contender with 25 kg / ha N).

Highest net return per rupees investment was obtained with Swaran Priya with N_4 (100 kg / ha N) application, followed by V_1N_5 (Swaran Priya with 125 kg / ha N), V_1N_3 (Swaran Priya with 75 kg / ha N), V_2N_4 (Arka Komal with 100 kg / ha N) application. Lowest cost: benefit ratio was obtained with Contender under N_1 (25 kg / ha N) application. More increase in yield as compared to expenditure at higher nitrogen levels resulted in more gross return and net profit. Tewari and Singh (2000) and Rahman *et al.* (2007) also reported increase in gross return, net return and C: B ratio with higher doses of fertilizers.

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Effect of different intercropping systems on growth and yield of rose (*Rosa indica*)

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ABSTRACT

The field experiment was conducted to find out the effect of intercropping on annual rose (*Rosa indica* L.) cultivation at Floriculture Research Farm, ASPEE College of Horticulture and Forestry, Navsari Agricultural University, Navsari, during 2014-15. The intercrops were African marigold, French marigold and Gaillardia. The experiment was laid out in a randomized block design with 10 treatments. The vegetative parameters of rose were significant at different stages. The plant height was significant at the end of experiment. While at the end of first intercropping season, at the time of planting of second intercropping season and at the end of experiment plant spread was recorded significant. The highest yield was obtained from sole rose (6.79 tonnes/ha), while least was found in rose + gaillardia 1:2 (4.77 tonnes/ha). The highest rose equivalent yield (11.2 tonnes/ha) was observed from the treatment T_2 (rose + African marigold 1:2). Whereas lowest (6.79 tonnes/ha) was recorded from the treatment T_7 (rose sole). In view of the LER, highest value was recorded from intercropping rose + French marigold 1:2 (1.54). However, lowest value (1.0) was recorded from sole rose. This shows profitability of intercropping over the sole cropping.

Key Words: Rose, African marigold, French marigold, Gailardia, Intercropping systems, Growth, Plant height, Plant spread, Equilent yield *etc.*

Intercropping is cultivation technique of two or more crops at the same time in the same field. When two or more crops with different rooting systems, a different pattern of water and nutrient demands and a different above the ground habits are planted together, water, nutrients and sunlight are used more efficiently. Therefore combined yields of crops grown as intercrops can be higher than the yield of the same crop grown as pure stand. Further in intercropping, risk for crop failure is spread over different crops and even if one crop fails there is still a harvest from the other crop. Since rose takes more time 3-6 months for commercial flower production, an experiment was conducted to find out the effect of intercropping on rose with African marigold, French marigold and gaillardia for generating extra income.

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MATERIALS AND METHODS

The field experiment was laid out at Floriculture Research Farm, Navsari Agricultural University, during 2014-15; The experiment was consisted of randomized block design with 10 treatments, *viz.* T_1, rose + African marigold (1:1); T_2, rose + African marigold (1:2); T_3, rose + gaillardia (1:1); T_4, rose + gaillardia (1:2); T_5, rose + French marigold (1:1); T_6, rose + French marigold (1:2); T_7, rose sole; T_8, gaillardia sole; T_9, African marigold sole; T_{10}, French marigold sole.

Two intercropping seasons were taken, first during winter and second during rainy season of 2014-15. Rose was selected as a main crop and its plants were planted at a spacing of 150 cm × 90 cm. While intercrops, viz. African marigold, French marigold and gaillardia were grown in inter-row spaces of rose with different ratios. Recommended dose of fertilizers for both rose and intercrops were applied during both the intercropping seasons. Effect of intercropping systems on growth and

yield characteristics of rose were recorded treatment wise and analyzed statistically.

RESULTS AND DISCUSSIONS

Statistical analysis of data revealed that plant height of rosa was significant. Maximum plant height was 108.64 cm from T_7 (sole cropping) (108.64 cm) which was statistically at par with T_1, T_2, T_5 and T_6. This may be due to non-existence of competition between rose and intercrops for nutrients, light, moisture and space which would have for the spurt in growth of pure rose. Similar results were reported by Lakshminarayanan *et al.* (2005) in leguminous vegetables intercropped with jasmine. Singh and Datta (2006) also reported that plant height of gladiolus grown in association with marigold did not differ significantly compared with the main crop. Plant spread in N-S direction exhibited significant difference due to the effect of treatments.

The maximum plant spread (117.93 cm) was noted from T_7 sole cropping which was statistically at par with T_1, T_2, T_5 and T_6. Whereas in plant spread in E-W direction, data were significantly highest in T_7, *i.e.* (111.04 cm), which was statistically at par with T_5 an T_6. While minimum plant spread were observed in T_4 in both the direction. This reduction mainly attributed to the competitive effects of intercrops.

During earlier stages of plant growth (height and spread), non-significant result were obtained due to different treatments may be attributed to delay root establishment of the main crop. At later stages, significant variation in growth among different treatments may be attributed to better and early root establishment and regular unintensified supply of essential nutrients at right time and at appropriate quantities. The results of our experiment are in agreement with those of Varghese *et al.* (1990) in cabbage, Natrajan (1992) in chilli and Mouneka and Asiegbu (1997) in okra.

The effect of intercropping systems on days taken to bud initiation of rose was found non-significant. Total number of flowers / plant was significantly maximum in T_7- rose sole by 399.67. However, minimum number of flowers being 301.27, was observed in T_4- rose + gaillardia 1:2. In terms of total weight of flower / plant, treatment T_7- rose sole reported significantly highest flower yield, *i.e.* 307.42 g / plant which was statistically on the same bar with T_5- rose + French marigold 1:1 being 274.49 g / plant. Whereas minimum yield (213.9 g / plant) was found in T_4- rose + gaillardia 1:2.

Maximum total yield (7.35 kg / plot and 6.79 tonnes / ha) was obtained from treatment T7- rose sole and minimum yield (5.16 kg / plot and 4.77 tonnes / ha) was recorded fromT_4- rose + gaillardia 1:2. The reduction in yield attributes under narrow spacing might be ascribed due to comparatively poor growth and development of individual plants owing to competition for resources like space, sun light, nutrients, moisture *etc*. Sarma *et al.* (1996) Islam *et al.* (2014) and Singh and Singh (2014) also reported higher yield in monoculture as compared to their corresponding intercropped yield.

The treatments of intercropping systems during advancement of age, intercrop suppressed the weed growth in inter row space of rose according to their density. Further, biomass of intercrop incorporated *in-situ* after harvesting got decomposed and served as reservoir of organic nutrients aiding in greater growth of rose, leading to more flower bud formation. Lakshminarayanan *et al.* (2005) and Agrawal *et al.* (2010) also reported that intercropping with proper planting density can increased the yield of main crop without interrupting the growth.

Table 1. Effect of different intercrops on growth of rose

Treatment	Plant height (cm)	Number of branches	Plant spread	
			N-S	E-W
T_1 : Rose + African marigold (1:1)	92.64	9.70	103.73	94.66
T_2 : Rose + African marigold (1:2)	91.79	9.56	101.90	91.27
T_3 : Rose + Gaillardia (1:1)	82.42	9.36	95.83	90.05
T_4 : Rose + Gaillardia (1:2)	81.47	9.10	86.53	86.76
T_5 : Rose + French marigold (1:1)	99.61	10.06	106.90	105.29
T_6 : Rose + French marigold (1:2)	97.24	9.80	104.23	101.17
T_7 : Rose (sole)	108.64	10.36	117.93	111.04
SEm ±	5.45	0.54	5.41	5.14
CD 5%	16.97	NS	16.67	15.82
CV	10.21	9.61	9.15	9.15

Table 2. Effect on intercrops on flowering characters of rose

Treatment	Days taken to flower bud initiation	Total number of flowers / plant / year	weight of flower (g / plant)	Total yield (kg / plot)	Total yield (tonnes / ha)
T_1 : Rose + African marigold (1:1)	22.56	347.26	250.88	6.09	5.63
T_2 : Rose + African marigold (1:2)	23.03	340.64	245.61	6.01	5.56
T_3 : Rose + Gaillardia (1:1)	23.06	311.40	220.05	5.29	4.89
T_4 : Rose + Gaillardia (1:2)	24.03	301.27	213.9	5.16	4.77
T_5 : Rose + French marigold (1:1)	20.90	362.77	274.49	6.59	6.10
T_6 : Rose + French marigold (1:2)	21.60	350.45	266.48	6.52	6.04
T_7 : Rose sole	20.66	399.67	307.42	7.35	6.79
SEm ±	1.29	10.29	11.65	0.32	0.29
CD (5%)	NS	31.71	35.90	0.98	0.90
CV	10.01	5.16	7.97	8.91	8.91

Table 3. Comparison of yield characteristics of African marigold under sole cropping, rose + African marigold 1:1 and rose + African marigold 1:2 during first and second intercropping season

Treatment	First season		Second season	
	Yield (kg / plot)	Yield (tonnes / ha)	Yield (kg / plot)	Yield (tonnes / ha)
T_9, Sole African marigold	14.56	11.23	12.39	9.56
T_1, Rose + African marigold (1:1)	4.10	3.80	3.86	3.58
T_2, Rose + African marigold (1:2)	7.68	7.11	6.25	5.78

The flower yield was maximum in treatment T_9- sole African marigold (14.56 kg / plot and 11.23 tonnes / ha), followed by treatment T_2- rose + African marigold 1:2 (7.68 kg / plot and 7.11 tonnes / ha) and treatment T_1 rose + African marigold 1:1 (4.10 kg / plot and 3.80 tonnes / ha) in first season of intercropping system. Moreover the same trend of result was observed in second season of African marigold as a intercropping. Maximum flowers were observed from sole cropping which might be due to lower plant population than that of sole cropping. Similar results in the direction of present results were also noticed by Mouneka and Asiegbu (1997) in okra, Das *et al.* (2008) in tamarind and Mahant (2011) in banana.

Higher flower yield was obtained from treatment T_{10}- sole French marigold (14.48 kg / plot and 13.40 tonnes / ha) as compared to treatment T_5- rose + French marigold 1:1 (4.46 kg / plot and 4.13 tonnes / ha) and treatment T_6- rose + French marigold 1:2 (9.49 kg / plot and 8.79 tonnes / ha) in intercropping system. Moreover, the same trend was observed during the second season of French marigold as a intercropping. Highest yield was found from sole cropping which might be due to lower population than that of sole cropping. Similar views in direction of present outcomes were also noticed by Das *et al.* (2008), Agrawal *et al.* (2010), Mahant (2011) and Singh and Singh (2014).

Table 4. Comparison of yield characteristics of French marigold under sole cropping, rose + French marigold 1:1 and rose + French marigold 1:2 during first and second intercropping season

Treatment	First season		Second season	
	Yield (kg / plot)	Yield (tonnes / ha)	Yield (kg / plot)	Yield (tonnes / ha)
T_{10}, Sole French marigold	14.48	13.40	12.47	11.55
T_5, Rose + French marigold (1:1)	4.46	4.13	4.024	3.72
T_6, Rose + French marigold (1:2)	9.49	8.79	7.96	7.37

Table 5. Comparison of yield characteristics of Gaillardia under sole cropping, rose + gaillardia 1:1 and rose + gaillardia 1:2 during second intercropping season

Treatment	During 1st season		During 2nd season	
	Yield (kg / plot)	Yield (tonnes / ha)	Yield (kg / plot)	Yield (tonnes / ha)
T_8, Sole Gaillardia	13.20	12.22	8.87	8.21
T_3, Rose + Gaillardia (1:1)	4.56	4.22	2.97	2.75
T_4, Rose + Gaillardia (1:2)	9.60	8.88	6.30	5.83

Table 6. Effect of intercrops on Land Equivalent Ratio and Land Equivalent yield of rose

Treatment	LER	Rose Equivalent Yield (t / ha)
T_1 : Rose + African marigold (1:1)	1.18	8.86
T_2 : Rose + African marigold (1:2)	1.43	11.2
T_3 : Rose + Gaillardia (1:1)	1.06	8.20
T_4 : Rose + Gaillardia (1:2)	1.40	10.28
T_5 : Rose + French marigold (1:1)	1.21	8.55
T_6 : Rose + French marigold (1:2)	1.54	11.09
T_7 : Rose sole	1.00	6.79
T_8 : Gaillardia sole	1.00	-
T_9 : African marigold sole	1.00	-
T_{10} : French marigold sole	1.00	-

The higher yield was obtained from T_8- sole gaillardia (13.20 kg / plot and 12.22 tonnes / ha), followed by treatment T_4- rose + gaillardia 1:2 (9.60 kg / plot and 8.88 tonnes / ha) and T_3- rose + gaillardia 1:1 (4.56 kg / plot and 4.22 tonnes / ha) in first season where as in second season the maximum yield (5.83 t / ha) was noticed with T_4- rose + gaillardia 1:2. This might be due to lower population than that of sole cropping. Nedunchezhiyan *et al.* (2002) reported similar findings in intercropping of spices with elephant foot yam.

The data of rose equivalent yield have been presented in Table 6. Maximum equivalent yield (11.20) was observed from T_2- rose + African marigold 1:2, while lowest equivalent yield (6.79) was recorded from treatment T_7- rose sole). The present outcomes are in agreement with those reported by Rahman *et al.* (2006), and Islam *et al.* (2014). They also reported higher equivalent yield in intercropping systems as compared to sole cropping. This variation in equivalent yield is due to difference in yield of intercrops as well as difference of the economic value of the intercrops.

The data associated to LER have been existing in Table 6. It is apparent from the data that LER was more than one in all intercropping treatments. Maximum LER (1.54) was found from T_6- rose + French marigold 1:2, followed by T_2- rose + African marigold 1:2, while lowest LER (1) was recorded from all the sole crops. This may be due to use of better resources *i.e.* space, sunlight, water, nutrition *etc*. The results are in agreement with those of (1996), Gawade *et al.* (2003), Lakshminarayanan *et al*. (2005), Rahman *et al*. (2006).

The highest net income was obtained from rose + African marigold 1:2 (₹ 7,11,980 / ha) followed by rose + French marigold 1:2 (₹ 6,65,802 / ha). The highest benefit : cost ration (4.32) was recorded from Rose + African marigold 1:2 followed by Rose + French marigold 1:2 (3.77). This may depend on higher equivalent yield and market price fetched during harvesting period.

There is a feasibility of intercropping of annual rose production during first year of its plantation. No adverse effect on growth and yield of rose was observed due to 1:1 planting ratio of different intercrops. In the performance of LER as well as profitability, rose + African marigold (1:2) was found to be best intercropping system, while gaillardia was least profitable. Thus, it can be concluded that African marigold is best intercrop in rose. Looking to profitability and their effect on main crop, it is recommended to take up intercropping of annual rose, while at the same time keeping in mind to choose such intercrops as per their objectives.

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Effect of integrated nutrient management on yield, quality and economic of chilli (*Capsicum annuum* L.)

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ABSTRACT

An experiment was conducted to find out the effects of integrated nutrient management on yield, quality and economics of chilli (*Capsicum annuum* L.) during 2012 *rabi* season at Research Farm, JNKVV, College of Agriculture, Tikamgarh, Madhya Pradesh. There were 10 treatment combinations (V_1I_1. V_1I_2, V_1I_3, V_1I_4, V_1I_5, V_2I_1, V_2I_2, V_2I_3, V_2I_4, V_2I_5) as V_1, Pusa Jwala; V_2, Garima 12 and INM facors, I_1- recommended dose of fertilizer (RDF) or the control (100:50:50 kg NPK/ha), I_2- RDF + FYM (10 t/ha), I_3 - RDF + vermicompost (2.5 tonnes/ha), I_4 - RDF + Vesicular arbuscul mycorrhiza (VAM) @ 2 kg/ha, I_5 - RDF + Azospirillum in factorial randomized block design with 3 replications. Application of RDF + vermicompost 2.5 tonnes/ha showed significant increase in fruit yield (271.5 g/plant) and fresh fruit yield of 6816 kg/ha. There was lowest fruit yield and fresh fruit yield of chilli (227.8 g/plant and 4218 kg ha, respectively) with recommended dose of fertilizer (RDF) or the control (100:50:50 kg NPK/ha). Combined application of RDF + vermicompost 2.5 tonnes/ha showed significant increase in ascorbic acid content (190.8 mg/100 g). There was highest net return (181607 Rs/ha), with benefit : cost ratio of 3.19. However, lowest ascorbic acid content (170.8 mg/100 g) and minimum net returns (95194 Rs/ha) were recorded in the control (100:50:50 kg NPK/ha) while there was minimum B:C ratio (3.19) with I_2.

Key Words: Farmyard manure, Vermicompost, VAM, Azospirillum, Ascorbic acid, Integrated nutrient management, Quality, Economics.

Chilli (*Capsicum annuum* L.) is an important spice-cum-vegetable crop cultivated extensively in India. Chilli production need to be increased primarily from enhancing the productivity with a combination of high-yielding varieties, standard cultural practices and balanced plant nutrition through integrated nutrient management (INM). Since chemical fertilizer alone are not be able to sustain the productivity, integrated use of all potential sources of plant nutrients seems to be the only option to maintain soil fertility and crop productivity (Paul *et al.*, 2013). Organic manures improve soil physical, chemical and biological properties and also moisture holding capacity of soil, thus result in enhanced crop productivity along with better quality of crop produce (Premsekhar and Rajashree, 2009). Jayanthi *et al.* (2014) found positive affect on soil quality, yield and quality characters of chilli with combined application of vermi fertilizer along with recommended dose of chemical fertilizer. Further, vermi fertilizer reduces 50% of RDCF to crop and enhances soil quality, yield and quality of chilli than chemical fertilizer alone. Further combined use of organics (farmyard manure, vermicompost, biofertilizers, panchagavya) along with inorganic fertilizers increased the nutrient-use efficiency, apparent nutrient recovery and available nutrient status of soil (Naidu *et al.*, 2009). Therefore, an experiment was conducted to identify/screen suitable genotype with better management practices for getting good quality fruits and higher fruit yield of chilli for getting maximum profit.

MATERIALS AND METHODS

The experiment was conducted at Research Farm, JNKVV, College of Agriculture, Tikamgarh, during 2012

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to find out the effect of varieties and integrated nutrient management on growth and fruit yield of chilli. The experimental site was clay loam, low in available N (266 kg N / ha) and high in available P_2O_5 (25.9 kg / ha) and K_2O (255 kg / ha). The experiment was laid out in a factorial randomized block design with three replications comprising 10 treatment combinations, *viz*. V_1I_1, V_1I_2, V_1I_3, V_1I_4, V_1I_5, V_2I_1, V_2I_2, V_2I_3, V_2I_4, V_2I_5, whereas, V_1, Pusa Jwala; V_2, Garima, 12 and INM facors, I_1, recommended dose of fertilizer (RDF) or Control (100:50:50 kg NPK / ha), I_2- RDF + FYM (10 tonnes / ha), I_3 - RDF + vermicompost (2.5 tonnes / ha), I_4 - RDF + vesicular arbuscular mycorrhiza (VAM) @ 2 kg / ha, I_5 - RDF + Azospirillum.

Organic manures were applied (on equal N basis) as per the treatment and incorporated into soil before sowing. One-third nitrogen was given before sowing as basal dose. Remaining two-third quantity of nitrogen was applied in two split doses, *i.e*. 30 and 60 days after transplanting (DAT). The seedlings were planted with a spacing of 60 cm × 45 cm. Five plants were randomly selected from each treatments and replication for the study. Immediately after harvesting their fresh yield was recorded. The weight of each picking was added to get the total green chilli yield. The data were recorded on fruit yield / plant (g) and fresh green fruit yield (kg / ha) and ascorbic acid content. Ascorbic acid content in chilli green fruits at 90 DAT was estimated by using 2, 6-dichlorophenol indophenol titration method. The required amount of N, P and K fertilizers were applied through urea, DAP and muriate of potash, respectively. Other cultural operations and plant-protection measures were followed as per the recommendations.

RESULTS AND DISCUSSION

The data on fruit yield and quality characters like ascorbic acid was influenced by different varieties and integrated nutrient management treatments. The data were statistically analyzed (Table 1). Integrated nutrient management practices exerted significant affect on fruit yield during both years. There was significantly highest fruit yield (259.6 g / plant) and total fresh fruit yield of 6193 kg / ha with genotype Garima-12 over Pusa Jwala. Among integrated nutrient management treatments, RDF (100:50:50 kg NPK / ha) + vermicompost 2.5 tonnes / ha recorded significantly higher fruit yield and total yield (271.5 g / plant and 6816 kg / ha, respectively), while, significantly lowest fruit yield and yield (227.8 g / plant and 4218 kg / ha, respectively), were recorded with the control. Interaction due to treatments and varieties for total green fruit yield was found non-significant. Fruit yield and interaction were found significant and highest fruit yield (282.2 g / plant) was recorded under the treatment combination of RDF + vermicompost 2.5 tonnes / ha with Garima-12, followed by combination of RDF + FYM 10 tonnes / ha with Garima 12 (255.7 g) (Table 1). The increase in fruit yield with application of RDF + vermicompost @ 2.5 t / ha (I_3) may be attributed to better growth in terms of plant height and number of branches, which reflected into improved yield components, *viz*. number of fruits / plant, fruit length, fruit girth and fruit yield / plant as compared to other INM treatments. The increased yield in I_3 was due to significantly more number of fruits / plant and fruit yield / plant respectively.

These parameters also showed similar trend to that of final green fruit yield. Increase in number of fruits /

Table 1. Effect of INM on yield and ascorbic acid content of chilli

Treatment	Yield and ascorbic acid content of chilli								
	Fruit yield (g / plant)			Fresh fruit yield (kg / ha)			Ascorbic acid content (mg / 100 g)		
	V_1	V_2	Mean	V_1	V_2	Mean	V_1	V_2	Mean
I_1	210.2	245.5	227.8	4123.00	4313.00	4218.00	173.3	168.2	170.8
I_2	240.2	255.7	247.9	6315.00	6819.00	6567.00	188.5	185.8	187.1
I_3	260.9	282.2	271.5	6526.00	7105.00	6816.00	191.9	189.6	190.8
I_4	220.7	235.9	228.3	5813.00	6217.00	6015.00	183.3	181.7	182.5
I_5	251.4	279.0	265.0	6177.00	6510.00	6344.00	185.1	183.4	184.2
	236.6	259.6		5791.00	6193.00		184.41	181.7	
	S.Em±	CD at 5%		S.Em±	CD at 5%		S.Em±	CD at 5%	
Variety (V)	0.44	1.32		47.2	141.2		0.32	0.96	
Treatment (I)	0.70	2.09		74.6	223.2		0.51	1.52	
Interaction (V x I)	0.99	2.95		105.43	NS		0.72	NS	

Table 2. Effect of INM on Economics of chilli

Treatment	Cost of cultivation (₹/ha)			Gross monetary return (₹/ha)			Net monetary return (₹/ha)			B:C ratio		
	V_1	V_2	Mean	V_1	V_2	Mean	V_1	V_2	Mean	V_1	V_2	Mean
I_1	52436	52436	52436	144305	150955	147630	91869	98519	95194	1.75	1.88	1:1.82
I_2	59436	59436	59436	144305	229845	225435	161589	170409	165999	2.72	2.87	1:2.79
I_3	56936	56936	56936	228410	248675	238543	171474	191739	181607	3.01	3.37	1:3.19
I_4	52896	52896	52896	203455	217595	210525	150559	164699	157629	2.85	3.11	1:2.98
I_5	53136	53136	53136	216195	227850	222023	163059	174714	168887	3.07	3.29	1:3.18

plant was due to production of more number of flowers, higher percentage of fruit set and reduced shedding of flowers and fruits, resulting in increased fruits.

Similar increase in fruit yield was observed in gangetic alluvial plain soils with 50% nitrogen received from vermicompost and 50% from urea (Pariari and Khan, 2013). Improved growth components under application of RDF + vermicompost @ 2.5 tonnes/ha (I_3) may be attributed to increase in availability of nutrients for a longer period and continuous supply of nutrients. This might have attributed to more availability and subsequent nutrient uptake, increasing the yield. The reasons for increased fruit yield in chilli was attributed to increased solubilization effect and availability of nutrients by addition of vermicompost and increased physiological activity leading to build-up of sufficient food reserves for developing sinks and better portioning towards the developing fruits.

This higher translocation was possible perhaps due to better sink capacity as indicated by more number of fruits and weight of fruits per plant. The results are in accordance with findings of Patil *et al.* (2004). Similar results were also reported by Subbaiah *et al.* (1982) in chilli. The increase in growth and morphological parameters in early stages of crop growth, indicate the efficiency of plant to trap available solar radiation efficiently which resulted in increased rates of assimilates which in turn used in fruit formation, thus ultimately increasing the yield per unit area. The results are in conformity with those of Swamy and Subba Rao (1992).

There was significant difference in ascorbic acid content due to integrated nutrient management treatments and varieties, whereas interaction effect was non-significant (Table 1). Among genotypes, ascorbic acid content was significantly higher in Pusa Jwala (184.4 mg/100 g) over Garima 12 (181.7 mg/100 g). Among treatments, RDF + vermicompost 2.5 tonnes/ha recorded significantly higher ascorbic acid content (190.8 mg/100 g) over all other treatments, followed by RDF + FYM 10 tonnes/ha (187.1 mg/100 g), whereas lower ascorbic acid content was recorded in the control (170.8 mg/100 g). The organically managed crop has usually higher ascorbic acid than conventional fertilized crop because when a plant exposed with more N, it increases protein production, reducing carbohydrates synthesis. Since ascorbic acid and acidity are synthesized from carbohydrates, its levels are also reduced. In organically managed soil, plants are generally exposed with comparatively low amount of N and several plant nutrients are released slowly over time. Therefore, organic crop would be expected to contain higher value of these quality traits and carbohydrates and less protein. Further, soil microorganism affects soil dynamics and plant metabolisms, ultimately resulting in plant composition and nutrition quality. Worthington (2001) and Bahadur *et al.* (2003) also recorded results. Increased in ascorbic acid content of fruit in these treatments could be attributed to combined application of organic and inorganic fertilizers which help in better uptake of NPK nutrients including micronutrients which in turn affect the quality traits in chilli. The results are in conformity with those of Grimstand (1990) and Asano (1994).

The cost of cultivation of ₹ 46,680/ha among different chillis varieties was the same as inputs used and cultural operations performed were similar in all varieties. There was highest gross monetary return of ₹ 248,675 recorded by combination V_2 I_3, *i.e.* Garima-12 × RDF + vermicompost (2.5 tonnes/ha) whereas it was lowest in V_1 I_1 and V_1 I_2 (₹ 144,305) (Table 2). It is evident that net monetary return was highest (₹ 191,739) with treatment combination of V_2 I_3 (Garima-12 × RDF + vermicompost), while least net return (₹ 91869) was recorded from V_1 × I_1 (Pusa Jwala × Control). The highest B:C ratio of 3.37: 1 was recorded under combination of V_2I_3 (Garima 12 × RDF + vermicompost @ 2.5 tonnes/ha), while it was minimum (1.75: 1) with the V_1 I_1 (Pusa Jwala x control). This might be due to lower yield and higher cost of cultivation compared to other treatments. The economic analysis of combined use of different fertility levels such as vermicompost

showed that maximum gross return was obtained under V_2 I_3 might be attributed due to highest fruit yield of chilli and maximum net profit as well as B:C ratio is due to increase in total fruit yield (Singh and Teena Rani, 2012).

CONCLUSION

The integrated nutrient management treatments rendered their significant effect on yield and quality characters as well as fruit yield of chilli. The treatment consisting of 100% RDF of NPK + vermicompost @ 2.5 tonnes / ha recorded maximum for all characters. The (control) was lowest performer. Further, if cost of vermicompost is reduced by its indigenous preparation by farmers themselves, the integrated application of vermicompost with fertilizers in equal proportion is beneficial.

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Effect of plant spacing on flower and seed production in different strains of gomphrena (*Gomphrena globosa*)

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ABSTRACT

The experiment was conducted to find out the effect of plant spacing on flower and seed production in gomphrena (*Gomphrena globosa* L.)" at the experimental farm of Department of Floriculture and Landscape Architecture, Dr. Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan, during 2013. The experiment was laid out in randomized block design (factorial) with four levels of spacings, *viz*. (20 cm × 15 cm, 20 cm × 20 cm, 20 cm × 25 cm, 20 cm × 30 cm) with three strains *i.e.* GGW (*Gomphrena globosa* 'White'), GGM (*Gomphrena globosa* 'Magenta') and GGP (*Gomphrena globosa* 'Pink'). Among different plant spacings, closest spacing of 20 cm × 15 cm gave best results for plant height (59.10 cm), earlier arrival of visible flower bud stage (52.00 days), flower yield/m^2 (1,253.13 g) and seed yield/m^2 (83.34 g). However, plant spread (44.88 cm), number of flowers/plant (72.11), flower diameter (2.31 cm), duration of flowering (96.55 days), weight of flowers/plant (65.79 g) and seed yield/plant (83.34 g) were maximum with 20 cm × 30 cm spacing. Among strains, GGM gave best results for plant height (54.65 cm), weight of flowers/plant (57.00 g), seed yield/plant (3.57 g), 1000 seed weight (3.49 cm), flower/yield m^2 (1,253.13 g) and seed/yield m^2 (75.14 g). Based upon the overall performance of different strains, a closest spacing of 20 cm × 15 cm was recommended under midhill zones of Himachal Pradesh.

Key Words: Plant spacing, Flower yield, Seed yield, Strains, Flower diameter

Globe Amaranth (*Gomphrena globosa* L.) or bachelor's button, a native to India, is one of the hardiest annual bedding plant known for its colourful inflorescence. The genus belongs to family Amaranthaceae and consists of about 100 species of half-hardy annual, biennial and herbaceous perennial, but only one species *Gomphrena globosa* with its cultivars is in general cultivation. It is grown in beds, borders, rockeries and pots. Pot grown plants are useful for indoor decoration. It is also grown for cut flowers which last for a long time. The dried flowers are used in flower arrangement and indoor decoration. Value-added products like pomanders, garlands, ornament etc. can be prepared from its flowers.

Plant density has been recognized as a major factor determining the degree of competition between plants and have profound effect on commercial cultivation of any ornamental crop. Since, sufficient information are not available on effect of plant spacing on flower and seed production in gomphrena, particularly in North Indian conditions, present studies were undertaken to find out optimum planting density for flower and seed production in its different strains.

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MATERIALS AND METHODS

The experiment was conducted at Department of Floriculture and Landscape Architecture, Dr Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh during 2013 (April-August). The experiment was laid out in a randomized block design (factorial) with four levels of spacings *viz.*, (20 cm × 15 cm, 20 cm × 20 cm, 20 cm × 25 cm, 20 cm × 30 cm) and three strains, *i.e.* GGW (*Gomphrena globosa* 'White'), GGM (*Gomphrena globosa* 'Magenta') and GGP (*Gomphrena globosa* 'Pink'). Well rotten farmyard manure (FYM) @ 5 kg/m^2 was mixed thoroughly in soil at the time of field preparation, thereafter, raised beds (1 m × 1 m) size were prepared and levelled properly. Full

dose of phosphorous (20 g/m^2) and potassium (20 g/m^2) and half dose of nitrogen (7.5 g/m^2) were incorporated into beds as a basal dose. The remaining half dose of nitrogen (7.5 g/m^2) was applied 30 days after transplanting. Nitrogen, phosphorus and potassium were applied through urea, single superphosphate and muriate of potash, respectively. Pinching was done 40 days after transplanting to encourage the emergence of lateral branches. Flower and seed yield parameters along with their quality characteristics were recorded. The data recorded on each parameter were analyzed by ANOVA technique as per Gomez and Gomez (1984). The treatment means were compared using critical difference values calculated at 5% level of significance. Standard error of difference between treatment mean and critical difference were worked out.

RESULTS AND DISCUSSION

There were maximum plant spread (44.88 cm), number of flowers/plant (72.11), flower size (2.31 cm) and weight of flowers/plant (65.79 g) was recorded with wider spacing of 20 cm x 30 cm (Table 1). Whereas maximum plant height was observed with closest spacing of 20 cm × 15 cm (59.10 cm). There was gradual decrease in plant height with increase in plant spacing. This might be due to decreased inter-plant competition at closer spacing and more availability of space for efficient utilization of light, nutrients and water at wider spacing. Mili and Sable (2003) also recorded maximum plant height at closer spacing of 30 cm × 30 cm compared with wider spacing in Calendula officinalis. Similar results were reported by Beniwal *et al.* (2003) and Ghosh and Pal (2008) in African marigold (Tagetes erecta) cv. 'Siracole'. Likewise Khobragade *et al.* (2012) also recorded maximum plant height with closer spacing in China aster *(Callistephus chinensis)*. Studies of Berimavandi *et al.* (2011) on effects of plant density and sowing date on growth, flowering and quantity of essential oil of *Calendula officinalis* L. demonstrated that maximum flower number/plant was obtained from density of 20 plants/m^2 (25.88 flowers/plant). On the other hand flower yield m^2 (1,391.11 g) and seed yield/m^2 (83.34 g) were recorded maximum with the closest spacing of 20 cm × 15 cm. These results might be due to increased plant population at closer spacing which ultimately increased flower as well as seed yield. Similar results of higher flower yield was recorded in golden rod at closer spacing (Ryagi and Nalawandi, 1996) and Ahirwar *et al.* (2012) in African marigold (Tagetes erecta). Whereas maximum seed yield (3.89 g/plant) and 1000 seed weight (3.73 g) were recorded with wider spacing of 20 cm × 30 cm. Enhanced production of individual plant was attributed to increased head diameter due to less competition and more number of vegetative buds at wider spacing. Seed yield was recorded maximum at wider spacing (60 cm × 60 cm) in garland chrysanthemum *(Chrysanthemum coronarium)* as compared to closer spacings (Dorajeerao and Mokashi, 2013). The results further support to those of Dorajeerao *et al*. (2012) in chrysanthemum *(Dendranthema grandiflora)*.

Minimum number of days for first flower bud appearance (52.00) and days to flowering (60.66) were observed with 20 cm × 15 cm spacing (Table 2). The maximum days taken for both the attributes were

Table 1. Effect of plant spacing on growth, flowering and seed production in gomphrena

Spacing (cm)	Plant height (cm)	Days taken for bud formation	Flower size (cm)	Weight of flowers/plant (g)	Flower yield/m^2	Duration of flowering	Seed yield/plant (g)	Seed yield/m^2 (g)	1000-seed weight (g)
20 × 15	59.10	52.00	1.64	38.26	1,391.11	93.00	2.85	83.34	3.16
20 × 20	54.58	52.88	1.82	48.51	1,219.26	94.88	3.69	72.08	3.33
20 × 25	53.14	54.11	1.97	57.81	1,143.55	96.00	3.69	67.88	3.47
20 X 30	50.21	55.33	2.31	65.79	1,049.60	96.55	3.89	63.40	3.73
Strains									
GGW	53.65	53.41	1.73	50.33	1,149.82	94.91	3.47	70.04	3.37
GGM	54.63	53.44	2.01	53.93	1.253.13	94.91	3.57	75.14	3.49
GGP	54.49	53.91	2.07	53.52	1,199.69	95.50	3.54	69.84	3.40
CD 0.05									
Spacing	0.83	1.08	1.17	2.44	72.33	0.62	0.26	1.85	0.04
Strains	0.72	NS	1.15	2.11	62.64	NS	NS	1.60	0.03
Spacing × Strains	1.44	1.88	NS	4.22	NS	1.08	0.46	NS	NS

NS*, Non-significant

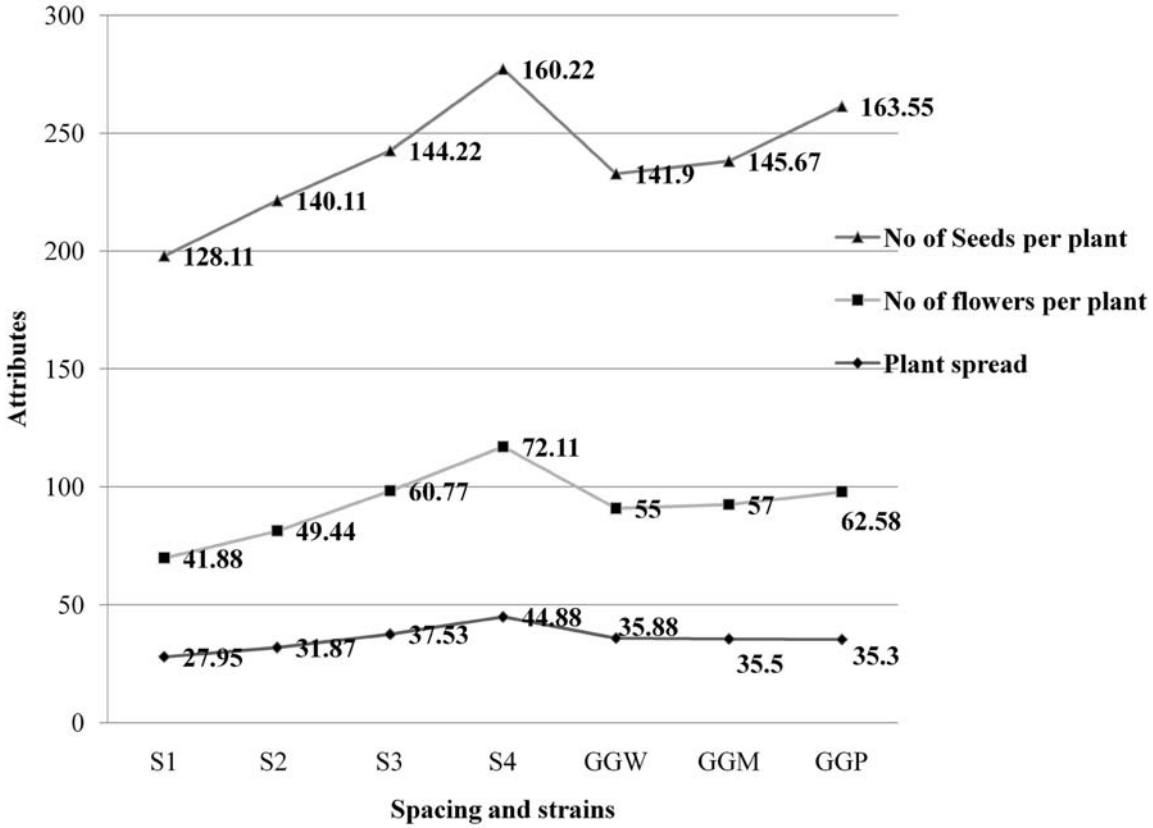

Fig. 1. Effect of spacing on plant spread, number of seeds/plant and number of flowers/plant in different strains

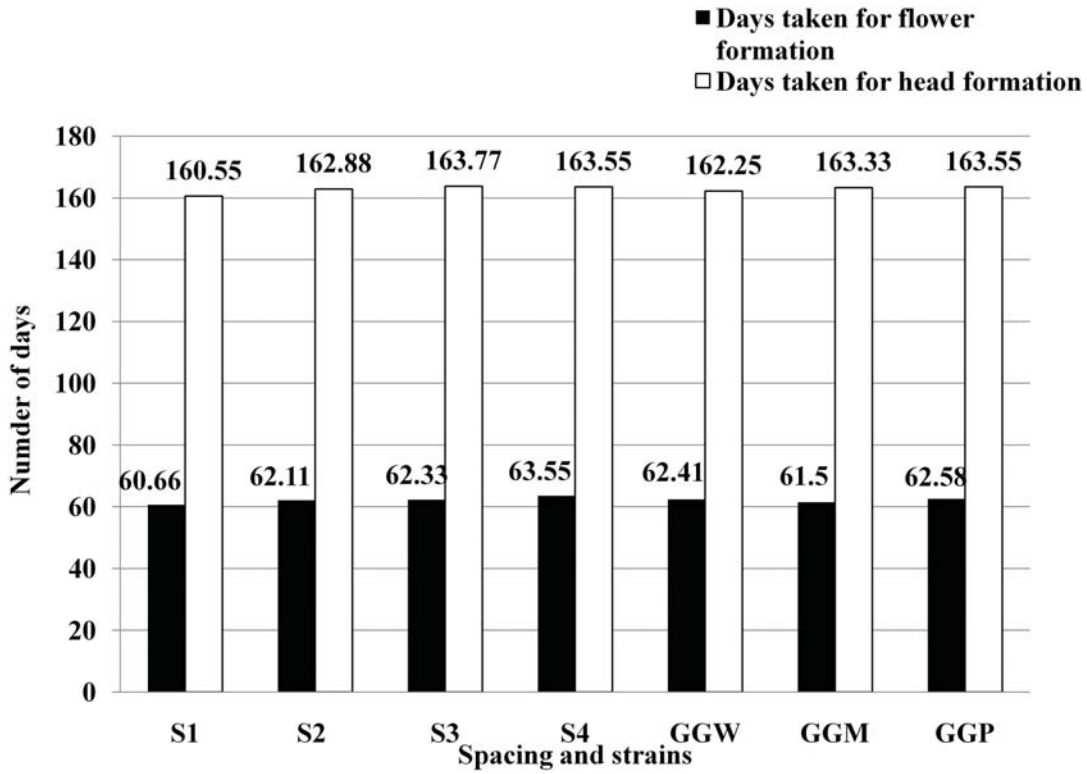

Fig. 2. Effect of spacing on days taken for flower formation and seed head formation in different strains

recorded at widest spacing of 20 cm × 30 cm. This might be due to reduction in growth at narrow spacing that directed the plants towards reproductive phase earlier than those plants at wider spacing. Plants transplanted at wider spacing of 20 cm × 30 cm gave best results for maximum duration of flowering (96.55 days). However minimum duration of flowering was observed with closest spacing of 20 cm × 15 cm (93.00 days). Early flower bud initiation was observed in *Calendula officinalis* at a spacing of 30 cm × 30 cm as compared to wider spacings (Mili and Sable, 2003). Similar results were reported by Chaudhary *et al.* (2007) in zinnia *(Zinnia elegans)*.

Amongst strains, maximum plant height (54.63 cm), weight of flowers / plant (53.93 g), flower and seed yield / plant (1,253.13 g and 75.14 g respectively) were recorded in GGM. On the other hand maximum flower size was recorded in GGP (2.07 cm). This might be attributed to their genetic makeup and differential response to prevailing climatic conditions. Effect on all other parameters was however found to be non-significant.

The interaction between plant spacing and strains was found to be significant on plant height, weight of flowers / plant, duration of flowering and seed yield / plant. Maximum plant height (60.50 cm) was observed with 20 cm × 15 cm spacing in GGM, whereas maximum weight of flowers / plant (68.97g), duration of flowering (98.33 days) as well as seed yield / plant (4.81g) were recorded with 20 cm × 30 cm spacing in GGP.

Thus, it can be concluded that the closet spacing of 20 cm × 15 cm, accommodating 35 plants / m^2 resulted in highest flower yield (GGW, 1368.38 g / m^2, GGM, 1500.38 g / m^2, GGP, 1304.58 g / m^2) and seed yield (GGW, 81.01 g / m^2, GGM, 86.25 g / m^2, GGP, 82.75 g / m^2) is recommended for different strains of gomphrena under mid hill conditions of Himachal Pradesh.

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Analysis of physico-chemical properties of jalpai (*Elaeocarpus floribundus* Blume.) grown in northern parts of West Bengal

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ABSTRACT

A study was undertaken to determine physio-chemical properties of fruits and to evaluate the best accession of jalpai (*Elaeocarpus floribundus* Blume.) during fruiting season of 2016 at Department of Post-harvest Technology, UBKV, Pundibari, West Bangal. The fruits were harvested from distinct locations of North Bengal and were brought to the lab for their biochemical analysis. ACC-11 had a very highest value of TSS (7.07° Brix), along with ACC-25 (6.9°Brix). The ACC-24 had high fruit weight (20.83 g) compared to other accessions. ACC-30 also showed a highest value of total sugar (7.50) and high reducing sugar content (2.35). ACC-20 not only had a high TSS value but also had higher amount of total sugar content (6.85). Similar trend was observed in ACC-29 where high TSS (18.7), high reducing sugar (2.17) and total sugar content (5.77) were recorded. Thus, ACC-11, ACC-20 and ACC-29, yield superior quality of jalpai, whereas ACC-30 produces fruits with high sugar and acid content.

Key Words: Accessions, Biochemical, Distinct characters, Physio-chemical, properties, TSS value, Total sugar

Jalpai or Indian olive (*Elaeocarpus floribundus* Blume. syn. *E. serratus*) belongs to family Elaeocarpaceae and is an important minor fruit grown in *terai* region of West Bengal, India. However, it is vastly, distributed in West Bengal, Asom, Tripura and Meghalaya. It is a medium to tall evergreen tree, maximum up to 20 m in height. Its trees are found in eastern Himalayas up to 3,000 feet and in the evergreen forests of North Kanara and western coast down to Travancore (CSIR, 2003). The total area under fruit crops is 172.70 thousand hectare with a total production of 2301.70 thousand tonnes in West Bengal (GWB, 2006). It is grown as homestead crop in shady or semi-shady condition with no or very few management practices. Flowers appear during April-May and fruits are harvested in October-November (Sankarsan *et al.*, 2006). The fruits are used in dysentery and diarrhoea. The leaves are used in rheumatism and as an antidote for poison (De and Parikh, 1985). There is a good demand for matured fruit in market for preparation of pickles and chutney (Bhowmick, 2011). There is no recommended packages of practices for cultivation of jalpai. It is locally propagated by seeds. The fruits are mainly marketed locally in North Bengal districts. A very small quantity is marketed in other parts of West Bengal. The average yield may vary from 40-60 kg fruits/tree/year. Information regarding flowering and fruiting characteristics of Indian olive is less in literature. Therefore, study was undertaken to evaluate physio-chemical properties of accessions collected from different locations of North Bengal.

MATERIALS AND METHODS

The fruits were collected from different distinct locations of North Bengal during fruiting season of 2016. The fruits collected from a location were of same tree. Clean fruits free from biotic and abiotic stress were collected. The fruit samples collected were in perfect maturity, neither unripe nor over ripe. The fruits were at first cleaned in water and then shade dried. The fruit samples were analysed for their physical and chemical properties. The individual plant was treated as ACC-1, ACC-2, ACC-3, ACC-4 to ACC-22 respectively. The design of experiment was randomized block design with 32 treatments and 3 replications.

Fruit skin colour was assayed by colour chart

designed by the Royal Horticultural Society, 5th edition (2007). The physical analysis of fruits like length and breadth was done using a standard 30 cm scale. The juice (in ml) content in fruit samples were estimated using standard Borosil glass measuring cylinder. The average weight of fruits was determined digitally by electronic weighing balance.

The pH of fruit samples were determined by digital pH meter manufactured by UTech. The weight of fruits, peel and pulp were taken by electronic weighing balance. The total soluble solid (TSS) was estimated by using the hand refractometer. Chemical assessment of fruits like titratable acidity (TA) and total sugar (TS) were done by method suggested by Association of Analytical chemists (AOAC, 1995).

RESULTS AND DISCUSSION

There was maximum fruit weight in ACC-24 (20.83 g) and minimum in ACC-28 (12.83) (Table 1). However, ACC-9 and ACC-11 were at par with ACC-24. Fruit length was maximum in ACC-24 (5.9 cm) and minimum in ACC-1 (3.47 cn). Fruit breadth was maximum in ACC-29 (2.97 cm) and minimum in ACC-1 (1.93 cm). Fruit shape varied from oval in ACC-1,

Table 1. Physical properties of different jalpai accessions (average of 3 replications)

Accession	Fruit weight (g)	Fruit length (cm)	Fruit breadth (cm)	Fruit shape	Fruit skin colour
ACC-1	13.37	3.47	1.93	Oval	GGN144B
ACC-2	15.53	4.47	2.6	Oval	GGN144B
ACC-3	13.67	4.13	2.53	Roundish	GGN144B
ACC-4	13.23	3.9	2.6	Oval	GGN144B
ACC-5	15.03	4.57	2.37	Oval	GGN144B
ACC-6	17.1	5.07	2.8	Oblong	GGN144B
ACC-7	17.27	5	2.6	Oval	GG143C
ACC-8	13.1	3.77	2.83	Oblong	GGN144B
ACC-9	19.5	5.27	2.73	Oval	GGN144B
ACC-10	19.23	5.03	2.67	Oval	GGN144B
ACC-11	12.73	3.9	1.97	Oval	GGN144B
ACC-12	13.9	4.5	2.47	Oblong	GG143C
ACC-13	17.37	5.17	2.9	Oval	GGN144B
ACC-14	16.9	4.83	2.47	Roundish	GG143D
ACC-15	13.8	4.2	2.5	Oval	GGN144B
ACC-16	18.63	5.33	2.77	Roundish	GGN144B
ACC-17	16.63	4.53	2.3	Oval	GGN144B
ACC-18	13.87	3.67	2.13	Oval	GGN144B
ACC-19	18.7	4.9	2.73	Oblong	GGN144B
ACC-20	18.23	4.67	2.53	Oval	GGN144B
ACC-21	13.2	4.07	1.97	Oval	GGN144B
ACC-22	14.73	4.53	2.47	Roundish	GG143C
ACC-23	18.33	5.63	2.8	Oblong	GGN144B
ACC-24	20.83	5.9	2.73	Oblong	GGN144B
ACC-25	13.77	3.57	1.97	Oblong	GGN144B
ACC-26	13.97	3.53	2.13	Oblong	GG143C
ACC-27	12.87	3.53	2.23	Oblong	GGN144B
ACC-28	12.83	4.43	2.73	Oval	GGN144B
ACC-29	18.7	5.5	2.97	Roundish	GG143D
ACC-30	17.47	5.33	2.8	Oblong	GGN144B
ACC-31	16.77	5.2	2.77	Oblong	GG143C
ACC-32	16.97	4.87	2.37	Oblong	GG143C
SEm.(±)	0.42	0.12	0.09	N/A	N/A
CD (5%)	1.19	0.34	0.27	N/A	N/A
CV	4.59	4.51	6.53	N/A	N/A

GG, green group

ACC-2, ACC-4, ACC-5, ACC-7, ACC-9, ACC-10, ACC-11, ACC-13, ACC-15, ACC-17, ACC-18, ACC-20, ACC-21 and ACC-28 to roundish oval in ACC-3, ACC-14, ACC-16, ACC-22 and ACC-29. Oblong-shaped fruits were observed in ACC-6, ACC-8, ACC-12, ACC-19, ACC-23, ACC-24, ACC-25, ACC-26, and ACC-27. Fruit skin colour varied from GGN144B in most of accessions and GG143C was observed in ACC-7, ACC-12, ACC-22, ACC-26, ACC-31, and ACC-32. (Table 1, Figs. 1, 2 and 3).

Maximum TSS (°Brix) was observed in ACC-11 (7.07) and minimum in ACC-4 (4.8°Brix). However, ACC-20 and ACC-25 was at par with ACC-11. Titratable acidity was maximum in ACC-3 (17.93) and minimum in ACC-9 (12.73). The ACC-3 was found at par with ACC-25. Total sugar was recoded maximum in ACC-30 (7.5) and minimum in ACC-2 (5.62). However ACC-3, ACC-4, ACC-12, 19, ACC-20 and ACC-31 was at par with ACC-30. Maximum reducing sugar was observed in ACC-13 (2.42) and minimum in ACC-2 (2.03) (Table 1).

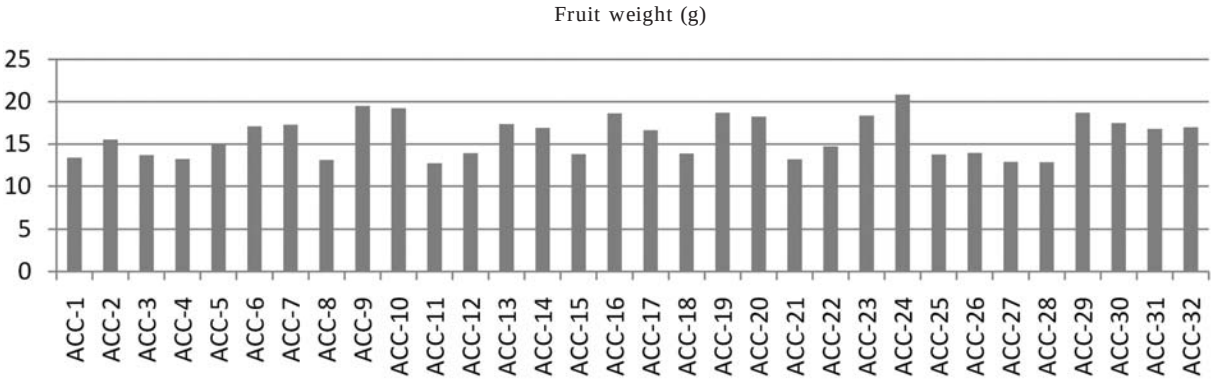

Fig. 1. Fruit weight of different accessions (average of 3)

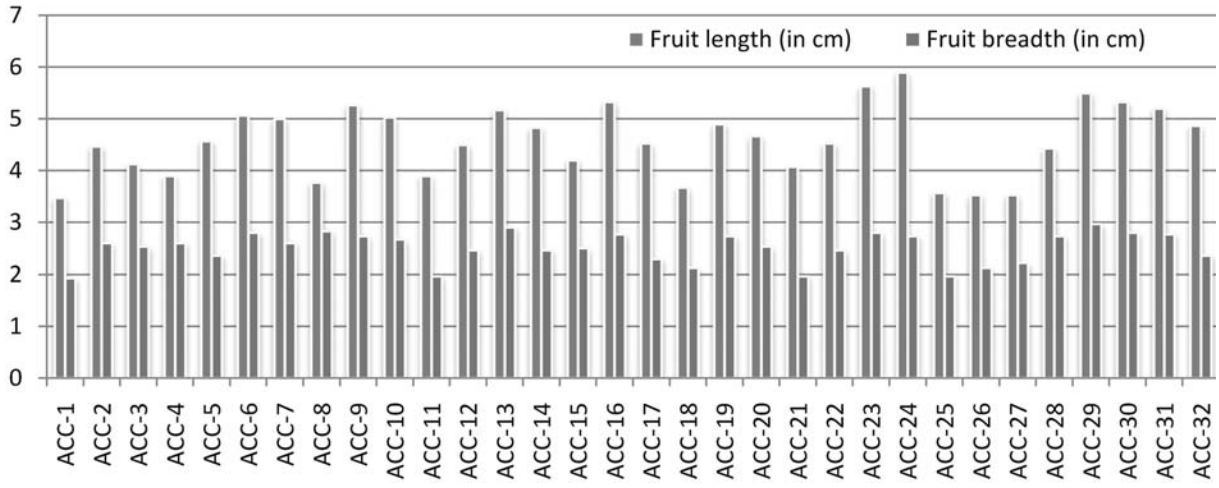

Fig. 2. Fruit length and breadth (cm) of fruits from different accessions (average of 3)

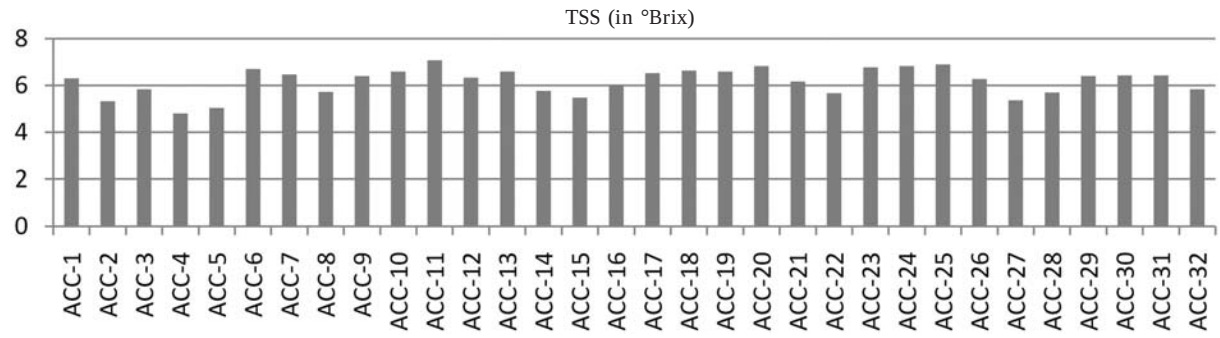

Fig. 3. TSS (°Brix) of fruits of different accessions (average of 3)

Table 2. Chemical properties of different Jalpai accessions from North Bengal (average of 3 replications)

Accessions	TSS (°Brix)	Titratable acidity (%)	Total sugar (%)	Reducing sugar
ACC-1	6.3	16.83	6.15	2.22
ACC-2	5.33	13.5	5.62	2.03
ACC-3	5.83	17.93	7.02	2.20
ACC-4	4.8	15.2	5.67	2.09
ACC-5	5.03	14.87	6.43	2.03
ACC-6	6.7	13.97	6.87	2.4
ACC-7	6.47	14.53	5.67	2.2
ACC-8	5.73	13.57	6.68	2.07
ACC-9	6.4	12.73	6.98	2.19
ACC-10	6.6	14.4	5.96	2.34
ACC-11	7.07	14.53	6.34	2.14
ACC-12	6.33	16.37	6.93	2.33
ACC-13	6.6	15.77	5.83	2.42
ACC-14	5.77	14.43	5.81	2.04
ACC-15	5.47	14.5	5.68	2.13
ACC-16	6.03	15.77	6.18	2.24
ACC-17	6.53	13.27	5.8	2.38
ACC-18	6.63	15.8	6.76	2.33
ACC-19	6.6	15.2	7.35	2.32
ACC-20	6.83	14.67	6.85	2.22
ACC-21	6.17	15.53	6.67	2.18
ACC-22	5.67	15.6	5.83	2.26
ACC-23	6.77	14.9	6.98	2.35
ACC-24	6.83	14.77	6.50	2.31
ACC-25	6.9	13.43	7.4	2.33
ACC-26	6.27	14.6	6.47	2.2
ACC-27	5.37	16.87	7.35	2.1
ACC-28	5.7	17.83	6.54	2.35
ACC-29	6.4	17.43	5.77	2.17
ACC-30	6.43	14.33	7.50	2.35
ACC-31	6.43	14.3	6.85	2.17
ACC-32	5.83	13.77	6.51	2.35
S.Em.(±)	0.1	0.18	0.13	0.04
CD (5%)	0.28	0.5	0.67	0.11
CV	2.75	2.01	3.47	3.13

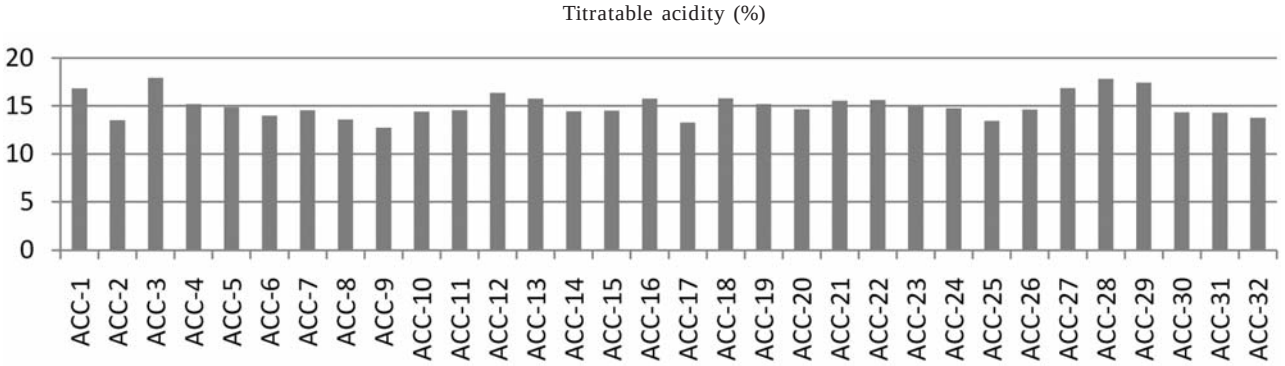

Fig. 4. Titratable acidity (%) of fruits of different accessions (average of 3)

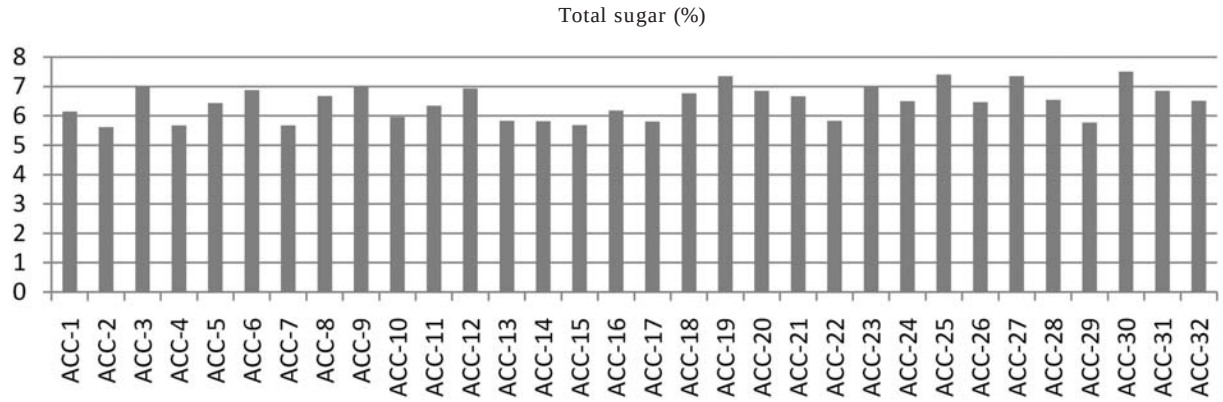

Fig. 5. Total sugar (%) of fruits of different accessions (average of 3)

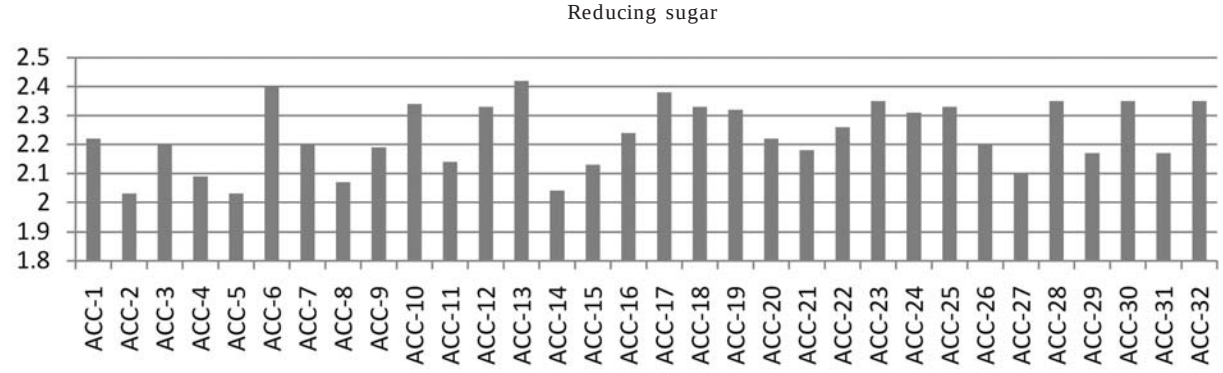

Fig. 6. Reducing sugar (%) of fruits of different accessions (average of 3)

Thus, it was found that ACC-11 had very highest value of TSS (7.07°Brix). ACC-25 had a high TSS 6.9°Brix. ACC-24 had high fruit weight (20.83 g) as compared to other accessions. ACC-30 also showed a highest value of total sugar (7.50) and high reducing sugar content (2.35). ACC-20 not only has a high TSS value but also higher amount of total sugar content (6.85). Similar trend was observed in ACC-29 where a high TSS (18.7°Brix), high reducing sugar (2.17) and total sugar content (5.77) were recorded. It can be established that ACC-11, ACC-20 and ACC-29 yields, superior quality fruits, whereas ACC-30 produces fruit with high sugar and acid content (Figs. 4, 5 and 6).

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Morpho-anatomical and molecular characters of *Bulbophyllum* and *Dendrobium* spp. found in Southern Ghats of India

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ABSTRACT

Anatomical and molecular analysis of genetic variability was investigated by using SDS-PAGE and RAPD markers in 6 epiphytic orchids, i.e. four species of *Bulbophyllum* and two of *Dendrobium*, collected from Southern Ghats of Sikkim, India. The *Bulbophyllum careyanum* (W.J. Hook.) Spreng., *B. maculosum* Schltr., *B. thomsonii* J. D. Hook., *B. tremulum* Wight., *Dendrobium anceps* Sw. and *D. crassinode* Benson and Rchb. f. were collected from East and West Sikkim. A low stomatal frequency and an extensive lignification on inner walls, and handle-cells were recorded in populations collected from East Sikkim (Pakyong) as compared to those from Central-Sikkim (Gangtok). The RAPD and protein profile data indicated intra-population diversity between these two sites which may be attributed to ecological and climatic conditions prevailing in both sites of North-East Himalayas.

Key Words: Orchids, Morpho-anatomy, Molecular, Orchids, Stomata frequency, RAPD analysis, Leaf epidermis, Pseudo-bulbs.

The North-Eastern Himalayas (Sikkim) is considered to be one of the biodiversity hot-spots in the world. The epiphytic orchids, *Bulbophyllum careyanum* (W.J. Hook.) Spreng., *B. maculosum* Schltr., *B. thomsonii* J. D. Hook., *B. tremulum* Wight, *Dendrobium anceps* Sw., and *D. crassinode* Benson & Rchb. f., were selected for the study. Habitat destruction, fragmentation of natural resources and illegal collections have jeopardized the size and frequency of orchid natural populations. Hence, there is an urgent need to evolve the conservation strategies for this important group of plants. The maintenance of genetic diversity among populations is very important for a long-term conservation programme (Avila-Diaz and Oyama, 2007). According to Besse *et al.* (2004) and Williams *et al.* (1990), RAPD (Random Amplified Polymorphic DNA), amplified by arbitrary primers, could be very useful genetic markers. Besse *et al.* (2004) studied genetic diversity in cultivated *Vanilla* by using RAPD markers. In orchids, genetic diversity has varied from, very low to high; wide spread species in general have higher level of variations than endemic species with a narrow geographic range and usually larger populations have more density (Avila-Diaz and Oyama, 2007; Gustafsson, 2000). The RAPD is a very powerful tool to estimate the range of genetic variability. Therefore, it is used to evolve conservation strategies of a particular species. Therefore, morphological and molecular diversity in *Bulbopyllum* and *Dendrobium* were studied throughout geographical distributions in North Eastern Himalayas (Sikkim) by SDS-PAGE (Sodium Dodecyl Sulphate polyacrylamide Gel Electrophoresis) and RAPD (Randomly Amplified Polymorphic DNA) analysis.

MATERIALS AND METHODS

Two sites in North-Eastern Himalayas (Sikkim), were selected for the study. The first site (P) is situated in the East Pakyong, Sikkim, and second in the Central Gangtok, Sikkim. The vegetation of Gangtok is dry and climate is monsoonal, whereas the Pakyong forests are dry deciduous and moist deciduous. At each site, three

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Table 1. Morphological characters of Bulbophyllum and Dendrobium (2010-2011)

Morphological character	Populations					
	P_1	P_2	P_3	G_1	G_2	G_3
Leaf						
Thickness of midrib region (mm)	15	14	16	17	19	21
Thickness of laminar region (mm)	10	09	08	12	13	15
Mid-rib vascular bundle length (mm)	15	14	17	21	23	25
Size of stomatal pore (mm)	05	06	07	07	09	11
Pseudobulb						
Vascular bundle size (mm)	07	08	06	09	08	12
Number of xylem caps (mm)	04	05	03	05	07	09
Size of water storage cells (mm)	07	09	06	09	11	15
Root						
Number of velamen layers	05	03	04	07	05	09
Lignification of Exodermis (mm)	06	05	08	09	12	11
Vascular cylinder size (mm)	11	10	13	13	15	14

populations were selected namely, P_1, P_2, P_3 and G_1, G_2 and G_3. Leaves, pseudobulbs and roots were sampled from these six populations growing on different host trees (Table 1). The materials were fixed in formalin-acetic acid-alcohol (FAA). The usual procedure of dehydration and embedding were followed (Berlyn and Miksche, 1976). Microtome sections were cut at a thickness of 8-10 µm and stained with safranin and fast-green.

Fresh leaves (2g) were crushed in extraction buffer containing 1.4 M NaCl, 20 mM EDTA (ethylene diamine tetracetic acid) 100 mM Tris-HCl (pH 8.0). 2% CTAB (N-Cety-N, N, N trimethyl ammonium bromide) and 0.2% mercaptoethanol with mortar and pestle and it was subjected to SDS-PAGE (Shi and Jackowski, 1998). Protein banding pattern was observed and also protein molecular weight marker ranging from 20- 97 KD was used for comparison.

A modified CTAB technique (Doyle and Doyle, 1987) was used for extraction of genomic DNA and PCR amplification. Only two random primers, sequencing 5' to 3', GGTGCGGGAA and CCCGTCAGCA, were used. The PCR was performed in a reaction volume of 25 µl containing 50 mM KCI, 10 mM Tris HCI (pH 9.0), 0.1% triton X-100, 1.5 mM $MgCl_2$, 100 µM each of dNTPs, 25 pmole primer, 100ng genomic DNA and 1 unit of Taq DNA polymerase.

Amplified products were resolved electrophoretically on 1.5% agarose gel run at 100V, visualized by staining with ethidium bromide. RAPD bands were scored as present or absent for each DNA sample and analyzed according to Nei and Li (1979) definition of genetic similarity, *i.e.* $s_{ij} = 2a/(2a+b+c)$, where s_{ij} is similarity coefficient between two individuals (*i* and *j*), *'a'* is number of bands present in both *i* and *j*, *'b'* is number of bands present in i and absent in *j*, and *'c'* is the number of bands present in *j* and absent in *i*. The matrix of similarity was clustered using UPGMA algorithm and the dendrogram was constructed.

RESULTS AND DISCUSSIONS

In *Bulbophyllum* species, leaves were thick, linear-oblong and leathery. Velamen roots were well developed, prominent pseudobulbs with furrows from all the samples. *Dendrobium* species possessed thin and linear leaves. Epidermal cells, relatively large on adaxial (upper) surface, were rectangular to polygonal in shape. Stomata are restricted to the abaxial surface (hypostomatic in distribution). The leaves are mostly hypostomatic in most of the orchids (Avadhani *et al.*, 1982). Rasmussen (1987) opined that hypostomaty is more frequent in mesophytic orchids and amphistomaty dominates in those of day and humid habitats. Paracytic stomata with two guard cells were observed in all the six populations. The minimum and maximum length of guard cells was 15.3 and 25.4 µm in P_2 and G_3 respectively (Table 2). The minimum and maximum size of stomatal pores were 37.8 and 59.3 µm in P_1 and G_2, respectively.

Thick cuticle was observed from the population of Gangtok, which is a drier area as compared to Pakyong. The populations collected from Gangtok showed prominent handle cells which help in preventing water loss. (Fig. 1b). The stomatal chambers were observed from all the samples (Fig. 1b). Mohana Rao and Khasim (1987) also observed extra vascular bundles (fiber

Table 2. Protein band pattern and molecular weight in six populations of *Bulbophyllum* and *Dendrobium* during 2010-2011

	Number of bands and molecular weight (KD)					
Population	1	2	3	4	5	6
P_1	75	65	48	36	28	42
P_2	72	68	58	42	39	51
P_3	81	72	63	48	31	39
G_1	85	78	73	72	69	75
G_2	79	81	85	68	59	63
G_3	83	93	89	75	68	69

bundles) in hypodermal position in some sarcanthine orchids. Khasim and Ramesh (2010) observed the vascular bundle size in *Vanda tessellata*. These cells are suggested to provide mechanical strength to the plant body as well as protect the leaf against desiccation. Mesophyll was homogenous and not differentiated into palisade and spongy parenchyma. The mid-rib vascular bundles were larger in size, and small and large laminar bundles were studied on its either side (Fig. 1a). There were about 5 to 9 layers of thick fiber caps present all around the midrib vascular bundles . Accordingly, the length and width of the mid-rib vascular bundles shows variations in all the six populations (Table 2); it was comparatively larger than from populations of Gangtok.

Stem / pseudobulb was thick, with furrows exclusively for water storage. Prominent water storage cells were found in populations collected from Gangtok, than those (Fig. 2d) collected from Pakyong. The minimum and maximum size of water storage cells was 15 mm and 22 mm in P_1 and G_3 respectively. Scattered vascular bundles were numerous in species collected from Gangtok (Fig. 2c), as compared to those collected from Pakyong (Table 2).

In trans-section, roots were circular in outline. The number of velamen layers showed slight variations amongst six populations. The minimum number was 8 in (Fig. 1e) P_2 and maximum as 12 in G_3. Cells in the outer most velamen layers were much thickened and

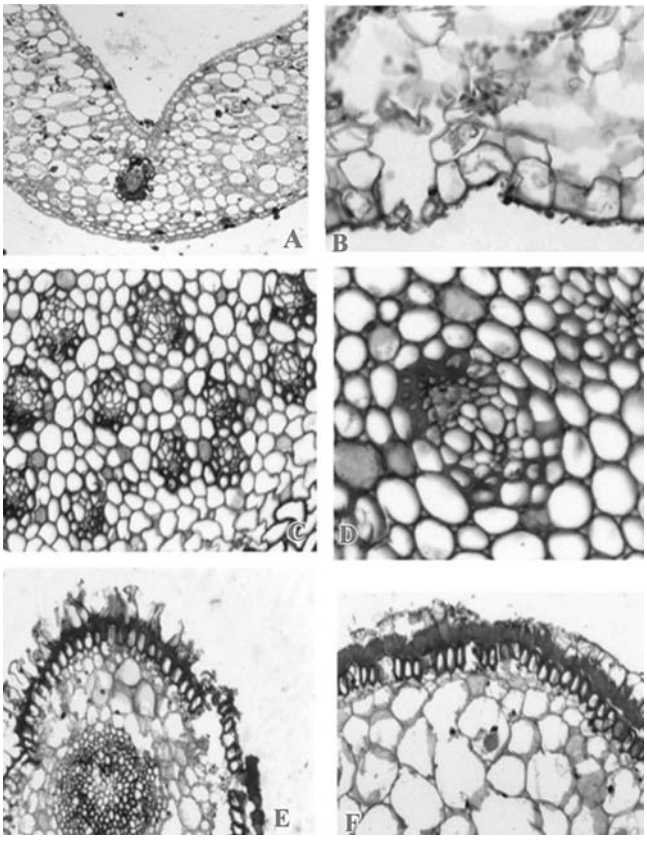

Fig. 1. a-f.Anatomical variations in leaf, pseudobulb and root samples from Pakyong

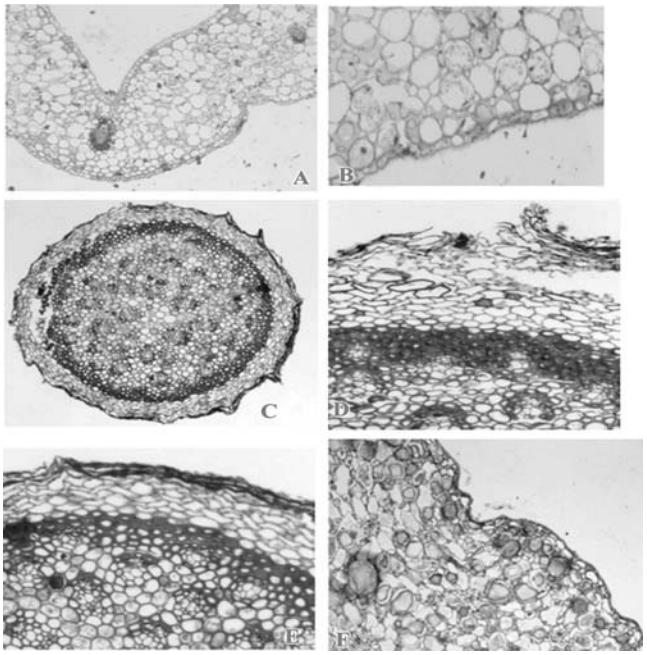

Fig. 2. a-f.Anatomical variations in leaf, pseudobulb and root samples from Gangtok

Table 3. Species with their host trees and their places of collection

Species	*Population	Host tree	Place of collection
Bulbophyllum careyanum	(P_1)	*Albizia gamblei*	Bhasme, Pakyong
B. maculosum	(P_2)	*Alnus nepalensis*	Sajong, Pakyong
Dendrobium anceps	(P_3)	*Terminalia elliptica*	Amba, Pakyong
Bulbophyllum thomsonii	(G_1)	*Phoenix sylvestris*	Nampung, Gangtok
B. tremulum	(G_2)	*Mangifera indica*	Lingzung, Gangtok
Dendrobium crassinode	(G_3)	*Syzygium cumini*	Rakdong, Gangtok

*The populations P and G indicate Pakyong and Gangtok

formed fiber mats, also known as tilosomes (Pridgeon *et al.*, 1983). Highest size of tilosomes was observed in G_3 whereas lowest was in P_1 and P_3 (Table 2).

The cortex was differentiated into outer, middle and inner zone. The exodermis was highly lignified all around. From the above data, it is evident that the host trees may play an important role in supplying minerals to the epiphytic orchids; the degree of supply of nutrients varies from one host tree to other. Pakyong forests are known as congenial for orchid growth as these have sufficient rainfall, warm, and humid conditions. Accordingly, roots may have undergone structural adaptations.

The SDS-PAGE protein profile showed six different bands of diverse molecular weight 73-83 KD that has been noted in different populations (Fig. 3). All bands are observed in G_1 and G_3 and few bands are observed

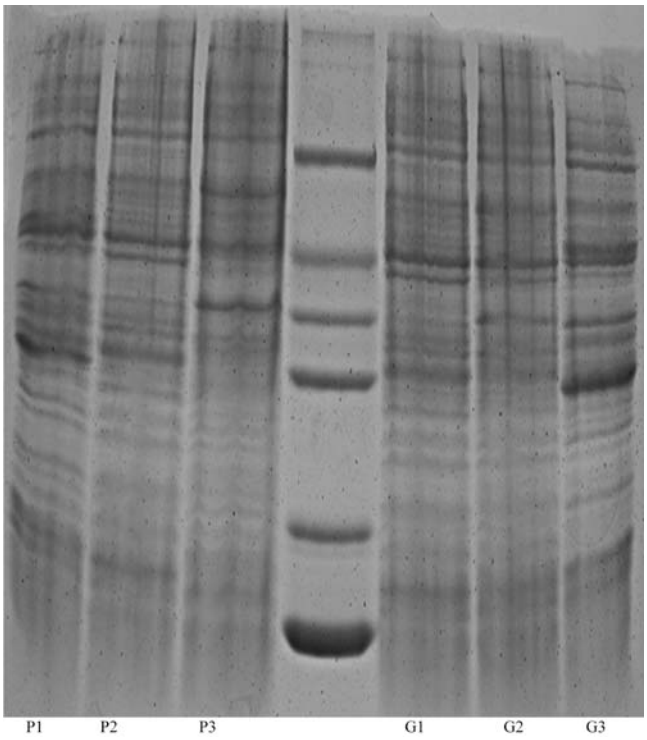

Fig. 3. SDS-PAGE Protein profile of *Bulbophyllum* and *Dendrobium*

P_1 and P_3. The protein bands are thick and prominent in G_1 and G_3 respectively. The protein bands varied considerably with respect to their staining intensities.

The RAPD amplification profile showed variability among six populations. The molecular weight of the bands is high in populations collected form Gangtok, than the populations collected from Pakyong (Fig. 4). The highest molecular weight observed in G_1 was 850 KD. In order to analyze the relationship among populations studied, UPGMA-based dendrogram was constructed using paired matrix values. The isolation by distance as well as climatic conditions brought about the considerable genetic variation (molecular and morphological) (Raymond and Rousset, 1995). According to Misra (1995), orchids are highly habitat specific, and therefore, they suffer very much due to the destruction of their delicate habitats. Basumatary *et al.* (2008) opined that the epiphytic orchids form a variety of associations in the ecosystem and the knowledge on their community dynamics has much significance in

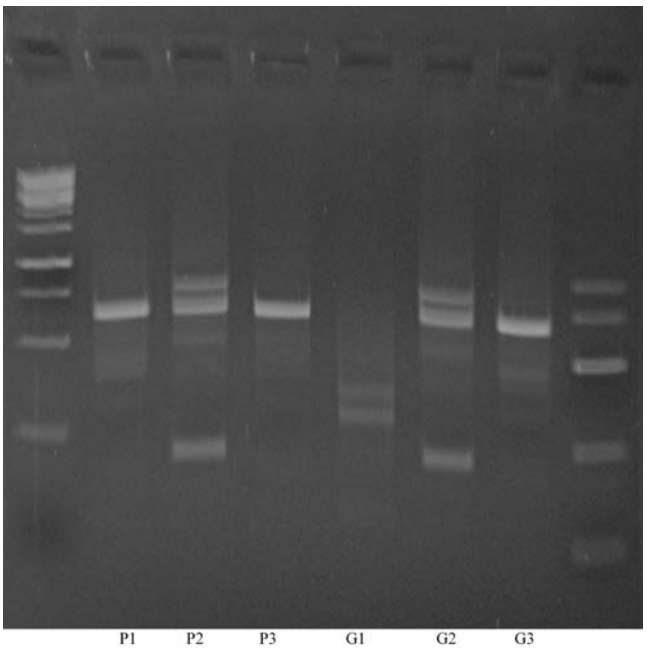

Fig. 4. RAPD analysis of *Bulbophyllum* and *Dendrobium*

formulating effective conservation measures. Therefore, apart from molecular analysis, studies on community dynamics and interaction with their host trees are equally essential before evolving the conservation strategies of epiphytic orchids.

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Evaluation of photosynthetic effeciency of elephant-foot yam *(Amorphophallus paeoniifolius)* to photon flux density and elevated CO_2

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ABSTRACT

An experiment was conducted to find out photosynthetic efficiency of elephant-foot yam (*Amorphophallus paeonifolius* Dennst.) at CTCRI, Thiruvananthapuram, during April-August 2017. In the search of photosynthetically efficient climate smart crops/varieties, net photosynthetic rate *(Pn)*, stomatal conductance *(g_s)* and intercellular CO_2 *(Ci)* were studied in three contrasting elephant-foot yam genotypes, *viz.* Sree Padma, Gajendra and Idukki local under ambient (400 ppm) and eCO_2 (eCO_2) (600, 800 and 1000 ppm) and *Pn* at photosynthetic photon flux densities (PPFDs), *viz*. 200, 400, 600, 800, 1000, 1200 and 1500 µmol/m^2/h at 30°C and 400 ppm CO_2 using portable photosynthesis system LI-6400, LICOR, USA. The maximum Pn of three genotypes was recorded at PPFD of 1500 µmol/m^2/s. The Pn steadily increased due to short-term (ten minutes) exposure at eCO_2 concentrations between 400 ppm and 1000 ppm in all genotypes. The genotypes had average Pn of 17.97, 25.07, 29.44 and 30.06 µmol CO_2/m^2/s at 400, 600, 800 and 1000 ppm CO_2 respectively. All of them had 56.71-82.51% hike in Pn at eCO_2 (1000 ppm) compared to ambient CO2 (400 ppm). However, per cent of increment in Pn at eCO_2 for every 200 ppm between 400 and 1000 ppm significantly declined (-4.81-9.03%) at 1000 ppm CO_2. The differences in Pn were statistically significant across all genotypes (P>0.05) and CO_2 concentration (P>0.001). However, interaction effect of genotypes and CO_2 concentrations on Pn was insignificant, whereas Pn had a quadratic relation with increase in CO_2 concentration (R^2= 0.932). The gs in all genotypes increased at 600/800 ppm eCO_2 concentrations but declined at 1000 ppm CO_2 compared to 400 ppm. All genotypes had average gs of 0.394 0.480, 0.491, 0.280 mol H_2O/m^2/s at 400, 600, 800 and 1000 ppm CO_2 respectively. However, per cent of increment in Ci at eCO_2 for every 200 ppm between 400-1000 ppm significantly declined (-36.40- -40.04%) at 1000 ppm CO_2. The differences in gs were statistically significant across elephant foot yam genotypes (P>0.05) and CO_2 concentrations (P>0.01). However, the interaction effect of genotypes and CO_2 concentration on gs is insignificant. The three elephant foot yam genotypes had the average *Ci* of 319.33, 487.07, 655.73 and 815.24 µmol CO_2/mol air at 400, 600, 800 and 1000 ppm CO_2 respectively. However, the per cent of increment in *Ci* at eCO_2 for every 200 ppm between 400-1000 ppm significantly declined (22.46-27.38%) at 1000 ppm CO_2. The differences in *Ci* were statistically significant across genotypes and CO_2 concentrations (P>0.001). However, the interaction effect of genotypes and CO_2 concentrations on *Ci* was insignificant. Statistically the net photosynthetic rate had a quadratic relation with the *Ci* (R^2= 0.710). The differences in total chlorophyll and protein content in the leaves of three elephant foot yam genotypes were statistically significant. Nevertheless, the gas exchange parameters were not influenced by the total chlorophyll and protein content.

Key Words: Elephant foot yam, eCO_2, Photosynthesis, Stomatal conductance, Intercellular CO_2, Climate change

The elephant-foot yam [*Amorphophallus paeoniifolius* (Dennst.), syn. *A. campanulatus* (Roxb.) BL. Ex Dence] is a herbaceous, perennial C_3 crop of the South-Eastern Asian origin. Its corms are usually eaten as a vegetable after boiling or baking. The rise in CO_2 concentration in atmosphere is an important change which can effectively

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influence the productivity of crop plants. The CO_2 can affect plant function mainly through its direct effect on photosynthesis and stomatal physiology. The C_3 plants respond to eCO_2 significantly. The photosynthetic enzyme Ribulose biphosphate carboxylase (Rubisco) has a low affinity for CO_2 on carboxylation and this reaction is not saturated at the current CO_2 concentration. Therefore, carboxylation of Rubisco respond to rising CO_2. Since photorespiration *(Pr)* decreases net efficiency of photosynthesis by 20-50% depending on temperature, and rising CO_2 50% reduces the oxygenase (photorespiration) reaction of Ribulose Biphosphate Carboxylase / Oxygenase (Rubisco) enzyme as CO_2 competes with O_2 (Lawlor and Mitchell, 2000).

The photosynthetic rates of C_3 plants were approximately doubled when plants were exposed to 700 compared to 380 ppm (Ainsworth and Long, 2005). Elevated CO_2 enhanced net photosynthetic CO_2 uptake and consequently increased biomass production and yield of C_3 crops (Rosenthal *et al.*, 2012; Cruz *et al.*, 2014). Being a C_3 plant it has advantage of responding positively to an increase in atmospheric CO_2, the photosynthetic response of elephant-foot yam to CO_2 concentrations and photon flux density has not yet been studied. Under controlled conditions, photosynthetic rate in sweet potato increased due to increase in Ci from 250 to 560 ppm and response was temperature dependant (Cen and Sage, 2005). In potato, tuber yield increased up to 1000 ppm (Wheeler *et al.*, 1994). Yields of wheat, rice, maize, soybean, cotton, castor bean, vegetable crops significantly increased at eCO_2 concentrations (Reddy and Hodges, 2000, Prasad *et al.* 2005, Chowdhury *et al.* 2005, De Souza *et al.* 2008, Vanaja *et al.* 2008, Razzaque *et al*. 2009, Singh and Jasrai 2012, O'Leary *et al*. 2015, Zhang *et al*. 2015).

Sweet potato storage root yield has been reported to increase up to 750 ppm CO_2 (Mortley *et al.*, 1996). Czeck et al. (2012) reported a significant increase in both above- and below-ground biomass at eCO_2 (1520 ppm) in sweet potato. The above-ground dry biomass increased by 43% for organic source of nutrients and by 31% for inorganic source of nutrients at eCO_2. The below-ground biomass increased by 61% in organic treatment and 101% increase in inorganic treatment, appreciably greater than above-ground biomass, attributing the importance of root crops under high CO_2 environment. Understanding genotypic responses to eCO_2 is therefore essential to identify traits for breeding varieties for changing climate. Therefore, photosynthetic response of three field grown elephant-foot yam genotypes to eCO_2 and PPFD was studied.

MATERIALS AND METHODS

The experiment was conducted during 2017 at the farm of CTCRI, Thiruvananthapuram, on three contrasting elephant-foot yam genotypes, *viz*. Sree Padma and Gajendra (varieties) and Idukki local (land race) grown under irrigated conditions during April-August 2017. There were three replications and each replication had 25 plants. The N: P_2O_5 and K_2O were applied @ 100:25:100 kg / ha as per the recommended package of practices. Plants were grown under open sunlight conditions with ≈12 hours sunlight per day under ≈1700 µ mol m^2 h^{-1} at 30°C ± 2°C during day time and 23°C ± 1°C during night time. Weed control mat was used to control weed growth in field. The diurnal changes of atmospheric CO_2 in field indicated a dip from ~470 ppm at 6.00 AM to ~380 ppm at 12.00 AM and ~400 ppm at 4.0 PM.

The net photosynthetic rate *(Pn)*, stomatal conductance *(g_s)* and sub-stomatal / intercellular CO_2 *(Ci)* under short-term exposure (10 minutes) to CO_2 cencentrations, *viz*. 400, 600, 800, 1000 ppm at 30°C and 1500 µ mol m^2 h^{-1} photosynthetic photon flux density (PPFD), and the *Pn* at different PPFDs, *viz*. 200, 400, 600, 800, 1000, 1200 and 1500 µ mol / m^2 / h at 30°C and 400 ppm CO_2 were recorded in leaves in controlled-climate cuvette using a LI6400 portable photosynthesis system, LI-COR Inc, Lincoln, USA. These parameters were recorded in the fully expanded first and second leaf 4 months after planting. In leaves total chlorophyll content was estimated according to (Lichtenthaller 1987) and the total protein content according to (Bradford 1966). The data were statistically analysed using SAS / Software Version 9.3, SAS Institute Inc., Cary, NC, USA 2010.

RESULTS AND DISCUSSIONS

Pn response to Photosynthetic Photon Flux Density

The *Pn* was recorded in leaves of all genotypes during short-term (10 minutes) exposure to CO_2 concentrations, *viz*. 400, 600 800 and 1000 ppm at 30°C and 1500 µmol / m^2 / s PPFD inside controlled-climate cuvette in a portable photosynthesis system. The *Pn* of all genotypes steadily increased due to increase in PPFD from 200 to 1500 µmol $m^{-2}s^{-1}$ (Fig.1). The differences in *Pn* among all genotypes were statistically insignificant across genotypes but the *Pn* significantly differed with PPFDs ($p<0.001$). However, interaction effect of genotypes and PPFDs on *Pn* was insignificant. Although maximum *Pn* of all the genotypes was recorded at PPFD of 1500 µmol / m^2 / s, the increase in *Pn* at PPFDs between 800 and 1200 µmol / m^2 / s were insignificant. Among 12 sweet potato genotypes, the maximum *Pn* was recorded at PPFD of 1500 µmol / m^2 / s and increase in *Pn* at PPFDs above 1000 µmol / m^2 / s were insignificant (Ravi *et al.*, 2017).

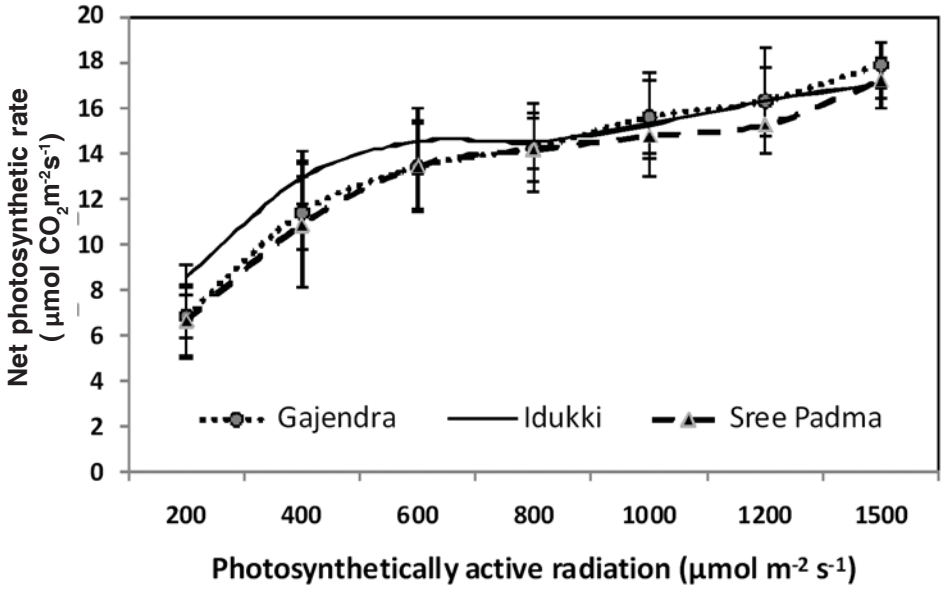

Fig. 1. Changes in net photosynthetic rate under different PPFD

Pn response to eCO_2

The *Pn* steadily increased due to short-term exposure to eCO_2 concentration from 400 ppm to 1000 ppm in leaves of all genotypes (Fig. 2). At 400 ppm CO_2, *Pn* varied from 15.70 µmolCO_2/m^2/s in genotype, Idukki local, to 19.17 µmol CO_2/m^2/s in Sree Padma. At 600 ppm CO_2, *Pn* varied from 24.17 µmol CO_2/m^2/s in Sree Padma to 26.39 µmolCO_2/m^2/s in the genotype Gajendra. At 800 ppm CO_2, *Pn* varied from 27.56 µmol CO_2/m^2/s in Sree Padma to 30.66 µmol CO_2/m^2/s in Gajendra. At 1000 ppm CO_2, *Pn* varied from 28.65 µmol CO_2/m^2/s in the genotype Idukki local to 31.49 µmol CO_2/m^2/s in Gajendra.

The per cent of increment in *Pn* at 600 ppm CO_2 as compared to 400 ppm CO_2, was minimum (26.05%) in Sree Padma and maximum (57.13%) in Idukki local. The per cent of increment in *Pn* at 800 ppm CO_2 as compared to 400 ppm CO_2, was minimum (43.72%) in Sree Padma and maximum (91.72%) in Idukki local. The per cent of increment in *Pn* at 1000 ppm CO_2 as compared to 400 ppm CO_2, was minimum (56.71%) in Sree Padma and maximum (82.51%) in Idukki local.

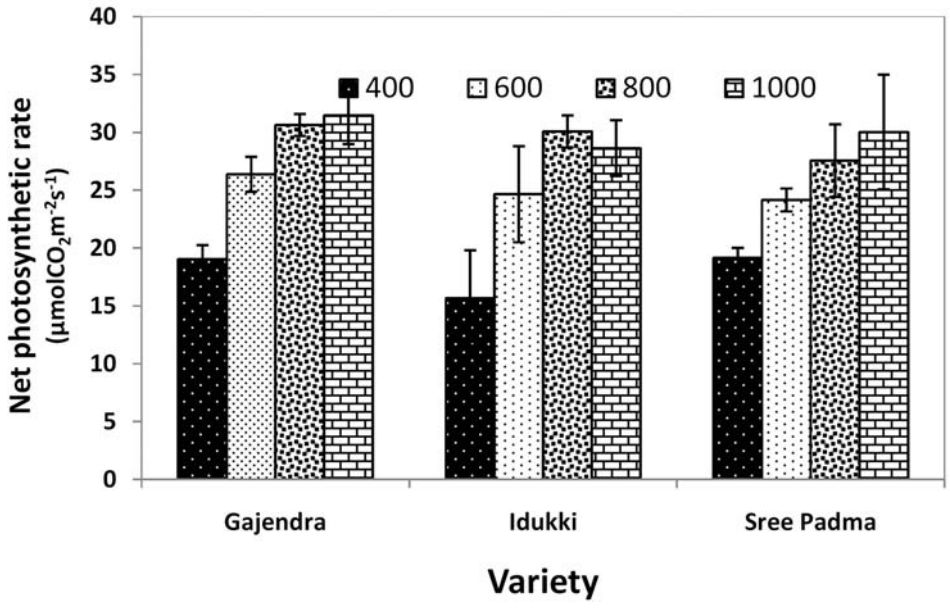

Fig. 2. Changes in net photosynthetic rate under different CO_2 concentrations. The error bars indicate St. Dev.

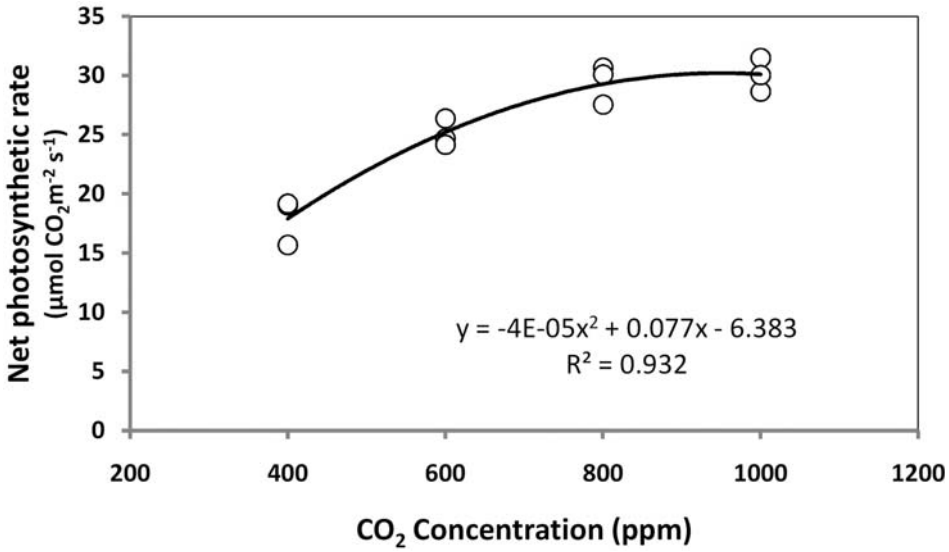

Fig. 3. Statistical relation between net photosynthetic rate and CO_2 concentration

The average *Pn* in all genotypes increased by 56.71, 65.29 and 82.51% at 1000 ppm CO_2 in Sree Padma, Idukki local and Gajendra respectively as compared to 400 ppm CO_2. The difference in *Pn* were statistically significant in all genotypes ($P>0.05$) and CO_2 concentrations ($P>0.001$). However, interaction effect of genotypes and CO_2 concentrations on *Pn* was insignificant, whereas *Pn* had a quadratic relation with increase in CO_2 concentration ($R^2= 0.932$) (Fig. 3). Among twelve sweet potato genotypes, *Pn* steadily increased due to short-term (ten minutes) exposure at eCO_2 concentrations between 400 ppm and 1000 ppm and had a quadratic relation with increase in CO_2 concentration (Ravi *et al.,* 2017).

Elevated CO_2 increased *Pn* by 54% in rice and ~33% in soybean at 700 ppm compared to 350 ppm CO_2 (Vu *et al.*, 1997), by 45% in sunflower at 700 ppm compared to 350% (Tezara *et al.*, 2002), by ~48% at 800 ppm compared to 400 ppm in C_3 plants (Allen and Prasad, 2004), by 66% in soybean at 1000 ppm compared to 400 ppm CO_2 (Prasad *et al.,* 2005), by 22 and 9% at 700 and 450 ppm compared to 350 ppm CO_2 in cotton (Reddy *et al.,* 2005), by 30-60% at 720 ppm compared to 370 ppm CO_2 in sugarcane (de Souza *et al.,* 2008), by ~70% at 550 ppm as compared to 370 ppm CO_2 in chickpea (Madan Pal and Sangeeta 2009). Compared to 389±40 ppm CO_2, eCO_2 increased the *Pn* by 11.7% at 550 ±60 ppm in mung bean leaf (Hao *et al.,* 2011).

The *Pn* significantly increased by 78% and 30% at 700 and 550 ppm CO_2 compared to 390 ppm CO_2 in blackgram under long-term exposure (Sathish *et al.*, 2014). Compared to 380 ppm CO_2, eCO_2 significantly increased net photosynthetic rate at 700 ppm in sunflower leaf under long-term exposure (Vanaja *et al.*, 2011). In oats *(Avena sativa)*, *Pn* significantly increased by 21-61% under long-term exposure to 600±50 ppm CO_2 as compared to 360 ppm CO_2 (Bhatt *et al.,* 2010). In rice *(Oryza sativa)* the *Pn* was significantly enhanced by 53-66% at eCO_2 (660 ppm) CO_2 compared to 330 ppm. Similarly, *Pn* was significantly enhanced by 48-94% under long-term exposure to eCO_2 700 ppm CO_2 compared to 350 ppm in soybean (Vu *et al.* 1997). The *Pn* significantly increased in maize under long-term exposure to eCO_2 at 450 and 550 ppm CO_2 compared to 390 ppm (Meng *et al.*, 2014).

Long-term exposure to eCO_2 (570±50 ppm CO_2) increased the *Pn* by 24.2 and 25.4% compared with ambient CO_2 (~360 ppm CO_2) and field grown rice plants (Razzaque *et al.* 2009). Long-term exposure to eCO_2 increased the *Pn* during flowering by 46 and 104%, while that during pod maturation by 23 and 14% than that in ambient CO_2 and field conditions, respectively in mung-bean plants (Chowdhury *et al.*, 2005). Long-term exposure to eCO_2 (550 ppm) significantly enhanced photosynthetic rate in rice compared to 370 ppm (Sujatha *et al.*, 2008). The *Pn* of wheat leaves had 31% enhancement under long-term exposure to 550 ppm CO_2 than at 380 ppm (Nie *et al.* 1995). In soybean, CO_2 enrichment between 550 and 700 ppm increased photosynthesis by up to ~30% (Bernacchi *et al.*, 2005, Prior *et al*. 2011).

Photosynthesis under eCO_2 (700 ppm) increased by 50, 60 and 78% under short-term (>1 week) exposure and by 14, 13 and 42% under long-term exposure in barley, cotton and soybean respectively (Ratnakumar *et al.*, 2011). Nevertheless, under controlled conditions, long-term exposure of cassava to eCO_2 at 750 ppm did not significantly enhance the *Pn* rate as compared to 390 ppm (Cruz *et al.*, 2016) or had insignificant effect on *Pn* at 550 ppm but significantly decreased *Pn* at 710

ppm as compared to 360 ppm (Gleadow *et al.*, 2009).

The per cent increment in *Pn* in leaves of all genotypes steadily declined with an increase in CO_2 concentration between 600 ppm to 1000 ppm. The per cent of increment in *Pn* at 600 ppm CO_2 compared to 400 ppm CO_2, was minimum (26.05%) in Sree Padma and maximum in genotype 38.49(%). The per cent of increment in *Pn* at 800 ppm CO_2 compared to 600 ppm CO_2 declined to minimum (14.02%) in Sree Padma and maximum (22.01%) in Idukki local. The per cent of increment in *Pn* at 1000 ppm CO_2 as compared to 800 ppm CO_2 was negligible and varied from minimum (-4.81%) in Idukki local to maximum (9.03%) in Sree Padma. Among twelve sweet potato genotypes, per cent of increment in *Pn* at eCO_2 significantly declined at CO_2 concentrations above 600 ppm (Ravi *et al.*, 2017).

The canopy photosynthesis of cotton plants gradually increased with increases in CO_2 above 350 ppm and reached maximum at 700 ppm and there was no further increase at 900 ppm (Reddy *et al.*, 2005). Similarly, canopy photosynthetic rates increased with increasing CO_2 from 160 to 500 ppm, but saturated at 500 ppm in rice (Baker *et al.*, 1990). The decrease in increment of *Pn* at short-term exposure to eCO_2 is attributed to decline in electron transport capacity, capacity of *Pi*-regeneration from phosphorylated photosynthetic intermediates, RuBP regeneration for Rubisco activity, decline in activation state of Rubisco and the balance between Rubisco and other processes limiting photosynthesis (Sage *et al.*, 1989, Makino and Mae, 1999).

Furthermore, unlike decline in *Pn* due to limitations in sink activity and decline in Rubisco activity under long-term exposure to eCO_2 reported in other crops, the decline in increment of photosynthetic rate recorded under eCO_2 in present study is not attributed to acclimation as the source and sink are simultaneously active in sweet potato. The total chlorophyll content in leaves of all genotypes varied from minimum (1.15 ± 0.10 mg / g fresh leaf) in Idukki local to maximum (1.67 ± 0.59 mg / g fresh leaf) in Sree padma. The differences in total chlorophyll content in leaves of genotypes were statistically significant. Nevertheless, Pn had no definite correlation with the total chlorophyll content. The long-term exposure of leaves to eCO_2 decreased or increased or had no effect on the total chlorophyll content (Bhatt *et al.*, 2010; Singh and Jasrai, 2012).

g_s response to eCO_2

The g_s increased between 400 ppm to 600 ppm CO_2 which then decreased at 800 ppm CO_2 in leaves of Gajendra, whereas gs in leaves of Idukki local and Sree Padma increased between 400 and 800 ppm CO_2 which then decreased at 1000 ppm CO_2 (Fig. 4). At 400 ppm CO_2, g_s varied from minimum 0.334 molH_2O / m^2 / s in Sree Padma to a maximum 0.435 molH_2O / m^2 / s in Idukki local. At 600 ppm CO_2, g_s varied from minimum 0.350 molH_2O / m^2 / s in Sree Padma to maximum 0.668 molH_2O / m^2 / s in Gajendra. At 800 ppm CO_2, g_s varied from minimum 0.380 molH_2O / m^2 / s in Sree Padma to a maximum 0.591 molH_2O / m^2 / s in Gajendra. At 1000 ppm CO_2, g_s varied from 0.242 minimum molH_2O / m^2 / s in Sree Padma to a maximum 0.354 mol H_2O / m^2 / s in Gajendra.

The per cent of increase in g_s at 600 ppm CO_2 compared to 400 ppm CO_2, was minimum (1.44%) in Idukki local and maximum (62.05%) in Gajendra. The g_s decreased by 43.38% in Gajendra, whereas g_s

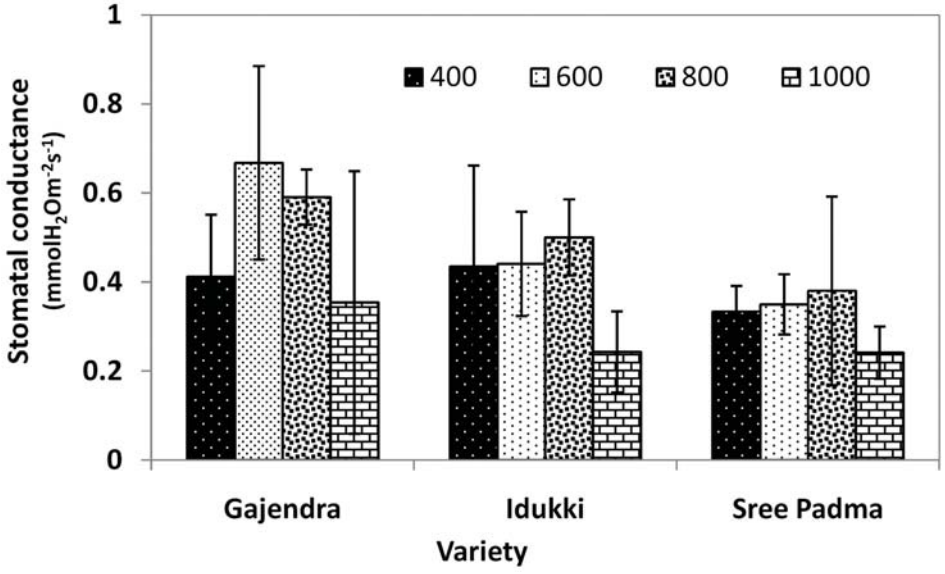

Fig. 4. Changes in stomatal conductance under different CO_2 concentrations. The error bars indicate St. Dev

increased by 13.92% and 15.12% in Sree Padma and Idukki local respectively at 800 ppm CO_2 compared to 400 ppm CO_2. The per cent of decrease in g_s at 1000 ppm CO_2 compared to 400 ppm CO_2, was minimum (-14.03%) in Gajendra and maximum (-44.04%) in Idukki local. The g_s decreased by -11.52% at 800 ppm CO_2 compared to 600 ppm CO_2 in Gajendra, whereas g_s increased by 13.48% and 8.64% in Sree Padma and Idukki local respectively at 800 ppm CO_2 compared to 600 ppm CO_2. The per cent of decrease in g_s at 1000 ppm CO_2 compared to 800 ppm CO_2, was minimum (-36.40%) in Sree Padma and maximum (-40.04%) in Gajendra.

The differences in g_s were statistically significant in all genotypes (P>0.05) and CO_2 concentrations (P>0.01). However, interaction effect of genotypes and CO_2 concentration on g_s is non-significant. There was a decrease in stomatal conductance (g_s) under eCO_2. During short-term exposure to eCO_2, stomatal aperture generally decreases in response to high CO_2 due to depolarization of membrane potential of guard cells and stomatal closure (Ainsworth and Rogers, 2007). Partial stomatal closure and associated decrease in stomatal conductance to H_2O is one of the most important responses to increasing CO_2. Among twelve sweet potato genotypes, g_s steadily decreased at eCO_2 concentrations between 400 and 1000 ppm (Ravi *et al.*, 2017). The g_s has been reported to be reduced by 21 - 50% at eCO_2 across plant species (Xu *et al.*, 2016). Under controlled conditions, long-term exposure of cassava to eCO_2 at 710/750 ppm significantly decreased g_s compared to 360/390 ppm (Gleadow *et al.*, 2009; Cruz *et al.*, 2016). The g_s decreased by 32% in cotton leaves under long-term exposure to eCO_2 (65 Pa CO_2) than at 35 *Pa* CO_2 (Harley *et al.*, 1992).

The g_s decreased by 31% at 450-550 ppm CO_2, 36% at 600-800 ppm CO_2 and 51% at > 850 CO_2 with respect to 330-360 CO_2 in soybean (Ainsworth *et al.*, 2002). The g_s decreased, on average, by 37% in eCO_2 at 720 ppm compared to 370 ppm in sugarcane (De Souza *et al.*, 2008). ECO_2 (700 ppm) decreased g_s of individual leaves by 43% at 26°C, 45% at 31°C, and 20% at 36°C compared to 350 ppm in cotton (Reddy *et al.*, 2005). In leaves of sunflower, g_s decreased by ~50% at 700 ppm as compared to 350 ppm CO_2 (Tezara *et al.*, 2002). Compared to 389±40 ppm CO_2, eCO_2 decreased g_s by 32% at 550 ±60 ppm in mung bean leaf (Hao *et al*. 2011). The g_s significantly decreased in sunflower at 700 ppm CO_2 as compared to 380 ppm (Vanaja *et al.*, 2011).

In leaves of Arabidopsis thaliana grown in ambient CO_2 (350 ppm) and measured at 700 ppm, g_s decreased by ~25% compared to 350 ppm because of partial stomatal closure CO_2 (Teng *et al.*, 2006). In leaves of Arabidopsis thaliana grown in eCO_2 (700 ppm), g_s decreased by ~25% at 700 ppm compared to 350 ppm CO_2 because of 19 and 14% decrease in stomatal density (SD) on adaxial and abaxial surfaces of leaves and 12 and 9% decrease in stomatal index (SI) on adaxial and abaxial surfaces of leaves (Teng *et al.*, 2006). In present study, elephant-foot yam was grown in ambient CO_2 (400 ppm), therefore there was decrease in g_s in its leaves during short-term measurements at eCO_2 (800 and 1000 ppm) compared to 400 ppm is attributed to partial closure of stomata and not due to a decrease in SD and the SI.

Ci response to eCO_2

The *Ci* in leaves of all genotypes steadily increased due to an increase in CO_2 concentration from 400 ppm to 1000 ppm. At 400 ppm CO_2, *Ci* varied from 279.00 µmolCO_2/mol air in Sree Padma to 345.00 µmolCO_2/mol air in Idukki local. At 600 ppm CO_2, *Ci* varied from 451.3 µmolCO_2/mol air in Sree Padma to 532.8 µmolCO_2/mol air in Gajendra. At 800 ppm CO_2, *Ci* varied from 615.3 µmol CO_2/mol air in Sree Padma to 689.2 µmol CO_2/mol air in Gajendra. At 1000 ppm CO_2, *Ci* varied from 756.3 µmolCO_2/mol air in Sree Padma to 877.9 µmolCO_2/mol air in Gajendra. Although, g_s significantly decreased at eCO_2 1000 ppm, decline in increment of net photosynthetic rate due to increase in CO_2 resulted in high intercellular CO_2 concentrations which in turn sustained greater net photosynthetic rates at eCO_2 compared to 400 ppm CO_2. Several studies reported greater *Ci* at eCO_2 levels.

In twelve sweet potato genotypes, *Ci* steadily increased due to short-term (ten minutes) exposure at eCO_2 concentrations between 400 ppm and 1000 ppm but the per cent of increment in *Ci* at eCO_2 significantly declined at CO_2 concentrations above 600 ppm (Ravi *et al.*, 2017). Compared to 389±40 ppm CO_2, eCO_2 increased *Ci* by 9.8% at 550 ±60 ppm in mung bean leaf (Hao *et al.*, 2011). The per cent of increment in *Ci* at 600 ppm CO_2 as compared to 400 ppm CO_2, was minimum (38.3%) in Idukki local and maximum (61.73%) in Sree Padma. The per cent of increment in *Ci* at 800 ppm CO_2 as compared to 400 ppm CO_2, was minimum (92.08%) in Idukki local and maximum (120.51%) in Sree Padma.

The per cent of increment in *Ci* at 1000 ppm CO_2 as compared to 400 ppm CO_2, the minimum (135.22%) in Idukki local and maximum (171.05%) in Sree Padma. The per cent of increment in *Ci* at 600 ppm CO_2 compared to 400 ppm CO_2, was minimum (38.30%) in Idukki local and maximum (61.73%) in Sree Padma. The per cent of increment in *Ci* at 800 ppm CO_2 as compared to 600 ppm CO_2 declined to minimum (36.35%) in Sree Padma and maximum (38.88%) in Idukki local. The per cent of increment in *Ci* at 1000 ppm CO_2 as compared to 800 ppm CO_2 further declined

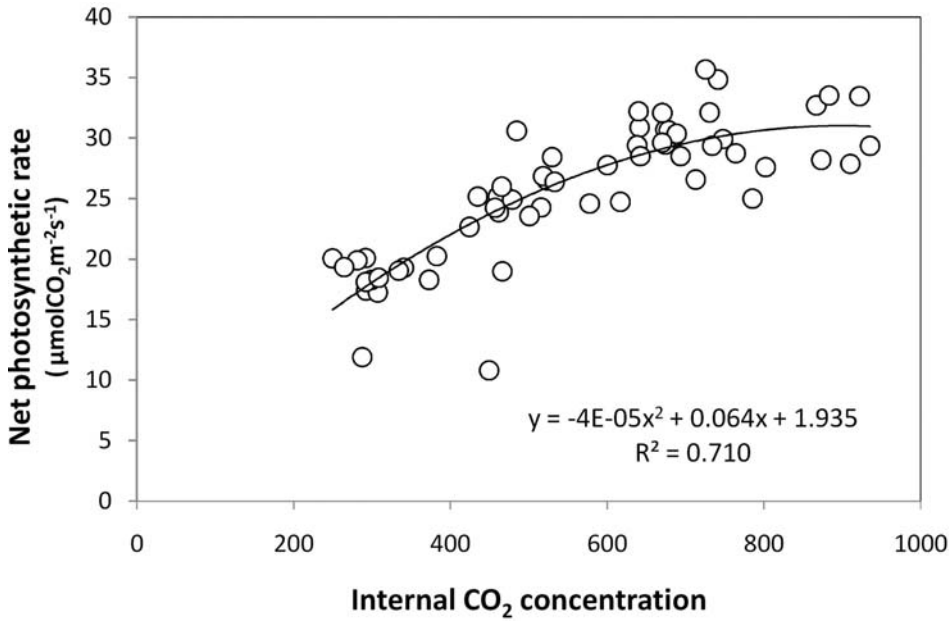

Fig. 5. Statistical relation between net photosynthetic rate and intercellular CO_2 concentrations.

to the minimum (22.46%) in Idukki local and maximum (27.38%) in Gajendra. Although eCO_2 significantly decreased g_s, the eCO_2 was adequate to sustain the *Ci* at high levels which resulted in decreased photorespiration *(Pr)* and increased *Pn*. The differences in *Ci* were statistically significant in all genotypes and CO_2 concentrations (P>0.001). However, interaction effect of genotypes and CO_2 concentrations on *Ci* was insignificant. Statistically, net photosynthetic rate had a quadratic relation with the *Ci* (R^2= 0.710) (Fig. 5) and is in agreement with Hikosaka *et al.* (2005).

The total protein content in leaves of all genotypes varied from minimum (3.71 ± 0.56 mg/100mg fresh leaf) to maximum (3.91 ± 0.32 mg/100g fresh leaf). The differences in total protein content in leaves of sweet potato genotypes were statistically significant. Nevertheless, *Pn* had no definite correlation with total protein content. Several reports indicate that long-term exposure to eCO_2 had decreased or increased or had no change on soluble protein content in the leaves (Bhatt *et al.*, 2010). Long-term exposure to eCO_2 reduced Rubisco content by 22% in rice and 8% in soybean (Vu *et al.*, 1997).

Long-term exposure to eCO_2 had no significant effect on Rubisco content in leaves of *Chenopodium album*, *Phaseolus vulgaris*, *Solanum tuberosum*, *Solanum melongena*, and *Brassica oleracea* (Sage *et al.*, 1989). Long-term growth of Arabidopsis at high CO_2 (1000 ppm) resulted in a ~35-40% decrease in expression of Rubisco protein and in the transcript of rbcL, gene encoding the large subunit of Rubisco, and 60% decline in mRNA of rbcS, the gene encoding the small subunit of Rubisco (Cheng *et al.* 1998).

There were significant differences in *Pn*, g_s and *Ci* across genotypes and CO_2 concentrations. These results under short-term exposure are similar to those reported in other crops under long-term exposure to eCO_2. The genotypes, Gajendra and Sree Padma, are promising for eCO_2 in the context of climate change and the mechanism needs to be elucidated. The difference in response of genotypes to eCO_2 warrants further investigation under long-term exposure to eCO_2 with respect to growth and corm yield.

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