REPRODUCTIVE BIOLOGY AND BIOCHEMICAL CHANGES ASSOCIATED WITH FLOWERING OF *DENDROCALAMUS STOCKSII* AND *OCHLANDRA TRAVANCORICA*

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By

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CERTIFICATE

This is to certify that the thesis entitled **"Reproductive biology and biochemical changes associated with flowering of *Dendrocalamus stocksii* and *Ochlandra travancorica*"** is an authentic record of research work carried out by Ms. Beena. V.B under my supervision and guidance in the Division of Sustainable Forest Management, Kerala Forest Research Institute, Peechi, Thrissur, in partial fulfillment of the requirement for the degree of Doctor of Philosophy in Environmental studies and no part of thesis has been presented for the award of any other degree, diploma or associateship in any university.

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DECLARATION

I hereby declare that the thesis entitled "**Reproductive biology and biochemical changes associated with flowering of *Dendrocalamus stocksii* and *Ochlandra travancorica***" is my own work and it contains no material previously published or written by another person nor material which has been accepted for the award of any other degree or diploma of the university or other institute of higher learning.

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I dedicate this thesis to SREE GURUVAYURAPPAN

V. B. Beena

CONTENTS

CHAPTER – I

INTRODUCTION

Bamboos are a group of woody perennial, evergreen to deciduous plants belonging to the grass family Poaceae, subfamily Bambusoideae, tribe Bambuseae. From the time immemorial, bamboo has been a part of human life due to its versatility which makes it a good raw material for numerous applications. Bamboo stands occupy an area of 36 million hectares worldwide which is equivalent to 3.2 per cent of the total forest area in the world. It is estimated that bamboo occupies over one percent of the tropical and subtropical forest area - over 22 million ha. Over 80 per cent of the total area covered by bamboo is located in Asia, 10 per cent in Africa and 10 per cent in America. About 30 per cent of bamboo may be classified as forest plantations vs. 3.8 per cent of woody plantations. According to the FAO/INBAR global thematic study, over 63 per cent of bamboo resources are privately owned with 36 per cent bamboo owned by Governmental entities. In comparison 80 per cent of all world forests are on public lands (Lobovikov, 2007).

In Asia, India is the one of the major bamboo producing countries (almost 11.4 million hectares) which accounts for roughly half the total area of bamboo reported for Asia. There are different reports on the number of genera and species of bamboos in India. As per the latest compilation 18 genera and 128

species has been reported (Seethalakshmi and Kumar, 1998). The 18 genera found in India are 1. *Arundinaria,* 2. *Bambusa,* 3. *Chimonobambusa,* 4. *Dendrocalamus,* 5. *Dinochloa,* 6. *Gigantochloa, 7. Melocanna, 8. Ochlandra, 9. Oxytenanthera, 10. Phyllostachys,* 11. *Pleioblastus,* 12. *Pseudosasa,* 13. *Pseudoxytenanthera,* 14. *Racemobambos,* 15. *Schizoztachyum,* 16. *Sinarundinaria,* 17. *Thamnocalamus* and 18. *Thyrsostachys*. Of the total species found in India about 20 are commercially used.

Kerala is one among the major diversity centers of bamboo in the country and 34 species of bamboos under seven genera have been recorded from this area. This comes to about 20 per cent of the total bamboo distributed in India and 95 per cent of the total species reported from peninsular India (Kumar and Ramesh, 2000). In addition to forest land, bamboo is found in homesteads also. The total standing crop of bamboo in homesteads was estimated as 13.61 million culms and its green weight was 0.331 million tonnes during 2004-2005 (Muraleedharan *et al.*, 2007). Whereas, the bamboo resource in the forest areas was estimated as 2.63 million based on the satellite imagery 1997.

Bamboo is a viable replacement for wood and is one among the strongest and oldest building materials ever used. Application of scientific and engineering skills on bamboo has led to an extended diversity of products ranging from domestic household items to industrial applications and generates income and employment. It contributes substantially to the ecological, economic and social

development. Ecologically, bamboo plays a critical role in soil and water conservation, the balance of oxygen and carbon dioxide in the atmosphere, lowers light intensity and protects against ultra violet rays. The inherent ability of different bamboo species to grow on marginal and wastelands makes it one of the preferred crops for greening the wastelands and degraded sites resulting in conservation of soil moisture and resulting in carbon sequestration.

The employment potential of bamboo is very high and the major work force involved is rural poor, especially women. In India, recently a rediscovery of the potential of bamboo for developing it as one of the sunrise industries resulted in launching of an integrated bamboo development programme by the Prime Minister on World Environment day - June 5, 1999 with a view to focus on the development of bamboo sector. Subsequently the Planning Commission, Government of India prepared an Action Plan to give maximum emphasis for promotion and development of bamboo during the Tenth plan. In countries like China and Vietnam, bamboo has been revered as brother and the farmer's friend. China during last two decades made tremendous progress in the value addition of bamboo which resulted in the economical development of bamboo rich counties like Anji. But in India, although it was involved in religion and culture from the time immemorial, bamboo industries except paper and pulp did not get sufficient attention. The progress achieved by China in short time, and the pressure from Northeast regions rich in bamboo and bamboo handicrafts

contributed for a major change in the perceptions about the potential of bamboo. With the involvement of international agencies like Asia Pacific Center for Transfer of Technology (APCTT), Food and Agricultural Organization (FAO), International Development Research Center (IDRC), International Network for Bamboo and Rattan (INBAR), International Plant Genetic Resource Institute (IPGRI), International Tropical Timber Organization (ITTO), United Nations Development Program (UNDP), United Nations Industrial Development Organization (UNIDO), World Bank, etc. a number of projects were under taken in bamboo growing areas. Substantial information on resource base, methods for cultivation, processing and improved utilization was generated as a result of these studies. The growing awareness about deforestation and its negative impacts on environment have also contributed to the growth of bamboo as wood substitute and eco-friendly material. Still there is a long way to go before policies and standards are set in place for the full integration of bamboo into the mainstream activity.

The demand for bamboo is growing more than its production and no doubt cultivation of bamboo in large scale is an immediate priority to support the developing industries with sufficient raw material. Lack of seed and/or other types of propagules could be a limiting factor for establishment of large - scale commercial plantations of the desired species. Some species *viz. Dendrocalamus stocksii*, *Bambusa balcooa* and *B. vulgaris* do not produce any seeds even after

profuse flowering (sterile). Flowering of bamboo has been a botanical enigma since time immemorial. Although three types of flowering (annual or continuous flowering, gregarious or periodic flowering, sporadic or irregular flowering) have been reported among bamboos, most of the woody bamboos that are commercially useful belong to the gregarious flowering group. Since gregarious flowering leads to the death of entire population, this may lead to the extinction of the sterile species. Attention is needed to find out the problems associated with sterility in bamboos and take necessary measures to promote seed production. Conventional methods of vegetative propagation like rooting of culm (stem) cuttings and rhizomes that are used in the absence of seeds have several limitations such as availability in limited number, transport problems and synchronous flowering (simultaneous flowering of the parent and offspring originated from the same clump). It adds to the necessity of having planting materials of bamboo from seed origin.

Reproductive biology, which includes the study of floral morphology, pollination leading to fertilization and seed production, can highlight the reproductive constrains of plants and are imperative for the conservation. Moreover, it can help in developing protocols combat the problems that impede regeneration. Literature on reproductive biology of bamboos is scanty. Studies on floral biology have been carried out in *Bambusa bambos (B. arundinaceae)*, *B. balcooa* Roxb., *B. vulgaris*, *Dendrocalamus giganteus*, *D. strictus* Nees., *Melocanna*

baccifera, *Ochlandra scriptoria*, *O. travancorica*, *Phyllostachys nidularia* etc (Nadguada *et al*., 1993; John *et al*., 1995; Banik, 1998; Ramanayake and Yakandawala, 1998; Koshy and Harikumar, 2001). It would seem that the cause of sterility in certain bamboo species is the non-availability of viable pollen grains and self-incompatibility (Koshy and Jee, 2001; Kondas *et al.*, 1973; Venkatesh, 1984).

In general, soon after gregarious flowering, the whole clump of the bamboo dies off leading to accumulation of large quantity of raw material. Proper cost-effective preservation methods for harvested bamboos are currently lacking. The seedlings take four to five years to reach harvestable stage. This gap adversely affects industries that are entirely depending on bamboo raw material. Absence of seed production even after profuse flowering and death of the flowered clumps are two major challenges which requires detail investigation.

Generally, the flowering of plants is associated with biochemical changes in the vegetative parts as more food resources are required for the seed production. In bamboo during flowering, biochemical changes occur in the culms and it is imperative to record the biochemical changes during and after flowering. The information on chemical composition during flowering, and subsequent death need to be studied to find out whether any leading to reversion. There is only limited literature available on such studies on bamboo.

In Kerala, gregarious flowering of *Dendrocalamus stocksii* (Munro) M. Kumar, Remesh & Unnikrishnan (Synonyms -*Pseudoxytenanthera stocksii* (Munro) Naithani, *Oxytenanthera stocksii* Munro) and *Ochlandra travancorica* (Bedome) Bentham (Gamble, 1896) occurred during 2003. *D. stocksii* popularly known as Konkan bamboo is distributed in Konkan area starting from North Kerala to Maharashtra. It is an important bamboo which is cultivated in homesteads. *O. travancorica,* reed bamboo endemic to Western Ghats, naturally distributed in the evergreen and semi evergreen forests of Kerala. It is an important raw material in weaving bamboo ply and pulp and paper industry.

The post flowering behaviour of these two species are entirely different. In *O. travancorica,* flowering is followed by abundant seed production and subsequent death of flowered clumps. Whereas, profuse flowering is found in *D. stocksii* but seed production is not yet reported (Seethalakshmi and Kumar, 1998). In this species, the flowered clumps do not die and is observed to revert back to vegetative phase. Hence, in the present study, the reproductive biology of the sterile *D. stocksii* was compared with that of seed setting *O. travancorica* to investigate the problems leading to lack of seed production. The biochemical changes associated with different stages of flowering in both species also was observed to know the chemical changes in these two species that has different post-flowering behaviour.

In this context, the objectives selected for the study were

1. To study the reproductive biology and post flowering behaviour of *Dendrocalamus stocksii* and *Ochlandra travancorica* and

2. To understand biochemical changes associated with flowering.

CHAPTER - II

REVIEW OF LITERATURE

Bamboo diversity and distribution

Over 110 genera and 1500 species of bamboo have been identified which are unevenly distributed in various parts of humid tropical, sub-tropical and temperate regions of the world (Subramaniam, 1998, Orhnberger and Goerrings, 1985). In India, there are 128 species of bamboos belonging to 18 genera, which include 87 naturally occurring and 41 introduced or under cultivation (Seethalakshmi and Kumar, 1998). Further six species were recorded as new from South India and Andaman Nicobar islands. Bamboos grow particularly well in the tropics and subtropics, but some taxa also thrive in the temperate climate of Japan, China, Chile and the USA (Grosser and Liese, 1971). Generally smaller bamboo species occur in high elevations or temperate latitudes, and the larger ones are abundant in the tropic and sub tropic areas (Lee *et al.,* 1994). Bamboo forests in India occupy approximately 10.03 million hectares (mha), which is about 12.8 per cent of the total forest area. Bamboo occurs in almost all the states of India, from the tropical to the temperate regions and the alluvial plains to the high mountains. It requires a temperature of 8 - 36º C with a minimum annual rainfall of 1000 mm and high atmospheric humidity for maximum growth. From

Kerala about 7 genera and 34 bamboo species have been reported (Kumar, Personal Com.).

Morphology and Vegetative growth

Different growth forms of bamboo occur in nature as trees, shrubs and climbers (Prasad and Gadgil, 1981). Bamboo plants consisted of roots, rhizome, culm (stem), branches, flowers and fruits. Roots are fibrous and form a dense network in the soil. Rhizome, the underground stem or the basal part of the stem has two parts viz., rhizome neck and rhizome proper. Based on the branching pattern basically two types of rhizomes have been reported – pachymorph (Sympodial, clump forming) and leptomorph (monopodial, runner or walking bamboo (McClure, 1966, Stapleton, 1997). Pachymorph rhizomes are short and thick, with larger diameter than the culm formed from upward curved apex of it. Leptomorph types of rhizome are an underground stem, which is long, slender, and smaller in diameter than the culm originated from it. A mixed type viz., amphimorph showing the characters of both pachymorph and leptomorph types are also found. Further classification based on short/long neck and single/tillering culms have been reported for pachymorph and leptomorph rhizomes, respectively (Judziewicz *et al.,* 1999).

Culm is the aerial, cylindrical vegetative axis having distinct nodes and internodes, connected to a rhizome network. The rhizome system constitutes the structural foundation of the plant and the culm is completely dependent upon

the nutrition provided by the rhizome and the older culms (Liese, 1985). The units which makes a culm consists of nodes, internodes, axillary buds, leaves and in some of the basal nodes, root primordia. The node is from where branch/leaf originate in the stem and because of this, morphology of node is complex. Internodes, the part between two successive nodes, may be solid as in *Dendrocalamus strictus*, or hollow with well developed lumen as in *Bambusa bambos* and the length of internode varies in different species. The growth pattern in bamboos is known as exponential i.e., each year a fresh set of vegetative shoots, originate from the base of the clump soon after monsoon showers. Mature culms are the result of a process of primary growth through which culm reaches maximum diameter, elongation of internode in a telescopic fashion and subsequent hardening through lignifications (McClure, 1966). Bamboo is reported as the fastest growing plant due to rate of growth of the new vegetative shoots. It is reported that the genus *Phyllostachys* in Japan grows more during the day (Ueda, 1960), whereas in the tropical regions, bamboos grow more during the night (Osmaston, 1918). An emerging culm reaches full height within 2-4 months (McClure, 1966, Ueda, 1960). Banik (1991) observed that in Bangladesh a bamboo clump starts producing culms generally either from May or June and may continue for up to 6-7 months.

Flowering and post-flowering behaviour

At the end of a pre-determined period of vegetative growth, flowering occurs in all the clumps originated from same seed lot and all propagules developed through vegetative propagation from their populations. The peculiarity of bamboos is in the extension of their vegetative growth phases, from few to many years (John and Nadgauda, 2001). The length of this vegetative growth process (also called the intermast period or flowering interval) differs among species (Guangchu, 2002). The period between two gregarious flowerings for a species over the same area is called its flowering cycle or physiological cycle. This cycle is more or less constant for a species in a given locality but differs in locations remote from one another and with well marked climatic and soil differences (Sharma, 1991). The flowering cycle of the priority species reported are given in Table 1.

Table 1. Flowering cycle of 16 priority species of bamboo

No	Bamboo species	Flowering cycle (years)	No	Bamboo species	Flowering cycle (years)
1	*Bambusa balcooa*	35 - 45	9	*Dendrocalamus asper*	30 - 40
2	*B. bambos*	30 - 40	10	*D.giganteus*	40
3	*B. blumeana*	45 – 100	11	*D.latiflorus*	60
4	*B. polymorpha*	35 – 60	12	*D. strictus*	25- 45
5	*B. textilis*	NR*	13	*Gigantochloa apus*	30 – 120
6	*B. tulda*	30 - 60	14	*G. levis*	NR*
7	*B. vulgaris*	80 – 88	15	*G. pseudoarundinacea*	NR*
8	*Cephalostachyum pergracile*	20	16	*Guadua angustifolia*	NR*

*Not reported

Bamboo flowering although amazing to scientists from time immemorial, detailed observations are limited, since flowering is rare in many species, historical records are fragmentary and not dependable as adequate verification is not possible (Campbell, 1985). From India, the major part of the literature on bamboo flowering is limited to the reports on incidence of flowering (Jansen 1976). Whether flowering is controlled by genetic make-up, physiological / biochemical parameters or environmental conditions is not clearly established. India has 70-72 species of bamboos with gregarious / mast flowering which is one of the highest concentration (Gadgil and Prasad, 1984).

According to Brandis (1906), bamboos have been classified into three categories based on flowering behaviour:

1. **Annual** flowering species, which flower every year and do not die,

2. **Sporadic or irregularly** flowering species, in which flowering occurs in isolated clumps in an area or isolated culms in a clump

3. **Gregarious/periodic** flowering species, in which whole clump flowers in an extensive area and die after seed setting.

Limited number of bamboo species exhibited persistent tendency to flower annually (Sharma, 1991). *Bambusa atra (Bambusa lineata)* is often described as constant flowering in the Andamans and Malaysia (Gamble, 1896; Rhind, 1945). For more than 100 years it has been flowering, but no seed set was observed. The culms after flowering do not die, instead remain healthy and green. Ramanayake (2006) also reported that there are other species with similar tendency to flower continuously without seed set. *Bambusa atra* and *Schizostachyum brachycladum* (introduced to Sri Lanka from Indonesia) constantly flower but with no seed set and continue to grow vigorously year after year. There are yet others such as the endemic species, *Ochlandra stridula* (bata) that flower and set seed annually.

Majority of commercial bamboo species belongs to gregarious/periodic flowering group. The approach of gregarious flowering is usually indicated by

the reduction or cessation of formation of new shoots, in the preceding year. A bamboo that flowers gregariously, usually exhibits sporadic flowering for a year or more before switching over to gregarious flowering. Both type of flowering may sometimes occur simultaneously. Bamboos would flower after reaching flowering maturity stage. Generally, flowering cycles of sympodial bamboos in the tropics have been reported between 20, 40 and 70 years (Gonzales *et al*., 1998). Some of the reports indicate that the site quality and climatic conditions influence the flowering. Delay in flowering was reported from better quality sites (Dwivedi, 1988). Bamboos with good seed setting are reported to flower earlier than those with poor seed setting capacity (Ueda, 1960). Growth regulators such as Coumarin and NAA treatments enhance the sprouting and flowering of sprouts developed from flowered bamboo cuttings. Although the age of the rhizome seems to have major influence, a short rainy season followed by a spell of severe drought also stimulated flowering (Adarsh kumar *et al.,* 1990). When the bamboo flowers gregariously, the flowering is so profuse that the whole plant is transformed into a gigantic inflorescence (Varmah and Bahadur, 1980). Gregarious flowering makes the seeds available in plenty, which necessitates certain management procedures to ensure restocking of an area after gregarious flowering. It occurs over large areas and has been attributed to several causes, such as drought. Although there are a few exceptions like *Dendrocalamus giganteus* (Seethalakshmi *et al.,* 2010a) gregarious flowering is usually followed by the death of the whole clump irrespective of age of the culm and new

rhizomes developed. Mortality appears to be more frequent under sub-optimal growing conditions, and when seed production is heavy, which may vary depending on the success of cross-pollination or other factors (Hughes, 1951; Janzen, 1976; Mc Clintock, 1979).

The exact physiological mechanism of bamboo flowering is not yet known. Different evolutionary hypothesis such as parental competition, consumer satiation and climatic periodicity have been put forward to explain bamboo flowering (Campbell, 1987). Clear-cut evidences are not available to substantiate any of the above hypotheses. Death of flowered bamboos in large populations is a cause of concern due to ecological, social and economic crises that set forth (John and Nadgauda, 2002).

Observations on post flowering behaviour of different bamboo species from various locations indicate that there are three categories (Sharma, 1991):

1. Flowering does not result in the death of either aerial or the underground parts. However, growth slows down during flowering e.g. some species of the genus *Arundinaria, Phyllostachys* and *Bambusa atra*.

2. Flowering results in complete death of aerial parts only, the rhizomes remaining alive, so that regeneration in such bamboo takes place from underground rhizomes e.g. *Arundinaria amabilis, A. simonii, Phyllostachys nidularia, Dendrocalamus giganteus.*

3. Flowering results in complete death of both aerial and underground parts (rhizomes) so that regeneration in such bamboo is only possible from seeds e.g. *Melocanna bambusoides, Thyrsostachys oliveri, Bambusa bambos, B. tulda, Ochlandra travancorica* etc**.**

Once the bamboo dies after flowering, the people whose livelihood depended on the bamboo resource are severely affected. The accumulation of dead and dried bamboos in large quantity invites forest fire in many locations. Mass mortality after flowering and seed set in bamboos generates a widespread and synchronous fuel load that significantly increases the potential for wildfire disturbance (Keeley and Bond, 1999). In addition, the dense monospecific "gregarious" aggregations of bamboo increase the effective target area to collect lightning strikes and thus increase the probability of ignition (Wardle *et al.,* 1997). Kadambi (1949) described, fierce forest fires resulted in the death of the bamboo (*Dendrocalamus strictus*) which was followed by copious natural regeneration. Every gregarious flowering and death of bamboos ends in causing disastrous fires whose ravage is often indescribable; wholesale destruction or at least crippling of the forest tree stand. Similar accounts of a linkage between bamboo mortality and wildfires are reported for other species in India (Bourdillon, 1895; Gadgil and Prasad 1984), Africa (Henkel, 1927), North America (Platt and Brantley, 1997) and South America (Kitzberger *et al.,* 1997). The land becomes bare as it takes time for the new generation to establish. Bare land could be

disastrous in mountainous areas leading to landslides (Ramanayake, 2006). Bamboo flowering invites many seed predators to the locality like birds, insects, rodents, elephants, human being etc. Increase of the population of predators result in shortage of food materials and famine in the flowered area in succeeding years. Flowering and death of *Melocanna baccifera* was reported by Banik (1998) and Ramanayake and Weerawardene (2003). From 2004 to 2008 gregarious flowering of this species occurred in the northeastern states of India (Jeeva *et al.,* 2009). Professional meetings were organized in India during last decade especially to address this issue (RFRI, 2002; NHU, 2010). Another problem which is prevalent in post flowering stages of bamboo groves is the growth of light demanding species leading to competition for the new generation of bamboo seedlings due to the opening of the canopy. Gregarious flowering and death of *Dendrocalamus giganteus* in Sri Lanka and *Melocanna baccifera* in India have been reported (Ramanayake and Yakandawala, 1998; Banik, 1998). Report on post flowering behaviour of *D. giganteus* from India have indicated that some of the clumps rejuvenate to vegetative phase (Seethalakshmi *et al.,* 2010a). Continuous observations have been made on flowering and seed set of bamboos from Southern part of India from Kerala Forest Research Institute (Seethalakshmi *et al.,* 2010b). About 22 species belonging to 4 genera including both endemic and introduced (*Bambusa bambos, B. tulda, B. striata, Dendrocalamus brandisii, D. giganteus, D. longispathus, D. sikkimensis, D. stocksii, D. strictus, Pseudoxytenanthera bourdillonii, P. monadelpha, P. ritcheyii, Ochlandra ebracteata, O.*

keralensis O. setigera, O. soderstromiana, O. spirostylis, O. sivagiriana, O. travancorica, O. travancorica var. hirsuta, O. scriptoria and *O. wightii)* were observed in bloom during 1980 to 2010 (Seethalakshmi *et al.,* 2010b).

Although gregarious flowering is observed in all the clumps originated from same seed lot there are different flowering cohorts for the same species of bamboo. Banik (1998) reported that *M. baccifera* exhibits more than one flowering cycle. *B. bambos* (L.) Voss the thorny bamboo has at least five cohorts within a small state of Kerala. Flowering occurred during the years 1982 (Peechi, Vazhachal); 1992 (Attappady, Wyanad); 1996 (Chinnar and homesteads in Trichur, Palakkad, Malappuram, Calicut Districts); 2001 (Attappady and Chinnar) and 2006 to 2010 (Wyanad, Vazhachal, Attappady and Edamalayar and in homesteads of Palakkad, Trichur and Malappuram Districts). In all the cohorts flowered clumps died after seed set. Similarly different flowering cohorts are observed for *Ochlandra travancorica* also in Kerala. While establishing large-scale plantations judicious mixing of different flowering cohorts will help to address the limitations of gregarious flowering, synchronous flowering and death etc (Banik, 2010).

Reproductive biology

An in-depth study of reproductive biology is necessary to understand various reproductive features and anomalies. The study can also help in developing certain protocols to combat the problems that impede regeneration (Moza and

Bhatnagar, 2007). Sexual reproduction is the only natural process that incorporates variability and ensures survival of species under adverse conditions. Studies on reproductive biology had been reported only for a few species of bamboos. It includes *Bambusa bambos*, *Dendrocalamus strictus* (Nadgauda *et al*., 1993), *B. vulgaris* (Koshy and Jee, 2001). *D. giganteus* (Ramanayake and Yakandawala, 1998; Seethalakshmi *et al.*, 2010a) *D. stocksii* (Beena *et al*., 2007) *Melocanna baccifera* (Banik, 1998; Ramanayake and Weerawardene, 2003), *Ochlandra travancorica* (Venkatesh, 1984), *O. scriptoria* (Koshy and Harikumar, 2001), *O. wightii* (Jijeesh and Seethalakshmi, Pers. Comm), *Pseudoxytenanthera monadelpha* (Jijeesh *et al*., 2009), *P. ritcheyi (*Beena *et al*., 2007) and *Thyrsostachys regia* (Alam, 1997). Observations on flowering and seed formation behaviour indicated three distinct groups.

1. Profuse flowering and abundant seed forming (eg. *Bambusa bambos, B. tulda, Dendrocalamus brandisii, D. longispathus, D. sikkimensis, D. strictus, Pseudoxytenanthera bourdillonii, P. ritcheyii, Ochlandra ebracteata, O. keralensis O. setigera, O. soderstromiana, O. spirostylis, O. travancorica, O. travancorica var. hirsuta, O. scriptoria* and *O. wightii)*

2. Profuse flowering and sparse seed forming (*D. giganteus, O. sivagiriana)*

3. Profuse flowering and sterile (sparse flowering species *B. striata*, *B. balcooa* and profuse flowering species (*D. stocksii*).

Flowering occurred during August to December and seed formation took about three to four months. Case studies revealed that seed fall started in February in *Bambusa bambos* (Seethalakshmi *et al.,* 2010c) and in different species of *Ochlandra* from March onwards (Seethalakshmi, 1993, Jijeesh *et al*., person. commun.). Observations on reproductive biology and seed setting have studied in *Dendrocalamus strictus* by Nadgauda *et al.,* 1993; *B. vulgaris* by Koshy and Jee (2001); *Melocanna bacciferra* by Banik (1998).

Dichogamy in which, the androecium matures 3-4 days before gynoecium is reported in many species like *Ochlandra*, *D. strictus*, (Venkatesh, 1984; Nadgauda *et al.,* 1993). The role of dichogamy in preventing self-pollination is ascertained by lack of seed formation in *O. travancorica* when inflorescences were enclosed in bags to exclude wind borne pollen from outside. Absence of seed set was also observed in isolated clumps growing in a location where wind was obstructed (Venkatesh, 1984).

In most of the species anemophylous pollination is reported. Production of abundant monoporate pollen was found in almost all the species observed. Although many honeybees have been found visiting the flowers, their role in pollination is not well understood. Visit of honey bees and ants was recorded in *Melocanna baccifera* at the time of anthesis and they are reported as pollinators (Banik, 1998). Honeybees assisted pollination was also recorded in *Phyllostachys nidularia* (Huang *et al.,* 2002). Studies on *M. baccifera* from Sri Lanka did not

report any insect visit (Ramanayake and Weerawardene, 2003). In related species *Ochlandra scriptoria* bees visiting flowers have been found to forage the anthers causing destruction to the pollen grains (Koshy and Harikumar, 2001).

Reason for poor seed setting / complete sterility has been studied in a few species. In *Bambusa vulgaris*, sterility is due to cumulative effect of a number of physical and physiological factors such as very low pollen viability and high pollen sterility (Koshy and Jee, 2001). Another sterile species is *Bambusa balcooa* which had a flowering cycle of 40 ± 5 years or a multiple of it (Banik and Alam, 1987). Flowering occurs sporadically in this species and no seeds are reported yet. Seed formation in *D. giganteus* is limited (Seethalakshmi *et al.,* 2010a) and observations elsewhere reported viable pollen and ovules but no mature seed (Islam *et al.,* 2000). Successful pollination is an essential pre-requisite for survival of plants in natural communities and is dependent on many biotic and abiotic factors. Poor pollen viability and slow growth of pollen tubes were observed in sterile species. Pollen viability is generally considered to indicate the ability of pollen grains to perform its function of the delivering the gamates to the embryo sac following compatible pollination (Shivanna *et al.,* 1991). Pollen viability is being evaluated by various staining techniques. It can be measured by *in vivo* as well as *in vitro* methods. Acetocarmine staining has been used for staining pollen grains of bamboos to check the fertility (Nadgauda *et al.,* 1997a; John and Nadgauda, 1997; Ramanayake and Weerawardene, 2003). In some

other plants, different types of stains are used to test the viability of pollen. Presence of dehydrogenase in viable pollen gives deep pink colour when stained by tetrazolium. Aniline blue was used to detect callose in pollen walls and tubes (Wang *et al.,* 2004). *In vitro* pollen germination is considered as an indication of the pollen viability *in vivo* (Stone *et al*., 1995, Tuinstra and Wedel, 2000). Sucrose, boric acid and calcium nitrate have been found to be the key substrates for pollen germination in some species (Wang *et al.,* 2004). Boron played a significant role in regulating pollen germination and pollen tube growth of *Picea meyeri* and its deficiency enhanced acidic pectin accumulation in pollen tubes, which might be associated with the increased content of carboxylic acid (Wang *et al.,* 2003). Pectin exists in the primary cell walls of higher plants as a major matrix component, and the wall in the tip region of the pollen tube is mainly composed of pectin layer. Loomis and Durst (1992) observed that when germinated pollen were transferred to boron-deficient medium, the pollen tubes would burst explosively at the outermost tips and suggested that boron may be interacting with other ions such as Ca^{2+} to form pectic network. Obermeyer *et al.,* (1996) reported that boron stabilizes the newly synthesized cell wall at the tube tip and thus prevented the tube from bursting due to its stimulatory effect upon ion transporters leading to an increase of pressure. Li *et al.,* (1996) demonstrated a relationship between the distribution of pectic materials and the growth patterns of pollen tubes. It is possible that the synthesis and modification of pectin are active processes that control the elongation of pollen tube. These suggest that the essentiality of boron

is directly associated with cell wall extensibility and consequently cell growth. Kong *et al.,* (2006) suggested that the protein phosphatase 1 or 2A, or both, is involved in the regulation of $Ca^{2}+$ uptake across the plasma membrane, in exocytotic activity and in the biosynthesis of cell wall components, all processes that occur in the tip region of pollen tubes and that control pollen tube development in *Picea wilsonii*. Wimmer and Goldbach (1998) demonstrated that Ca^{2+} functioned as a stabilizing factor for the forming of boron complex in cell wall.

In a few species such as *D. strictus*, *Pseudoxytenantera monadelpha* and *Ochlandra travancorica* pollen fertility was determined and *in vitro* pollen germination techniques were standardized. In *D. strictus*, 98 per cent pollen fertility was observed both by staining and *in vitro* germination method with pollen tube length 15-20 times than the diameter of mature pollen grains. The germination medium was modified Brewbacker containing one per cent glucose. *In vitro* pollen germination studies in *O. travancorica* indicated 90 per cent germination in the media with one per cent sucrose. *In vivo* germination also was found to be better. In *Pseudoxytenantera monadelpha,* a species which appeared sterile, pollen viability was poor (Jijeesh *et al.,* 2009). In all the above species pollination was anemophylous.

In-vitro flowering in bamboos was first reported in seedlings in embryogenic calli of *Dendrocalamus strictus* and *Bambusa bambos* (Rao and Rao, 1990) and in shoot

cultures of *Bambusa bambos* and *Dendrocalamus brandisii* (Nadgauda *et al.,* 1990). These reports have been an excitement and was followed by the *in vitro* flowering experiments on shoot cultures of *Dendrocalamus hamiltonii* (Chambers *et al.,* 1991); *Bambusa vulgaris, Dendrocalamus strictus* and *D. giganteus* (Rout and Das, 1994); in shoot cultures of *Dendrocalamus asper* (Arya and Arya, 1996); in somatic embryos of *Bambusa oldhamii* (Chang *et al.,* 1997). *In vitro* flowering experiments were done also in adult bamboos in shoot cultures of *Bambusa nana, B. bambos, B. multiplex, B. brandisii, Dendrocalamus membranaceus* and *Cephalostachyum pergracile* (Prutpongse and Gavinlertvatana, 1992); in shoot cultures of *Bambusa ventricosa* (Gielis, 1995); In shoot cultures of *Bambusa ventricosa, Bambusa multiplex, Phyllostachys* sp. and *Chusquea* sp. (Gielis *et al.,* 1997); In shoot cultures of *D. brandisii, D. latiflorus* and No. 7 hybrid (Guangchu and Yuxia, 1998). Gielis (1998) suggested that of the above identified possibilities, the most promising ones are those involving hybridization under controlled conditions. However detailed studies and results are very scarce. Most often the development of pseudospikelets yields normal florets with male and female reproductive organs. These organs are smaller than those observed under *in vivo* conditions (Rao and Zamora, 1995; Nadgauda *et al.,* 1997b). The main problems for *in vitro* hybridization seem to be pollen viability and synchronisation of flowering. Pollen viability is always much lower *in vitro,* possibly due to defects in wall formation (Nadgauda *et al.,* 1997b). Seed setting has been observed (Nadgauda *et al.,* 1990; Rout and Das, 1994) but at low percentages, and

apparently only when many flowers were open at a particular moment (Nadgauda *et al.,* 1997b). Gielis (1998) also suggested that it is necessary to intensify research in this field and many hurdles still need to be taken before the methods really become applicable at agricultural scales.

Rejuvenation after flowering

Death of flowered bamboo is attributed to reproductive exhaustion caused by the movement of food reserves from the vegetative parts for seed formation. If however, the rhizome is left with sufficient resources, the clump is able to recover although all aerial culms that flower die (Ramanayake, 2006). The experiments on *Phyllostacys vivax*, which flowered in China from 1969 to 1976 showed some positive results with regard to artificial rejuvenation (Hsiung *et al.,* 1981). The rhizomes (without culms) from the flowering stand were dig out and cut into 30-50 cm sections. These were dipped in gibberellic acid solution (100 mg/l) for 5 hours and then buried in a cutting bed. When new shoots emerged, they were sprayed with the same solution every two weeks. After 3 months, only 36 per cent of the culms from treated rhizomes flowered, while 64 per cent of the culms from untreated controls were observed in flower indicating that the treatment is positive. After a year, the treated culms produced more normal, non-flowering culms than the controls. In China, vigorous groves of bamboo, over 200 years old, have been maintained by carefully controlling their flowering behaviour (Wang and Shen, 1987). Whenever old culms show first

signs of flowering, they are immediately cut off and large quantities of nitrogen are applied to the underground parts to circumvent flowering.

Chemical composition of bamboos

When the new shoots are formed in bamboo, critical bio-chemical processes are initiated. These lead to rapid growth, as well as concurrent hardening, as the shoot elongates and turns into a woody culm. Young bamboo shoots are edible and have high nutritional value, low in fat, and it is a good source of fibre. They are rich in vitamins, cellulose and amino acids.

Bamboo tissue contains several inclusions, which can also be called as extraneous materials or extractives. These can be organic compounds, which may be extracted by cold or hot water or by organic solvents or inorganic materials, which result in ash on burning. A waxy layer often covers the surface of the bamboo culms, and it is common and can be felt especially on young culms. Mc Clure (1966) refers to white exudates on the culm surface of several species. The "Dowga" wax of *Bambusa blumeana* hinders the penetration of chemicals during the pulping process (Beri *et al.,* 1967).

The results of an assay of vitamin B12 indicate that edible bamboo shoots do not contain vitamin B12, but some compounds showing the vitamin B12-like activity (known as the alkali-resistant factor) (Emi *et al.,* 2005). According to Monsalud and Nicolas (1958), bamboos contain more ash, silica and pentosans than woods.

It was reported that the Philippine bamboos had higher ash and silica content than those of Asian bamboos, but lower lignin content than the Indian species (Semana *et al.,* 1967). Espiloy (1983) found that the silica content increases in a linear fashion from second internode of the butt portion (1.6 per cent) to internode 30 (9.9 percent) in *B. blumeana*. It is reported that free glucose was found generally lower in the upper parts of the culms, the starch content was highest in the mid height and it decreased almost linearly in the lower and the higher sections and regarding the radial distribution, the free glucose and starch contents were higher in the inner part of the culm and the free glucose was found associated with parenchyma cells (Okahisa *et al.,* 2007). Starch grains were found within the parenchyma cells and in other cells. Assessment of Yoshimoto and Morita (1985) was that 5-year old Moso bamboo (*Phyllostachys pubescens)* contained fructose, sucrose and glucose as free sugars and the seasonal fluctuation of these sugars was reported (Morita, 1985 and Ninomiya *et al.,* 1998). Mollison (1993) suggests that many bamboos have poisonous hydrocyanic acid content. Bhat *et al.,* (2005) studied the distribution of starch content across the culm wall of *Bambusa bambos* (L.) Voss and reported that maximum starch content was observed in the inner portion of the distal part of the culm. In contrast, it was lowest in the outer part of the basal portion of the culm.

Chemical Changes associated with flowering

Even though, a number of studies are available on the biochemical constituents of bamboo, literature on the biochemical changes associated with flowering of bamboos is scanty. According to Srinivasa *et al.,* (1974) accumulation of the chemicals like starch in the rhizomes, sugars and other substances in the plant tissues cause flowering. The accumulation of these chemicals is influenced by soil and climatic conditions. But, Liese (1998) reported the complete exhaustion of starch preceding the flowering. Garg *et al.,* (1998) studied the chemical changes during flowering and death in *D. strictus* and revealed increase in the level of reducing sugars and total carbohydrates during flowering which reduced significantly at the end of flowering. However, the level of the α-cellulose and lignin reduced during flowering which increased at the end of flowering. Reduction in the level of hemicellulose and marginal change in the ash content after flowering was also noticed. There was a high reduction in the moisture level reflecting osmotic shock. Kao (1972) reported that physiological changes such as reduction in starch and nitrogen content correlated with flowering and seeding.

According to Mac Donald (1969) and Liese (1985), the chemical composition varies according to the individual characteristics of the species, age and position of the culm, its geographical position and other related factors. The chemical analysis during flowering and death brings out the consumption of the reserved

food in the rhizomes of the clump leading to death. Since there is no seed setting consumption of necessary materials is less and new shoots are produced leading to rejuvenation of flowered clumps in *D. stocksii*. Even though biochemical changes during these stages play an important role, studies regarding this are scanty. Gregarious flowering often followed by death leads to accumulation and wastage of culms. The present study gives information of the variation in chemical changes.

CHAPTER - III

MATERIALS AND METHODS

Materials

Dendrocalamus stocksii (Munro) M. Kumar, Remesh & Unnikrishnan which belongs to the flowered, sterile and rejuvenating group and *Ochlandra travancorica* (Bedome) Bentham (Gamble, 1896) which belongs to the flowered, seed setting and drying group of bamboo were selected for the study. *D. stocksii* is a medium sized bamboo species endemic to Western Ghats. It is found mostly in cultivation in homesteads. *O. travancorica* is a shrubby reed like bamboo is also endemic to Western Ghats, naturally distributed in the evergreen and semi evergreen forests of Kerala.

Gregarious flowering of *D. stocksii* occurred during 2004-2005 confined to the areas, Chattanchal, Cherkkala, Poinachi, Monacha, Periya, Neeleswaram, Cheruvathur etc., in Kasaragode district. Three rhizomes of the felled culms were uprooted from Chattanchal and Monacha areas for transplantation. Three other flowered clumps in these areas were selected randomly and tagged for field observations.

O. travancorica also flowered gregariously during the same period in different areas under the Forest Divisions of Munnar and Kollam, viz. Neryamangalam,

Kulathupuzha, Shendurney Wildlife Sanctuary, Marappalam, Sasthanada, Shankily, Arippa etc. It was found growing extensively both as pure and mixed vegetation in Kulathupuzha Forest Range and Shendurney Wildlife Sanctuary. Three rhizomes of the flowered plants were uprooted from Arippa and Sasthanada. Three other flowered clumps in these localities were randomly selected for taking observations in the field.

The uprooted rhizomes of both species were transplanted to the nursery in the campus of Kerala Forest Research Institute, Peechi, Kerala and allowed to grow till they flowered. These transplanted plants of *D. stocksii* and *O. travancorica* flowered from October and November 2005, respectively. The average minimum & maximum temperature of 23.36°C and 32.22°C, respectively in the nursery during the period from May 2005 to September 2006. Reproductive biology of the two species was studied on these plants and compared with those in their natural habitat.

Methodology

Reproductive biology and post flowering behaviour

Reproductive biology of the two species were studied by frequent observation of the flowered areas, collecting and fixing the sample specimens for the detailed study on floral morphology, collection of pollen, pollen viability tests, fertilization and observation of post flowering behaviour. For determining the nature of flowering of both bamboo species, total number of flowered clumps in

flowering localities was counted. If flowering occurs in almost all the clumps of the same origin in a locality, it was considered as gregarious and if flowering occurs only in patches it was considered as sporadic.

Clump and culm characteristics

A detailed survey was conducted in the areas where flowering occurred to study the characteristics of flowering clumps and culms within the clumps, for both the species. Fifteen clumps at random were selected and the average number of culms/clump and number of flowered and non - flowered culms/clump were recorded.

F**loral morphology**

Fifteen clumps were selected at random, from which hundred flowers were also collected at random for the detailed observations on floral morphology. The specimens were fixed in FAA (Formalin-Acetic-Alcohol – 5 ml formalin, 5 ml glacial acetic acid, 90 ml 50% ethyl alcohol). The type of inflorescence, number of florets/spikelet, number of flowers with emerged out anthers/stigma, length of floral parts, number of anthers, time of anther emergence, type of anther dehiscence, type of pollination, quantity of pollen produced, diameter of pollen grains and length of pollen tube were recorded. Diameter of the pollen grains and pollen tube length was measured using image analyzer (Leica Q 500 MC) under 40x magnifications.

Pollination

Mode of pollination

Time of anthesis as well as anther emergence was noted. Type of dehiscence was verified by observing the dehisced anthers under dissection microscope (10x).

Mode of pollination was studied by using two methods.

1. Observation of insects visiting anthers and stigma of the flowers during anthesis to find out their role in pollination.

2. Adhesive tapes were fixed near the flowers in such way that pollen carried by the wind stick to the tape and after anthesis and the tape was observed under microscope to find out the role of wind in pollination.

Estimation of pollen production

In order to find out the quantity of pollen grains produced, flowers with anthers were collected just before dehiscence and kept in separate pieces of papers. When the anthers dehisced, each flower was dabbed to collect pollen grains and the pollen grains collected from each flower was weighed. This weight was divided by the number of anthers, from which the pollen grains were collected. Average amount of pollen grains produced by one anther was calculated as follows,

$$\text{Weight of pollen grains in one anther} = \frac{\text{Total weight of pollen grains (mg)}}{\text{Total number of anthers}}$$

Pollen viability

Pollen viability is the key factor in fertilization and seed formation. Pollen viability was tested by both staining and *in-vitro* pollen germination methods.

Staining method

Pollen viability was tested by staining method at the time of anthesis as well as five minutes after anthesis. Pollen grains dusted directly to the cavity slide containing solution of acetocarmine (one per cent) were observed under compound microscope (Radford *et al*., 1974; Nadgauda *et al*., 1997a; Ramanayake and Weerawardene, 2003; Koshy and Harikumar, 2001). The pollens that are stained well were considered as viable and the shriveled as non-viable. Four microscopic fields of view were selected at random and counted stained, shriveled and total number of grains per field of view and the viability was expressed in percentage.

Pollen germination *in- vitro*

In-vitro pollen germination tests were conducted with five different germination media consisting of sucrose, boric acid and calcium nitrate in different proportions (Wang *et al*., 2004). The composition of different germination media are given in Table 2.

Table 2. **Composition of different media tested for pollen germination**

Composition	**M1**	**M2**	**M3**	**M4**	**M5**
Sucrose (g)	10	10	10	0	10
Boric acid (g)	0.01	0.01	0	0.01	0
Calcium nitrate (g)	0.03	0	0.03	0.03	0
Distilled water (ml)	100	100	100	100	100

Fresh mature anthers were collected and dusted over clean Petri dishes containing germination medium. While dusting, pollen grains from different anthers were mixed to account for the variation. Pollen grains were transferred to cavity slides for observation under high resolution. One hour after inoculation, number of pollen grains germinated and total number of grains per field of view were recorded. Pollen grains that produce pollen tubes longer than the diameter of the grains only were considered as germinated (Tuinstra and Wedel, 2000). Diameter of the pollen grain and pollen tube length were determined using image analyzer (Leica Q 500MC) in millimeter under 40x magnification. Pollen germination was calculated in each medium and expressed in percentage as proposed by Guangchu (2002). Sixteen microscopic fields at random were selected for taking observations.

$$\text{Germination percentage} = \frac{\text{Number of pollen grains germinated in 16 fields}}{\text{Total number of pollen grains in 16 fields}} \times 100$$

Stigma receptivity

To study stigma receptivity, three methods were followed

1. The emerged out stigma was observed with hand lens to find exudations and colour changes. The observation was done in fifteen flowers selected at random.

2. Just before stigma emergence, twenty flowers were selected at random. Fresh pollen grains were applied with a brush on the stigma of ten flowers during exudation and ten flowers after drying up of exudation. These flowers were then covered with small paper bags and seed setting was observed.

3. Stigma collected a few minutes after hand pollination was stained with lactophenyl cotton blue and observed under microscope for germinated pollen grains entangled among stigmatic hairs.

Hybridization and seed set

The effect of artificial pollination on formation of seed was studied by dusting the pollen on the receptive surface of stigma using a brush at the time of exudation. Hand pollination was also done by applying artificial germination medium in which maximum pollen germination recorded *in-vitro* over the stigma. Pollinated flowers were marked and bagged with cloth bags. Inter-

generic hybridization between the two genera (*O. travancorica* and *D. stocksii*) was done by dusting the pollen of *O. travancorica* on stigma of *D. stocksii* to see whether seed formation occurs. About 25 healthy flowers at the time of stigma emergence were used for this study.

Post flowering behaviour

The flowered clumps of *D. stocksii* and *O. travancorica* were observed continuously for three years to study the post flowering behaviour. Clumps after flowering were examined regularly to see whether any production of new shoots and rejuvenation to vegetative phase occurs. To observe seed formation, polythene sheets were spread under the flowered clumps and the fallen mass was winnowed to separate the seeds. The area under flowered clumps was observed for a period of one year for appearance of wildlings. The chaff was spread in a nursery bed and watered regularly to see whether any seedling is found.

Biochemical changes associated with flowering and seed set

Sample collection

Samples were collected according to the flowering behaviour of the plant. Survey of the flowered areas of both the selected species was done. Clumps with uniform physical characters such as total number of culms / clump, no of culms flowered and type of flowering were selected. One clump, each was randomly

selected from three different localities to conduct the biochemical analysis. Within these clumps with 25 to 35 culms, culms having 2 - 5 inflorescences were marked and divided in to groups, each group consisting of three culms. Each group was collected in alternate months as different stages.

Selection of clumps

The clumps of *D. stocksii* were selected from the homesteads of three localities viz. Chongla, Poinachi and Periya in Kasaragode district in Northern Kerala. Since this species reverted to vegetative phase after flowering, samples consisted of six different stages consisting of three stages during flowering and three stages during rejuvenation (Table 3).

Table 3. Different stages of collection of the samples

Species	Stages	Time of sample collection	Description
D. stocksii	1	May, 2005	The clumps were in bloom during these stages.
	2	August 2005	
	3	November 2005	
	4	February 2006	The clumps were reverted back to its vegetative phase after flowering
	5	May 2006	
	6	August 2006	
Ochlandra travancorica	1	February 2005	The clumps were in bloom, setting seeds and started dying
	2	April 2005	
	3	June 2005	
	4	August 2005	Complete death

The clumps of *O. travancorica* were selected from three different localities viz. Arippa, Sasthanada and Kulathupuzha in Kulathupuzha forest range, Kollam

district. Samples of *O. travancorica* were taken for only four stages including one stage during post flowering. By this time the culms were completely dried.

Collected culms were split vertically into two parts and each one was cut into small pieces; oven dried and powdered using Wiley Mill to pass through 1 mm sieve. The ground material was mixed thoroughly to improve the homogeneity of the sample. Another part of the split culm was stored in deep freezer. The powdered samples in labeled bottles were kept air tight. The sample materials for extraction were kept in deep freeze below 4º C.

Biochemical parameters

Biochemical parameters like starch, reducing sugar, phenol, protein, amino acids, crude fat, fatty acids and vitamins in the culms of *D. stocksii* and O*. travancorica* were estimated at different stages during and after flowering.

Starch

Starch content was estimated as per the method of Humphreys and Kelly (1961). Sample, in triplicate (0.4g) were dried for 72 hours in desiccator containing concentrated sulphuric acid. To the sample in a 50 ml beaker, add 4.7 ml of 7.2 M perchloric acid and kept for 10 minutes. The contents were transferred into a 50 ml volumetric flask and made up to the volume with distilled water. After centrifuging, 10 ml aliquot were placed in a 50 ml volumetric flask together with a drop of phenolphthalein and made alkaline with 2 N sodium hydroxide. Then 2 N acetic acid was added until the colour was discharged. This was followed by

the addition of 2.5 ml acetic acid, 1.5 ml of 10% weight over volume potassium iodide and 5 ml 0.01 N potassium iodate. Samples were kept for 15 minutes and the colour was read at 650 μm using a spectrophotometer. Similarly a reading of the blank sample was also taken. The starch content was then calculated by applying the formula:

$$\text{Percentage starch} = \frac{0.368 \times (\text{E reading} + 0.008)}{\text{Oven dried weight of sample}} \times \frac{50 \times 100}{100}$$

Where E = difference of absorption between sample and blank.

Reducing sugar

Reducing sugar was estimated by Nelson-Somoyogi method (Sadasivam and Manickam, 1996). Sugars were extracted from 100 mg sample with 10 ml hot 80% ethanol and the supernatant was evaporated by keeping it on a water bath maintained at 80ºC. The residue was dissolved in 10 ml water and pipetted out in to test tubes (0.1 ml and 0.2 ml). Working standard was prepared from stock standard (100 mg glucose in 100 ml distilled water) by 1:10 dilution with distilled water. Pipetted out 0.2 ml, 0.4 ml, 0.6 ml, 0.8 ml and 1 ml of the working standard solution into a series of test tubes and the volumes of all the test tubes were made up to 2 ml with distilled water. All the test tubes were kept in a boiling water bath for 10 minutes after adding 1 ml of alkaline copper tartrate reagent. After cooling the tubes, 1 ml of arsenomolybdic acid reagent was added

and made up to the volume (10 ml) with water. After 10 minutes the colour was read at 620 nm and from the graph drawn, the amount of reducing sugars present in the sample was calculated.

Absorbance corresponds to 0.1ml of test = X mg of glucose

10ml contains $= \frac{X}{0.1} \times 10$ mg of glucose

= % of reducing sugars

Phenol

Phenol was estimated following the method suggested by Sadasivam and Manickam (1996). From 1 g of the dried sample, total phenol was extracted with 80% ethanol. The homogenate was centrifuged at 10,000 rpm for 10 minutes. The supernatant collected was evaporated to dryness. This residue was dissolved in 5 ml distilled water and pipetted out 0.2 ml to 2 ml into test tubes and each tube was made up to 3 ml with water. Added 0.5 ml Folin-Ciocalteau reagent and after 3 minutes mixed thoroughly by adding 2 ml of 20% Na_2CO_3. The test tubes were kept in boiling water for exactly one minute, cooled and the colour intensity was read at 650 nm. From the standard graph the amount of phenol was calculated and expressed as mg/100 g material. Working standard was prepared by diluting 10 times the standard which contains 100 mg catechol in 100 ml water.

Protein

Protein was estimated by Lowry's method (Sadasivam and Manickam, 1996). Proteins were extracted from 500 mg of the dried sample using 5-10 ml tris HCl buffer (pH 7.8). Centrifuged and the supernatant was used for the estimation. Pipetted out, 0.1 ml and 0.2 ml of the extract in to two test tubes. Protein stock standard solution was prepared by dissolving 50 mg bovine serum albumin in distilled water and made up to 50 ml in a standard flask. Working standard was prepared by diluting 10 ml of the stock solution to 50 ml with distilled water. From the working standard, 0.2 ml, 0.4 ml, 0.6 ml, 0.8 ml and 1 ml was pipetted out in to a series of test tubes. The volume of all test tubes were made up to 1 ml with distilled water, mixed well and allowed to stand for 10 minutes by adding 5 ml of alkaline copper solution. This was followed by the addition of 0.5 ml of Folin-Ciocalteau reagent, mixed well and incubated at room temperature in the dark for 30 minutes. The colour developed was read at 660 nm using a spectrophotometer and the amount of protein in the sample was calculated from a standard graph.

Absorbance corresponds to 0.1ml of test = X mg of protein

10ml contains $= \frac{X}{0.1} \times 10$ mg of protein

= % of protein

Amino acids

Total amino acids were determined as per the procedure by Ishida *et al*., (1981). For analysis, 100 mg of the sample was taken in a heat stable test tube. Added 10 ml 6 N HCl and sealed the test tube after filling with pure nitrogen gas and kept at 110ºC for 24 hours to liberate free amino acids. The contents were filtered into a round bottom flask through Whatmann No. 42 filter paper and washing the filter paper 2-3 times with distilled water. The filtrate was flash evaporated to remove all trace of HCl, and redissolved in 10 ml of 0.05 M HCl.

The sample was filtered through a 0.45 μm membrane filter and injected (20 μl)in to the amino acid analyser (HPLC-LC 10 AS) equipped with cation exchange column packed with a strongly acidic cation exchange resin i.e., styrene di vinyl benzene co polymer with sulphinic group. The column used was Na type i.e. ISC-07/S 1504 Na having a length of 19 cm and diameter 5 mm.

The HPLC was equipped with Shimadzu FL 6A fluorescence detector and Shimadzu CR 6A Chrompac recorder. The mobile phase of the system consisted of two buffers, Buffer A (sodium citrate, pH to 3.2) and Buffer B (sodium citrate – boric acid, pH 10). A gradient system was followed for the effective separation of the amino acids. The oven temperature was maintained at 60ºC. The total run was programmed for 62 minutes. The amino acid analysis was done with non-switching flow method and fluorescence detection after post-column

derivatization with o-phthalaldehyde. In the case of proline and hydroxyl proline, imino group was converted to amino group with hypochlorite.

Standard amino acid (Sigma chemical Co., St. Lousis, USA) was run to calculate the concentration of amino acids in the sample. Equipment was calibrated using standards before starting the analysis.

Quantification of amino acids

The standard and the sample were analyzed under identical conditions. The elution time of the amino acids of the sample was compared and identified with those of the standard. Quantification of amino acid was done by comparing the respective peak areas in the chromatogram of the sample and the standard. The amino acid content was calculated using the formula,

The amount of each amino acid was expressed as g /100g sample.

$$\text{mg amino acid / g sample} = \frac{\mu\text{mol} \times \text{mol.wt} \times \text{volume made up} \times 1000 \times 100}{1000 \times 1000 \times 20 \times \text{wt of sample}}$$

Estimation of tryptophan

Since the amino acid tryptophan is not stable to acid digestion in the presence of even trace amounts of oxygen, it is estimated separately by alkali digestion as per the method of Sastry and Tummuru (1985). From the dried sample, 0.3 g was hydrolyzed with 10 ml of NaOH for 24 hours in a sealed tube with pure nitrogen.

The hydrolysate was neutralized to pH 7 with 6 N HCl using phenolphthalein indicator. The volume made up to 25 ml with distilled water was then filtered through Whatman No.1 filter paper and the filtrate was used for the estimation.

To test tubes containing 4 ml of 50% H_2SO_4, 0.1 ml of 2.5% sucrose and 0.1 ml of 0.6% thioglycolic acid were added and the tubes were kept for 5 minutes in water bath maintained at 45 - 50ºC and cooled. Pipetted out 0.6 ml and 0.8 ml filtrate of the sample to tube and allowed to stand for 5 minutes after the volume was made up to 5 ml with 0.1 N HCl. A set of 0.1 ml to 0.8 ml standard tryptophan (10 µg/ml solution) was run in a similar way. The colour was read at 500 nm in a spectrophotometer and the amount of tryptophan was calculated from the graph and expressed as g /100g sample.

Fat

Crude fat in different stages of culms was determined by Soxhlet method (Sadasivam and Manickam, 1996). Fat content from 5 g of dried sample was extracted with petroleum ether for 16 hours in a Soxhlet apparatus. The extracted fat was dried and weighed after removing the solvent by evaporation. The amount of fat in the sample was calculated using the formula

$$\text{Fat content in the sample (\%)} = \frac{\text{Weight of fat (g)}}{\text{Weight of sample (g)}} \times 100$$

Fatty acids

The fatty acid profile of the samples was evaluated after cold extraction of fat (Folch *et al.,* 1957). From the dried sample, 12 g was subjected to fatty acid extraction using chloroform-methanol mixture (2:1). The extract was transferred to a separating funnel, add 20% of water into it and left over night for separation of phases. The lower chloroform fraction was collected in round bottom flask through filter paper containing anhydrous sodium sulphate was evaporated to dryness in a flash evaporator. The extract in the round bottom flask was made up to 10 ml by using chloroform. From this, 9 ml was taken for the analysis of fatty acids.

Analysis of fatty acids

Analysis of fatty acids was done as suggested by Gas chromatography method (AOAC, 1975). To the extract (9 ml), was added 5 ml of methanolic NaOH and refluxed for 5-10 minutes by passing nitrogen gas. Added 6 ml BF_3 solution and refluxed for further 6 minutes. Cooled and added 6 ml saturated NaCl solution. The contents were transferred to separating funnel and extracted with 30 ml petroleum ether (b.p. 60 - 80ºC). The upper layer was washed with water, filtered in to a flask through filter paper containing anhydrous sodium sulphate and evaporated to reduce the volume.

Methyl ester of the fatty acids thus obtained was separated by gas liquid chromatography (Varian CP 3800.USA) equipped with a capillary column (30 m long and 0.54 mm diameter) and a flame ionization detector (FID) in the presence of hydrogen and air. The carrier gas was nitrogen and the flow rate was 4 ml/min and the oven temperature started from 150ºC to 200ºC at 4ºC/min. Fatty acids separated were identified by comparing the retention time of standard FAME and quantified using the Star software (Varian). Individual fatty acids were expressed as a percentage of total fatty acids.

Vitamins

Analysis of Fat Soluble Vitamin

Fat soluble vitamins were determined as per Pozo *et al*., (1990). Appropriate volume (4 ml) from the extract was concentrated in a Heidolph Vacuum flash evaporator. Then, 15 ml of ethanol and 1.5 ml of KOH (20%) solutions were added to it. It was refluxed for 30 minutes in a boiling water bath; the solution was cooled and transferred to a separating funnel. Distilled water was added to it and extracted with 20 ml of petroleum ether. Upper petroleum ether layer was collected. The extraction was repeated. The petroleum ether extract was washed with water and the petroleum ether layer was dried by passing through Na_2SO_4. The solution was concentrated and made up to 10 ml with petroleum ether. It was injected into HPLC.

Chromatographic Conditions

A Merck-Hitachi LaChrom HPLC with L 7000 Quarternary gradient pump and L7400 progrmmable UV deterctor, was used for the vitamin determination. Column used was LiChrospher C18 Reversed Phase 250 x 4 mm column with the particle size of 5µ. Chromatographic separation made use of continuous gradient elution with 0.1% Tri fluro acetic acid (TFA) in HPLC grade water (eluent A) and 0.1 % TFA in acetonitrile (eluent B). The gradient started at 25% of 0.1% TFA in acetonitrile and was increased to 100% in 2nd minute. The total separation time was less than 20 minutes and the gradient was run for 15 minutes to ensure full separation. Detection was performed using a programmable variable wavelength detector, Vit A at 325 nm, Vit D at 264 nm, Vit E at 294 nm and Vit K at 325 nm. The HPLC gradient profile used for the separation of vitamin A, D, E and K and the retention time are given below (Table 4 and Table 5).

Table 4. Chromatographic conditions for fat soluble vitamins in HPLC

Time (min)	Mobile phase composition		
	0.1% TFA in HPLC grade water (%) (A)	0.1% TFA in acetonitrile (%) (B)	Flow rate (ml/min)
0	75	25	1.0
2	0	100	1.0
8	0	100	0.50
16	0	100	1.0
24	75	25	1.0

Table 5. Retention time of fat soluble vitamins in HPLC

Retention time	Vitamin (minute)
Vitamin A	14.25
Vitamin D	20.6
Vitamin E	18.5
Vitamin K	14.25

Retention time of each vitamin was noted after injecting known concentration of vitamins. Peak area of the vitamin standards was used to calculate concentration of vitamins in the sample by comparing the respective peak area by the following equations.

For vitamin concentration in sample:

$$\text{Concentration of vitamin (ppm)} = \frac{A \times B \times D}{C}$$

Where,

A = Peak area of the vitamin in the sample

B = Concentration of standard vitamin injected into HPLC

C = Peak area of vitamin standard

D = Dilution factor

Analysis of Water Soluble Vitamins

Water soluble vitamins was estimated by slightly modifying the method of Cho *et al*., (2000). Four g of the sample was homogenized with 1% Meta-phosphoric acid. The extract was filtered and the residue was repeatedly extracted. The filtrate was made up to a known volume and injected into HPLC.

Chromatographic Conditions

A Merck-Hitachi LaChrom HPLC with L 7000 Quarternary gradient pump and L7400 progrmmable UV detector, was used for the vitamin determination. Column used was Waters Xterra C18 Reversed Phase 2.1 x 150 mm column with the particle size of 5µ. Chromatographic separation made use of continuous gradient elution with 0.1% TFA in HPLC grade water (eluent B) and 0.1%TFA in acetonitrile (eluent B). The gradient started at 25% of 0.1% TFA in acetonitrile and was increased to 100% in 2nd minute. The total separation time was less than 20 minutes and the gradient was run for 15 minutes to ensure full separation (Table 6). Detection was monitored at 205 nm. The retention is given below (Table 7).

Table 6. Chromatographic conditions for water soluble vitamins in HPLC

Time (min)	Mobile phase composition		
	0.1% TFA in Acetonitrile(%)(A)	0.1% TFA in HPLC grade water(%) (B)	Flow rate (ml / min)
0	75	25	1.0
2	0	100	1.0
8	0	100	0.50
16	0	100	1.0
24	75	25	1.0

Table 7. Retention time of water soluble vitamins in HPLC

Vitamins	Retention time (minute)
Thiamin(b1) (ppm)	6.47
Nicotinic acid(b3) (ppm)	5.31
Pantothenic acid(b5) (ppm)	10.18
Pyridoxine(b6) (ppm	9.13
Folic acid (b9) (ppm)	12.87

Estimation of Vitamin Concentration

Concentration of vitamin (ppm) = $\frac{A \times B \times D}{C}$

Where,

A = Peak area of the vitamin in the sample

B = Concentration of standard vitamin injected into HPLC

C = Peak area of vitamin standard

D = Dilution factor

Statistical Analysis

Statistical packages SPSS and SAS 10 for windows were used for statistical analysis. Analysis were done to find out variations in biochemical contents during and after flowering in two species, variations due to interaction effects of location, variations between stages during and after flowering and within culms in a group. Analysis of variance was done and DMRT was performed for data with significant F-values.

CHAPTER- IV

REPRODUCTIVE BIOLOGY AND SEED SET

Flowering history, floral morphology, pollination, fertilization, seed set and post flowering behavior of two species were studied. *D. stocksii* (Fig 2. a) represented a non seed-setting and *O. travancorica* (Fig 3. a), a seed- setting species.

Flowering history

The reports on flowering of the two bamboo species are given below (Table 8). Gregarious flowering of *D. stocksii* was first observed in 1884 in North Kanara followed by 1994 in Silent valley and 2003-2006 in northern Kerala. Since the flowering reports were from different locations a definite flowering cycle could not be arrived at. But this indicated that different flowering cohorts occur in nature for this species.

Gamble (1896) has reported a flowering cycle of 7 years for *O. travancorica*. Flowering occurred during the years 1868, 1875, 1882 and 1905. Later reports are from southern Kerala during 1976, 1984, 1988, 1992, 1993 and 2003-2006. The seedlings originated from the flowering of 1984 at Vazhachal, transplanted at the campus of Kerala Forest Research Institute, Peechi were also observed and no flowering was found till February 2011 indicating that flowering cycle is more than 27 years. During the study period (2004-2010) flowering was observed in southern Kerala under the Forest Divisions of

Kollam and Munnar, viz. Neryamangalam, Kulathupuzha, Shendurney wild life sanctuary, Marappalam, Sasthanada, Shankily, Arippa etc. It is found growing extensively both as pure and mixed vegetation in Kulathupuzha forest range and Shendurney wild life sanctuary. This clearly indicates that *O. travancorica* has at least 5 flowering cohorts in Kerala.

Table 8. Flowering records of *D. stocksii* and *O. travancorica*

Species	Year of flowering	Location	Reference
D. stocksii	1884, 1888	North Kanara	Blatter, 1929
	1994	Silent Valley	Sequiera and Kumar, 1995
	2003-2006	Northern Kerala	Beena *et al.,* 2007
	2003-2006	KFRI Peechi, Bambusetum	Beena *et al.,* 2007
O. travancorica	1868, 1875, 1882, 1905	-	Gamble, 1896
	1976	Southern Kerala	Asari, 1976
	1984	Southern Kerala	Venkatesh,1984
	1988,1992,1993	Southern Kerala	Seethalakshmi, 1993
	2003-2006	Southern Kerala	Seethalakshmi, 2010b

Nature of flowering, clump and culm characteristics

Observations at Chattanchal, Monacha, Chongla, Poinachi and Periya in Kasaragode district, 75 per cent clumps of *D. stocksii* initiated sporadic flowering during October 2004, which turned to gregarious and was peak in

April 2005. During June 2005 with the onset of monsoon, new shoots started sprouting up. Production of flower was decreased in 75 per cent flowered culms and remained vegetative with a few leaves. After the monsoon, these culms turned to reproductive phase again and finally died. New culms sprouted up during June 2005 produced no flowers and the whole clump gradually reverted to vegetative phase. The selected clumps of *D. stocksii* consisted of 32 ± 6 culms with mean height of 9 ± 3 m and 2.5 - 4 cm diameter. Among the 4 clumps in the bambuseta of KFRI Field Research Centre, Velupadam which was collected from Thalasseri and planted during 1992 also flowered along with the mother plants indicating the synchronous flowering nature of *D. stocksii*.

In *O. travancorica* almost all places in Kulathupuzha forest range, Shendurney Wildlife Sanctuary both sporadic and gregarious flowering was observed from December 2004 onwards. Gregarious flowering was peak during February 2005 and produced 50 ± 6 seeds per culm from June when rainy season began. The selected clumps of *O. travancorica* consisted of 30 ± 5 culms with height of 3 - 6 m and 5 ± 2 cm diameter.

Table 9. Clump and culm characteristics of flowered *D. stocksii* and *O. travancorica*

Particulars	*D. stocksii*	*O. travancorica*
Height of culms (m)	9 ± 3	3 – 6
Diameter of culms (cm)	2.5 - 4	5 ± 2
No of culms / clump	32 ± 6	30 ± 5

Floral Morphology

The inflorescence of *D. stocksii* is a large panicle with sessile spikelet each consisting of 1-2 florets, arranged as globose heads having an average diameter of 2.5 cm and 3-5 cm distance. Whereas, the inflorescence of *O. travancorica* is subverticillate, spicate panicle, with one flowered, sessile spikelets in the axils of bracts. The details of floral morphology are presented in the Table 10.

Table 10. Details of floral characters in *D. stocksii* and *O.travancorica*.

Particulars	***D. stocksii***	***O. travancorica***
Number of spikelets per head	38.9 ± 4.2	6 ± 1.8
No of florets per spikelet	1-2	1
Number of spikelet with exposed stigma per head	5.1 ± 1.97	2.2 ± 1.38
Number of spikelet showing anthers per head	3.9 ± 1.37	2.7 ± 1.3
Length of spikelet (cm)	1.36 ± 0.09	3.48 ± 0.35
Length of palea (cm)	1.28 ± 0.15	3.61 ± 0.2
Length of lemma (cm)	1.15 ± 0.12	3.78 ± 0.3
No of anthers	6	54.5 ± 4.3
Length of anthers (cm)	0.53 ± 0.05	1.57 ± 0.23
Length of filaments(cm)	0.5 ± 0.01	3.5 ± 0.13
No of filaments	6	54.5 ± 4.3
Length of pistil(cm)	1.12 ± 0.1	3.02 ± 0.2
Colour of stigma	purple	white
Diameter of pollen grains (mm)	0.17 ± 0.01	0.21 ± 0.02
Length of pollen tube (mm)	0.12 ± 0.09	1.11 ± 0.25

Number of spikelets per head in *D. stocksii* was six times more compared to *O. travancorica*. Length of spikelets, palea, lemma and pistil of *O. travancorica* was three times more when compared to *D. stocksii*. No of anthers in *D. stocksii* were 6 in number where as *O. travancorica* flowers contained ten times more anthers in number (Fig 2. c and Fig 3. b). Length of anther and filament of *D.*

stocksii was smaller when compared to *O. travancorica*. Length of pollen tube in *D. stocksii* was smaller than its diameter whereas, it was more than 2 times in *O. travancorica*. Dichogamy and protogyny was observed in both species, where the gynoecium matured 3 - 4 days before androecium and this prevented self-pollination.

Pollination

Anthers emerged out from 6 A. M in the morning and dehisced by 11 A. M in *D. stocksii*, whereas anthers of *O. travancorica* emerged out at 9 A. M and dehisced by 11 A. M liberating a large number of pollen grains. In *D. stocksii*, only 15 per cent of anthers dehisced and others dried out without shedding pollen. The type of dehiscence in both species was longitudinal.

Mode of pollination

Examination of the adhesive tapes attached near the flowers with emerged out anthers after anthesis in *D. stocksii* showed less than 4 per cent of pollen grains attached to it while 96 per cent of pollen grains were found attached to the tapes near the anther dehisced flowers of *O. travancorica*. This indicated the possibility of anemophilous pollination in both species.

Insect visit was not observed in the flowers of *D. stocksii* where as Dammer bees (*Trigonia irridipennis*) visited anthers of *O. travancorica* even before they fully emerged out. However, they did not visit stigma.

Pollen produced per anther

Pollen grains of both species are monoporous, having single pore through which single pollen tube is produced. The pollen production was negligible in D*. stocksii* while *O. travancorica* produced 1.51 ± 0.13 milligram pollen grains per anther.

Pollen viability

Staining method

Fresh pollen grains of *D. stocksii* at the time of anthesis showed 90 per cent viability and two minutes after it reduced to about 2 per cent. Pollen grains of *O. travancorica* showed 99 per cent viability both at anthesis and after five minutes of dehiscence. Comparatively the percentage and duration of viability of the pollen was much higher in *O. travancorica***.**

***In- vitro* germination of pollen grains**

The pollen germination of *D. stocksii* and *O. travancorica* in different medium are presented in Table 11.

Table 11. Percentage of pollen germination of *D. stocksii* and *O. travancorica*

Medium	*D. stocksii*		*O. travancorica*	
	Germination percentage	Time taken for germination (minutes)	Germination percentage	Time taken for germination (minutes)
M1	11	20	62.16 ± 1.8	20
M2	2	20	53.5 ± 3.27	30
M3	0	-	9.26 ± 3.4	70
M4	0	-	0.00	0
M5	0	-	95.8 ± 2.97	30

M1- Sucrose (10 g), Boric acid (0.01 g), Calcium nitrate (0.03 g), H_2O; M2- Sucrose (10g), Boric acid (0.01 g), H_2O; M3- Sucrose (10 g), Calcium nitrate (0.03 g), H_2O M4- Boric acid (0.01 g), Calcium nitrate (0.03 g), H_2O; M5- Sucrose (10 g), H_2O

In *D. stocksii* percentage of pollen germination was generally low and germination occurred only in M1 and M2. Maximum germination obtained was 11 per cent in M1 and the next was 2 per cent. In both the media it took 20 minutes.

In *O. travancorica* pollen germination was observed in all the media except M4. Maximum germination was observed in M5 (95.8 ± 2.97 per cent) followed by M1 (62.16 ± 1.8) and M3 (53.5 ± 3.27). The duration taken for germination was also different ranging from 20 to 70 minutes.

Stigma receptivity

Stigma of both the species emerged out in the morning by 9 A. M. In *D. stocksii*, it was purple in colour at emergence, after emerging out it remained darker with oily exudation for 10-15 minutes and dried off. Seeds were not

produced when fresh pollen grains were dusted on stigma. When stained with lactophenyl cotton blue after hand pollination, pollen grains found entangled among the hairs of stigma, but they did not germinate. In *O. travancorica* stigma was white in colour, produced colourless exudation giving a shining appearance in the sunlight. During anthesis, lumps of pollen grains were attached to the stigma, which remained exposed for about 20 to 30 minutes. Seeds were produced by all flowers when fresh pollen grains were applied during the exudation of stigma and bagged whereas; no seeds were produced when pollen grains applied on the stigma after the drying up of exudation. When the stigma of these flowers was stained with lactophenyl cotton blue, number of germinated pollen grains was less than 5 per cent.

Hybridization

Attempts for intergeneric hybridization using *D. stocksii* as female parent and *O. travancorica* as male did not give any positive result when fresh pollen grains of both species were applied with and without artificial germinating media. In *O. travancorica* 98 per cent flowers produced healthy seeds when fresh pollen grains of the same species was applied during exudation on stigma with out applying artificial germination media. Seeds were not produced when artificial media were applied on stigma before pollination.

Post flowering behavior

After gregarious flowering, all the flowered culms of *D. stocksii* died and new culms sprouted from the same rhizome during June 2005 produced no

flowers. By August 2006, 98 per cent of new culms were vegetative. The clump appeared as vegetative without any symptoms of previous flowering. Seeds or wildlings were not observed in the ground area under flowered clumps even after monsoon showers. While winnowing the mass collected from the clumps and ground area around the clumps no seeds were found. When the fallen mass was put in water the whole chaff floated up indicating that no fertile seeds are available. Not even single wildlings could be located under the flowered clumps indicating complete absence of seed set.

Each culm of *O. travancorica* produced 50 ± 6 seeds and the whole clump died after seed setting. Seed production and dispersion occurred mostly during May and June. Vivipary was observed. The vigorous growing seedlings fell on to the ground by external forces got attached in the soil. The newly germinated seedlings gave the appearance of green carpet to the forest floor. By December 2006, almost all the flowered clumps dried (Fig 2 & 3).

BIOCHEMICAL CHANGES

In order to find out the biochemical changes occurring in bamboo culms during flowering and post flowering, a series of biochemical analysis were carried out. Variation in the quantity of starch, protein and fat, reducing sugar, amino acid, fatty acid and vitamin content of the culms at different stages of flowering were determined for both the species. The variation estimated during and after flowering was compared not only between species but also due to location within the species. The present study gives information of the nutrients exhausted during flowering, seed set and rejuvenation.

CHAPTER - V

STARCH, REDUCING SUGARS AND TOTAL PHENOL

Starch

In *D. stocksii*, highest percentage of starch was found during the first stage of flowering (10.947) which decreased to 9.508 and 7.719 during second and third stages, respectively. When the clumps started to revert back into vegetative phase in the initial stage (fourth), the lowest level of 4.729 gradually increased to 4.796 and finally 5.038 as the vegetative growth was resumed. Increase in the percentage of starch was observed during the reversion from reproductive phase to vegetative phase. In *O. travancorica*, when the culms began to bloom, the starch percentage of the culms was the highest (4.974). As the seed set and drying of clumps started (third and fourth stage) the starch content of the culms decreased. Lowest percentage of starch was observed (1.955) when the culms completely died (Fig 4).

There was significant variation in starch content of the culms due to species ($p=0.05$) and different stages of flowering ($p=0.01$). The variation in starch level was not significant due to locality. The interaction effects location x species as well as culm within stage x species were not significant (Appendix I).

Reducing sugars

In *D. stocksii* the percentage of reducing sugars ranged from 1.916 to 2.824 during different stages of flowering. Initially the reducing sugar content of the culms was lower when compared to second stage of flowering (2.824). As

the clumps rejuvenated, the quantity of reducing sugars decreased and the least amount of reducing sugars of 1.916 was observed at stage six. In culms of *O. travancorica* reducing sugars increased as the flowering initiated and it was the highest during second stage (1.998) which was followed by a gradual decrease and was completely exhausted during seed set and death (0.049) (Fig 4).

The effect of different stages with in species and the interaction effect of culms with in stage and species were significant in the reducing sugar content of the culms (P=0.01) (Appendix II).

Total phenol

The percentage level of total phenol in *D. stocksii* decreased (24.8) after flowering during rejuvenated stage. In *O. travancorica* it was reverse. During flowering period phenol content was less and the percentage level increased (48.87) during seed set. Phenol content of the culms varied significantly at different stages (p=0.01) (Fig 4). The total phenol varied significantly between different stages in both species (Appendix III).

CHAPTER - VI

PROTEINS AND AMINO ACIDS

Protein

The protein content in the flowering culms of *D. stocksii* was lower when compared to the fresh culms originated after reversion to vegetative phase. The highest percentage of protein was observed at rejuvenated stage (4.665). In *O. travancorica* percentage protein was highest during flowering and decreased during seed set and death. Highest protein content was observed during the onset of flowering i.e., the first stage (2.647) and the lowest was observed after complete death of the culms (1.969) (Fig 5). The variation in the protein content due to species and different stages was significant (p=0.01) in both species. Significant variation was not observed in the protein content of the *D. stocksii* culms during the stages of flowering (first three stages) (Appendix III).

Amino acids

Quantification of different amino acids was carried out in the flowered and rejuvenated culms (stages 1 and 6) of *D. stocksii* and the flowered and seed setting culms (stages 1 and 3) of *O. travancorica*. Of the 18 amino acids analysed, 16 were present in both the species (1. asparagine, 2. threonine, 3. serine, 4. glutamine, 5. proline, 6. glycine, 7. alanine, 8. valine, 9. iso leucine, 10. leucine, 11. tyrosine, 12. phenylalanine, 13. histidine, 14. lysine, 15.

arginine and 16. tryptophan) and two amino acids (cystein and methionine) were absent.

Statistical analysis of the data revealed significant variation in different amino acids due to species, location and different stages. The variation in 12 amino acids viz., 1. asparagine, 2. threonine, 3. serine, 4. glycine, 5. alanine, 6. valine, 7. leucine, 8. tyrosine, 9. phenylalanine, 10. lysine, 11. arginine and 12. tryptophan was significant at one percent level in different stages of flowering. The variations due to location, species and location x species were not significant. Another two amino acids, iso leucine and histidine in the culms also varied due to different stages and between species at five percent level. While, the glutamine content of the culms varied among species only. The proline content of the culms varied due to different locations only (p=0.05) (Appendix IV to XX).

Amino acid composition during flowering and rejuvenation in *D. stocksii*

There were changes in amino acid composition of the culms associated with flowering and rejuvenation. Tyrosine was found only in vegetative phase. The level of other five amino acids increased during the rejuvenation phase than that of the flowering stage (glutamine, proline, valine, arginine, and tryptophan). Of these, the percentage of increase of tryptophan and arginine was prominent (77.8 and 51.6 respectively). Lysine was not detected during rejuvenation indicating that it is completely used up. The level of other 9 amino acids decreased during rejuvenation (asparagine, threonine, serine,

glycine, alanine, isoleucine, leucine, phenylalanine, histidine). The percentage of decrease varied between 6 and 66. Maximum percentage of decrease (66) was seen in the levels of phenyl alanine. On comparison of amino acids at flowering and rejuvenated stages, the amino acids viz. asparagine, threonine, serine, glycine, alanine, valine, leucine, tyrosine, phenyl alanine, lysine, arginine and tryptophan were significantly different at one percent level.

Amino acid composition during flowering and death in *O. travancorica*

All the amino acids except asparagine were higher during flowering phase in *O. travancorica*. The percentage of decrease varied from 3 to 76. Maximum percentage of decrease was seen in lysine and tryptophan (76 and 71.8 respectively) followed by arginine (34.8) and histidine (24.2). The percentage of decrease between 10 and 20 was observed in the level of five amino acids viz., valine (12.4), proline (12.6), alanine (13.6), phenyl alanine (16.7), and threonine (17.4). The decrease was not prominent in others viz., serine (0.6), leucine (3.1), iso leucine (3.4), glutamine (4.2) tyrosine (5) and glysine (5.4).

On comparison of amino acids at flowering and seed setting stages, the amino acids viz. threonine, glycine, alanine, valine, lysine, arginine and tryptophan were siginificantly different at one percent level (Fig 5 to 10).

CHAPTER - VII

FAT, FATTY ACIDS AND VITAMINS

Fat

The pattern of utilization of crude fat was completely different in two species. In *D. stocksii*, the average fat content ranged from 0.442 to 0.224 percent during different stages of flowering. It decreased during flowering and reversion to vegetative phase. But in *O. travancorica*, fat was completely utilized during flowering and fruiting. The lowest fat content was observed during death of the culms. The average fat content of the culms ranged from 0.2835 to 0.137 per cent (Fig 11). The crude fat content of the culms varied significantly due to different stages and interaction effects of culms with in a stage x species (p=0.01) only (Appendix XXI).

Variation in fatty acids at different stages of flowering

Quantification of the different fatty acids was carried out. Of the 20 fatty acids analysed two were not detected in both the species (C10 - capric acid and 20:1cis-11-eichosenoic acid). Others were present in different quantities (C11 - undecanoic acid, C 12 - lauric acid, C 13 - tridecanoic acid, C 14 - myristic acid, C 15 - pentadecanoic acid, C 15:1 cis-10-pentadecanoic acid, C 16 palmitic acid, C 16:1 palmitoleic acid, C 17 heptadecanoic acid, C 18 stearic acid, C 18:1n-9c oleic acid, C 18:2n-6t linolelaidic acid, C 18:3n-3 linolenic acid, C 20 archidic acid, C 20:5n-3 cis-5,8,11,14,17-eicosapentaenoic acid, C 22 behenic acid, C 22:2 cis-13, 16-docosadienoic acid, and C 24 lignoceric acid). The analysis of variance was conducted only for six (C 14 - myristic acid, C 15:1 cis-10-pentadecanoic acid, C 16 palmitic acid, C 18 stearic acid, C 20 archidic acid, and C 24 lignoceric acid) as traces

of others were detected. Individual fatty acids were expressed as a percentage of total fatty acids. Analysis of variance revealed significant variation in myristic acid, pentadecanoic acid and stearic acid content at different stages at one percent level and archidic acid and lignoceric acid content at five percent level. The archidic acid content of the culms also varied due to location at 5 per cent level (Appendix XXII to XXVII).

Changes in fatty acid composition in *D. stocksii*

Eight fatty acids were found to increase during rejuvenation. The percentage of increase varied from 5.6 to 83.6. Maximum increase was observed for C 17 heptadecanoic acid (83.6) and C 18:1n-9c oleic acid (83) followed by C 14 - myristic acid (72), C 20:5n-3 cis-5,8,11,14,17-eicosapentaenoic acid (67.7) C 18 stearic acid (60.9), and C 15 - pentadecanoic acid (58.6). Marginal increase was recorded in C 16 palmitic acid (5.6) and C 16:1 palmitoleic acid (10.5). Two fatty acids viz., C11 undecanoic acid and C22 behenic acid were detected only during flowering phase. The other eight fatty acids recorded a decrease during rejuvenation phase. The percentage of decrease varied from 19.1 to 86. Maximum decrease was observed in C 22:2 cis-13, 16-docosadienoic acid (86), followed by C15:1 cis-10-pentadecanoic acid (69.6). Decrease between 30 to 45 per cent was seen in the level of four fatty acids viz., C 20 archidic acid (19.1), C18:2n-6t linolelaidic acid (28.9), C12- lauric acid (34), C13- tridecanoic acid (34), C 18:3n-3 linolenic acid (41), and C 24 lignoceric acid (43.7) .

Changes in fatty acid composition culms of *O. travancorica*

Compared to *D. stocksii* fatty acid utilization was more in *O. travancorica*. Only marginal increase in the percentage of four fatty were observed in post flowering phase and they are C 20 archidic acid (3.3), C 18 stearic acid (4.1), C 22 behenic acid (6.4) and C 18:2n-6t linolelaidic acid (8), On the contrary considerable decrease in the quantity of seven fatty acids viz., C 18:1n-9c oleic acid (65.8), C 13 - tridecanoic acid (56), C 12 - lauric acid (55.6), C 22:2 cis-13, 16-docosadienoic acid (52.4), C 14 - myristic acid (50), C 15 - pentadecanoic acid (40), C 24 lignoceric acid (38.9) and slight decrease of C 16 palmitic acid (7.5) were observed after seed formation. Six fatty acids viz., C11 - undecanoic acid, C 15:1 cis-10-pentadecanoic acid, C 16:1 palmitoleic acid, C 17 heptadecanoic acid, C 18:3n-3 linolenic acid and C 20:5n-3 cis-5,8,11,14,17-eicosapentaenoic acid were absent after seed formation (Fig 11).

VITAMINS – FAT AND WATER SOLUBLE

Four fat soluble vitamins viz., Vitamin A, D, E and K and seven water soluble vitamins viz., ascorbic acid, nicotinic acid, thiamine, pyridoxine, folic acid, riboflavin, pantothenic acid were analyzed in both species at two stages. Statistical analysis of the data on fat soluble vitamins revealed significant variation in different stages at one percent level. The vitamin D content of the culms varied also due to species ($p=0.05$) but the interaction effect of species and flowering stages was not significant (Appendix XXVIII to XXXI). Analysis of the data on water soluble vitamins showed significant differences in nicotinic acid, thiamine, pyridoxine, folic acid, riboflavin and pantothenic acid of the culms at different stages ($p=0.01$) of flowering. Significant

variation was not observed due to location or species. Since ascorbic acid content was negligible it was not included in the statistical analysis (Appendix XXXII to XXXVII).

Fat soluble vitamins during flowering and rejuvenation in *D. stocksii*

Except vitamin K, other three fat soluble vitamins decreased during rejuvenation phase. Maximum percentage of decrease was observed in vitamin E (80.3) followed by vitamin A (62.5) and vitamin D (11.7). On the other hand vitamin K which was found in traces during flowering recorded an increase of 97.5 per cent during rejuvenation. Statistical analysis revealed that the difference in the levels of vitamin D, vitamin E and vitamin K of the species varied at one percent level at different stages.

Fat soluble vitamins during flowering and seed setting culms of *O. travancorica*

Generally fat soluble vitamins, except vitamin D recorded a decrease during seed setting stage. The percentage of decrease was maximum for vitamin A (97.9) followed by vitamin K (61.6) and vitamin D (57.7). Vitamin D showed a slight increase of 3.7 per cent. Analysis of variance showed that the levels of vitamin D, vitamin E and vitamin K contents of the species varied at one percent level at different stages (Fig. 12 and 13)

Water soluble vitamins in flowering and rejuvenating culms of *D. stocksii*

Of the seven water soluble vitamins nicotinic acid (81.7) showed maximum decrease followed by pyridoxine (51), riboflavin (31.7), ascorbic acid (25.4) and thiamine (24.3). Comparatively the percentage of decrease of pantothenic acid (18.9) and folic acid (15) was low. Statistical analysis showed that the nicotinic acid, thiamine, pyridoxine, folic acid, riboflavin and pantothenic acid of the culms significantly varied (p=0.01).

Water soluble vitamins in flowering and seed setting culms of *O. travancorica*

Highest percentage of decrease was recorded in ascorbic acid (89.9) followed by thiamine (72.78), pyridoxine (73.9) and nicotinic acid (41.8). Both pantothenic acid and folic acid showed narrow decrease (16.7 and 15.6 respectively). Statistical analysis showed that nicotinic acid, thiamine, pyridoxine and folic acid varied significantly variation (p=0.01). Whereas, there was no significant variation in riboflavin and pantothenic acid (Fig. 14 to 16).

CHAPTER – VIII

DISCUSSION

Flowering of 22 sympodial bamboo species belonging to four genera was observed during last three decades in southern India. Among them, three different groups viz., sterile, profuse and sparse seed forming were reported (Seethalakshmi *et al.,* 2010b). Simultaneous flowering of two species - *Dendrocalamus stocksii* and *Ochlandra travancorica* belonging to two genera, representing two different groups with regard to seed set (sterile and profuse seed forming) provided a good opportunity to make a comparative assessment of various factors affecting flowering and seed production. They also showed two types of post flowering behaviour (seed set and death, sterile and rejuvenation) making it possible to assess some of the bio-chemical changes in both situations.

Flowering, reproductive biology and post flowering behaviour

History revealed that flowering has been reported from various locations previously. Only one of the populations is flowering currently, while others are in vegetative stage. This indicated that both the species have different flowering cohorts in Kerala. One flowering cohort/flowering genotype is considered as single population originated from one seed lot of same species. Even if they are geographically separated all the clumps originated from this population will flower together and it is known as a flowering genotype/flowering cohort. Occurrence of different cohorts have already

been reported among various species like *Melocanna bambusoides*, *Bambusa bambos* etc.(Banik, 1998; Seethalakshmi *et al*., 2010c). It is highly advantageous for a plant group like bamboos in which synchronous flowering of parent and offspring resulting in death of the entire population has been observed. In the context of enhancing bamboo resources by expanding the area under cultivation with new plantations, availability of planting stock from a mixture of cohorts having a flowering interval of at least five years between them (block planting or mosaic planting) can minimize the gap in supply of raw material to industries due to the gregarious flowering and death. Mapping of flowering cohorts and documentation of planting stock produced from different cohorts are essential to select suitable planting material and predict flowering. The present study has helped to document one flowering cohort each of the two species and a tentative assessment about the different cohorts from the flowering history.

Information on flowering was insufficient to find out the flowering cycle. However, the observation on *O. travancorica* clumps planted in the KFRI campus clearly indicated that the flowering cycle is not seven years as documented earlier (Gamble, 1896). The seedlings that originated from the 1984 flowering are yet to flower. The period of flowering which was between September to April and the appearance of flowering such as sporadic nature first and then becoming gregarious within a period of two years etc. are in full agreement with previous reports (Banik, 1998; Koshy and Harikumar 2001; Beena *et al.,* 2007; Seethalakshmi, 2009). Seeds of *O. travancorica*

had been reported as recalcitrant, with a viability of about 45-60 days and there was no dormancy (Seethalakshmi, 1993). Season of seed fall (April – May) coincided with the pre-monsoon showers, favoring profuse natural regeneration. The sprouts originated from culm cuttings / rhizome taken from flowered culm / clump planted in the nursery also turned into an inflorescence which gave a convenient system for observations on reproductive biology.

There was considerable variation in reproductive parts of both the species. Inflorescence of *D. stocksii* consisted of more number of flowers than that of *O. travancorica* but flowers were smaller. Although two species belonged to different genus, there was some similarity. Spikelets of both species are bisexual, dichogamous and protogynous. The maturation period of female and male phase of each floret ensured that pollination can only happen through pollen grains from other flowers. *D. stocksii* produced abundant number of flowers but there were no sufficient pollen grains while *O. travancorica* produced less number of flowers each having numerous anthers with large quantity of pollen grains.

Comparison of reproductive biology between species revealed considerable variation in percentage of anthesis, quantity and quality of pollen grains, distribution of pollen, preference of insects like bees, receptivity of stigma, rate of growth of pollen tube etc. *O. travancorica,* the seed setting species, had ten times more anthers (6 and 60, respectively). Exertion of reproductive structures started in the cool morning hours, which was in full blossom in the

bright light between 9 to 11 A.M. In previous reports also, the timing of anthesis and of exertion of stigma are dependent on environmental conditions such as air temperature and relative humidity, and both occur in the morning hours (Nadgauda *et al.,* 1993 and John *et al.,* 1995). Majority of anthers (85 per cent) dried off in *D. stocksii* before dehiscence without shedding the pollen grains. Since pollination was anemophilous, lack of production of sufficient viable pollen and release to atmosphere appeared to be limiting factors for seed formation. It was again confirmed by the difference in the quantity of pollen adhered to cellophane tapes hung near stigma.

Observations on pollen viability also showed considerable variation between two species. The duration of pollen viability was less than five minutes in *D. stocksii* and more than 30 minutes in *O. travancorica* which was clearly indicated by staining methods (90 per cent initially and 2 per cent after five minutes for *D. stocksii;* 99 per cent both at anthesis and after five minutes for *O. travancorica*).

Pollen grains of species such as *Pseudoxytenanthera ritcheyi*, *Ochlandra travancorica*, and *Ochlandra scriptoria* were found to be attracted by dammer bees on which they voraciously fed. Insect visits were not observed in *D. stocksii*, which produced only negligible amount of pollen, while lot of bees visited O*. travancorica* during anthesis, but not interested in stigma indicating lack of direct role in pollination. Indirect role in pollination by insect visit resulting in release of large quantity of pollen in a short time that appeared to

enhance the chance of pollination has been suggested by other investigators (Huang *et al.*, 2002).

According to Tuinstra and Wedel (2000), pollen grains with pollen tube smaller than its diameter were not considered as germinated. Even though 11 per cent pollen grains of *D. stocksii* germinated *in-vitro*, pollen tube produced was approximately equal to or slightly longer than its diameter. Only two per cent germination was recorded in the medium containing sucrose and boric acid.

Percentage of pollen germination of various species in different germination media varied. Highest pollen germination of *O. travancorica* was shown in medium containing only sucrose with in 10 to 15 minutes. Less and slow germination was seen in the medium containing sucrose and calcium nitrate where as the rate and percentage was high in the medium containing sucrose and boric acid indicating that boric acid has some effect on pollen germination as suggested by Wang *et al*., (2003). In *P. ritcheyi*, which showed close similarity to *D. stocksii*, highest germination percentage (96.3) was observed in the medium containing sucrose, boric acid and calcium nitrate followed by sucrose and calcium nitrate.

There was difference in the receptivity of stigma for different species. Stigma of *D. stocksii* was purple and had oily exudation, but the rate of pollen germination on the surface of stigma was low and rate of growth of pollen tube was very slow. Attempts for hybridization with pollen grains of *O.*

travancorica were not successful. The result was the same even when artificial germination medium was applied for pollination indicating non-receptivity of stigma. From the observations made on *D. stocksii*, representing a sterile species of bamboo, it is clear that some of the factors leading to sterility in bamboo are small quantity of pollen production, low viability, lack of release of sufficient quantity of pollen due to lack of proper anthesis and the non-receptivity of stigma. On the contrary in *O. travancorica,* representing a profuse seed forming species, abundant pollen was produced, viability was about 99 per cent, good release due to high percentage of anthesis and highly receptive stigma. Even hand pollination with pollen grains of same species produced seeds. This species usually grows in moist areas and the fluid secreted on the stigma remained for comparatively long duration without drying to receive the pollen grains. However hybridization with pollen grains of *D. stocksii* was not successful. Seed set was not observed even when germination media was applied on the surface of stigma.

Post flowering behaviour of both the species was also different. In *D. stocksii,* flowering continued for about three years. All the flowered culms dried. The new shoots originated from the rhizome from third year onwards appeared completely vegetative. The capacity for reversion to vegetative phase is very advantageous in bamboo. From plantation of such species there will not be a gap in supply of raw materials to industries. Since it is not setting seeds, culms can be harvested during the flowering phase without waiting for seed fall as prescribed in felling cycle. The new shoots originated from the old

rhizome are of the same size as pre-flowering ones and hence harvesting can be continued without much gap. *D. stocksii* is found in cultivation in the laterite soils of Konkan areas of southern India. Establishment of large-scale plantations with the rhizomes/culm cuttings of clumps that are capable of rejuvenating and intensive management of such plantation will provide raw material for long period. In *O. travancorica* all the flowered culms dried after profuse seed formation. New shoot formation was not observed from the flowered clumps. Profuse natural regeneration was found in the flowered locations. In short, both the species survived after flowering. In *D. stocksii* it was through rejuvenation after flowering and in *O. travancorica* through seedling population.

Biochemical changes during flowering and post-flowering stages

There are limited observations on the bio-chemical changes occurring in bamboo during post-flowering stages. Observations on *D. strictus* showed that death after flowering is due to excessive deprivation of reducing sugars and moisture content leading to loss in vitality and osmotic shock along with toxicity generated due to enormous increase in lignin content (Garg *et al*., 1998). There are only a few species that are reverting to vegetative phase after flowering (Ramanayake and Yakandawala, 1998; Beena *et al.,* 2007; Seethalakshmi *et al*., 2010a). In species that are reverting to vegetative phase after flowering observations on biochemical changes are lacking. Due to simultaneous flowering of both the species some observations were made during this investigation. The chemical composition in bamboos is known to

vary according to the individual characteristics of the species, age and position of the culm, location and other related factors (Mac Donald, 1969; Liese, 1985; Bhat, 2005).

The percentage of variation of different parameters such as starch, reducing sugars, phenols, proteins and crude fat showed marked difference between two species (Table, 12).

Table 12. Comparison of starch, reducing sugar, total phenol, protein and fat during flowering and post flowering stages of *D. stocksii* and *O. travancorica*

No.	Parameters	Variation between flowering and post flowering stages (+/- in percentage)	
		D. stocksii	***O. travancorica***
1	Starch	- 53.978	- 81.320
2	Reducing sugars	- 21.475	- 97.407
3	Total Phenols	- 24.806	+ 48.878
4	Proteins	+ 13.531	- 25.600
5	Crude Fat	- 49.321	- 51.590

In starch, reducing sugars and crude fat reduction, occurred in both the species but the percentage of reduction was more in *O. travancorica*. The reduction of starch, reducing sugars and proteins was in the order of 83.3, 97.4 and 25.6 in *O. travancorica* while it was only 53.9, 21.4 and 13.5 in *D. stocksii*. Another interesting feature was the difference in pattern of utilization of phenol. While 24.8 per cent decrease was observed in *D. stocksii* about 48.8 per cent increase was observed in *O. travancorica*. Quantity of protein increased in *D. stocksii* while decrease up to 25 per cent was seen in *O. travancorica*. There was not much difference in crude fat. Since there is no seed setting,

consumption of food reserves is less and rhizome is likely to have sufficient nutrient reserves to produce new vegetative shoots in *D. stocksii*. Higher amount of food utilization for seed formation may be draining the nutrient reserves in *O. travancorica* which subsequently leads to the death of flowered clumps. Earlier report on *D. strictus*, a profuse seed setting and dying species also showed reduction in starch and reducing sugars and an increase in lignin content (Garg *et al.*, 1998). The variation with in species due to location is not significant.

Amino acid profile of flowered and rejuvenated culms (stages 1 and 6) in *D. stocksii* and the flowered and seed setting culms (stages 1 and 3) in *O. travancorica* was investigated. There was difference in the percentage of amino acids between species during flowering and post-flowering period (Table 13).

Table 13. Variation in amino acid profile during flowering and post-flowering

Amino acids	**Variation between flowering and post-flowering stages ***		**Amino acids**	**Variation between flowering and post-flowering stages ***	
	D. stocksii	***O. travancorica***		***D. stocksii***	***O. travancorica***
Tyrosine	+ 100	- 5	Asparagine	- 10.6	+ 2.2
Tryptophan	+ 77.8	- 71.8	Histidine	- 4.9	- 24.2
Arginine	+ 51.6	- 34.8	Alanine	- 6.5	- 13.6
Proline	+ 11.5	- 12.6	Serine	- 15.2	- 0.6
Valine	+ 6.5	- 12.4	Leucine	- 14.5	- 3.1
Glutamine	+ 5.8	- 4.2	Glycine	- 12.7	- 5.4
Lysine	- 100	- 76.0	Threonine	- 9.5	- 17.4
Phenylalanine	- 66	- 16.7	Iso leucine	- 6.3	- 3.4

* + / - in percentage

All the amino acids except asparagine showed a decrease in the level in *O. travancorica* during post flowering stage. Lysine and tryptophan showed maximum decrease followed by arginine. The difference in histidine, phenyl

alanine, proline, valine, threonine and alanine was between 10 and 25 per cent. Leucine, isoleucine, glutamine, tyrosine and glycine showed small variation from 3 to 10 percentage. The amino acid profile of *D. stocksii* was different from that of *O. travancorica*. Tyrosine appeared only during post-flowering phase. Considerable increase was seen in the levels of tryptophan and arginine. Other three amino acids viz., proline, valine and glutamine also increased in small levels varying from six to twelve per cent. All other amino acids showed decrease, lysine was completely absent during post-flowering phase. Cystein and methionine was totally absent in both species.

The role of amino acids in synthesis of protein, growth regulating substances, enzymes etc. is well established. L - tryptophan is the precursor for auxin synthesis. The increase in the levels of tryptophan in *D. stocksii* and decrease in *O. travancorica* clearly indicated that tryptophan synthesis occurred during rejuvenation. Similarly L - arginine induces synthesis of flower and fruit related hormones and 34 per cent of it are used up during seed formation while synthesis occurred during rejuvenation. The role of L - proline in fertility of pollen is well established. L - proline & hydroxy proline act mainly on the hydric balance of the plant strengthening the cellular walls in such a way that they increase resistance to unfavorable climatic conditions. L - lysine, L - glutamic acid increase the pollen germination and growth of pollen tubes. Glycine and glutamic acid are fundamental metabolites in the process of formation of vegetable tissue and chlorophyll synthesis and are also known to be very effective chelating agents. L - alanine, L - valine & L -

leucine improves quality of fruits. L - histidine helps in proper ripening of fruits. Indications from the observations of amino acid profile of two species are that the synthesis of amino acids which facilitates vegetative growth occur in *D. stocksii* and more utilization of amino acids that are in involved in fruit formation in *O. travancorica.* This appears to be the pioneering study comparing the amino acid profile of a flowering, sterile and rejuvenating and flowering seed setting and dying bamboo species. More observations from other species are required to make definite conclusions about the role of amino acids in bamboos with different post-flowering behaviour.

Variations in some of the fatty acids were seen between flowering and post-flowering stages in both the species (Table 14).

The level of eight fatty acids viz., C14 myristic acid, C15 pentadecanoic acid, C16 palmitic acid, C16:1 palmittoleic acid C17 heptadecanoic acid, C18 stearic acid, C18:1n-9c oleic acid, C20:5n-3 cis-5,8,11,14,17-eicosapentaenoic acid increased during post flowering phase of *D. stocksii.* Only three fatty acids, C18 stearic acid, C20 archidic acid and C22 behenic acid increased in *O. travancorica*.

Table 14. Variation in fatty acid profile during flowering and post-flowering phase in *D. stocksii* and *O. travancorica*.

No.	Fatty acid	Variation between flowering and post-flowering stages *	
		D. stocksii	***O. travancorica***
1.	C10 capric acid	Nil	Nil
2.	C11 undecanoic acid	- 100	- 100
3.	C12 lauric acid	- 34	- 55.6
4.	C13 tridecanoic acid	- 34	- 56
5.	C14 myristic acid	+ 72	- 50
6.	C15 pentadecanoic acid	+ 58.6	- 100
7.	C15:1 cis-10-pentadecanoic acid	- 69.6	- 100
8.	C16 palmitic acid	+ 5.6	- 7.5
9.	C16:1 palmitoleic acid	+ 10.5	- 100
10.	C17 heptadecanoic acid	+ 83.6	-100
11.	C18 stearic acid	+ 60.9	+ 4.1
12.	C18:1n-9c oleic acid	+ 83	- 65.8
13.	C18:2n-6t linolelaidic acid	- 28.9	+ 8
14.	C18:3n-3 linolenic acid	- 41	- 100
15.	C20 archidic acid	- 19.1	+ 3.3
16.	C20:1cis-11-eichosenoic acid	Nil	Nil
17.	C20:5n-3 cis-5,8,11,14,17-eicosapentaenoic acid	+ 67.7	-100
18.	C22 behenic acid	- 100	+ 6.4
19.	C22:2cis-13, 16-docosadienoic acid	- 86	-52.4
20.	C24 lignoceric acid	- 43.7	-38.9

* +/- in percentage

Very long chain fatty acids (VLCFAs) are essential components for eukaryotes. They are elongated by the elongase complex in the endoplasmic reticulum and are incorporated into four major lipid pools (triacylglycerols, waxes, phospholipids, complex sphingolipids). Modifications of the nature and levels of VLCFAs in waxes, phospholipids and complex sphingolipids

have effects on embryo, leaf, root and flower development. VLCFAs phospholipids and sphingolipids are not only involved in membrane structure and dynamics regulating cell size but also in division and differentiation (Bach and Faure, 2010). An in-depth study is required to find out the contribution of fatty acids during flowering and seed set in plants.

There was difference in the level of fat and water soluble vitamins in both the species. Of the four fat soluble vitamins, two Vitamins A and E reduced in both species although the percentage of reduction was different (Table 15).

Table 15. Variation in Vitamins during flowering and post-flowering

1. Fat soluble	**Variation between flowering and post-flowering stages ***		**Water soluble continued**	**Variation between flowering and post-flowering stages ***	
	D. stocksii	***O. travancorica***		***D. stocksii***	***O. travancorica***
Vitamin A	- 62.50	-97.90	Thiamine	- 24.3	- 72.78
Vitamin D	- 11.70	+3.70	Pyridoxine	- 51	-73.9
Vitamin E	- 80.30	-57.70	Folic acid	- 15	-15.6
Vitamin K	+ 97.50	-61.60	Riboflavin	- 31.7	-8.9
II. Water soluble			Pantothenic acid	- 18.9	-16.7
Ascorbic acid	- 25.4	-89.9	* + / - in percentage		
Nicotinicacid	- 81.7	-41.8			

In the levels of Vitamin D, reduction was found in *D. stocksii* while slight increase was recorded in *O. travancorica.* Maximum variation was in the case of Vitamin K. It increased up to 97.5 per cent in rejuvenated culms of *D. stocksi,* whereas, reduction up to 61.6 per cent was recorded in *O. travancorica.*

Of the water soluble vitamins, ascorbic acid content was negligible in both species. In the other six water soluble vitamins decrease was observed for five in both the species (Table 15). The pattern of utilization of pyridoxine and

thiamine was different. Pyridoxine, one of the six specific compounds of Vitamin B6 **(**pyridoxine, pyridoxal, pyridoxamine, and their respective phosphates), serves as coenzyme and is involved in the metabolism of protein and carbohydrates. Their phosphates are the most active components in numerous reactions involving amino acid and protein metabolism. Although its role is well established in animals, the biosynthesis pathway and the function in plants are not well elucidated. Investigation with PDX1 gene from *Arabidopsis* showed that the gene expressed in all plant parts examined and its expression level was not significantly regulated by abiotic stress or the phytohormone abscisic acid. The PDX1 protein was mainly associated with the plasma membrane and endomembranes, implying a potential involvement of vitamin B6 in membrane function. Further, pdx1 mutants are hypersensitive to osmotic and oxidative stress suggesting that vitamin B6 may represent a new class of antioxidant in plants (Chen and Xiong, 2005). The decrease of pyridoxine in the rejuvenating species *D. stocksii* and decrease in the other may be due to its involvement in metabolism of protein and carbohydrates. Thiamine or vitamin B-1, is believed to induce systemic acquired resistance (SAR) in plants. Korean researchers found that thiamine-treated rice and other vegetable crop plants showed increased resistance to fungal, bacterial and viral infections. Thiamine also activates the SAR-related gene in tomato, tobacco and cucumber plants (Pyung Ahn *et al*., 2005) The decrease of thiamine in *O. travancorica* may be an attempt of the plant to

increase the resistance to infections with micro-organism during death after seed set.

To sum up, the observations made on starch, reducing sugars, proteins, phenols and crude fat clearly indicate the utilization of more food reserves in seed forming *O. travancorica.* The difference in the amino acid profile suggested that the metabolism may be different in both the species. The limited observations made on fatty acids and vitamins are insufficient to make any definite conclusion about the role played by these components during the post flowering phase. More information from species that show similar post-flowering behaviour is required. At present in bamboos, opportunities for such studies are scarce since populations with known age and known flowering cycle are only a few. Only if flowering could be predicted at least one-year in advance preparations for in-depth study can be made.

CHAPTER - IX

SUMMARY

1. Flowering, reproductive biology, seed set and post-flowering behaviour of two bamboo species viz., *Dendrocalamus stocksii* and *Ochlandra travancorica* was studied.

2. Since the two species showed two types of post-flowering behaviour, biochemical changes associated with post flowering stages were also observed.

3. In *D. stocksii* flowering was observed during 2003-2006 in northern Kerala, all the flowered culms died, formation of new vegetative shoots was observed from the rhizome indicating that this species has the rare capacity to rejuvenate from rhizome after flowering.

4. In *O. travancorica* flowering was observed during 2004-2006 in southern Kerala, flowered clumps produced large quantity of seeds and died.

5. In both species flowering occurred only in some of the populations indicating that there are different flowering cohorts for the same species.

At least five flowering cohorts were observed for *O. travancorica* and two flowering cohorts were seen for *D. stocksii.*

6. The data available on flowering and the current observations were insufficient to find out the correct flowering cycle of both species. However the observations on clumps of *O. travancorica* originated from the flowering of 1984 revealed that flowering cycle is more than 27 years and the present report of 7 years are not correct.

7. Observations on reproductive biology showed that both species are dichogamous with protogyny. The stigma matured four to five days before androecium. Seed set was observed only in *O. travancorica*.

8. There was difference in the reproductive structures like size and number of spikelets, anthers etc. between species. Difference was also observed in the quantity and quality of pollen produced, pollen viability *in vivo* and *in vitro*, receptivity of stigma in both species.

9. Of the two species, *O. travancorica* produced better quality and quantity of pollen, viability was nearly 99 per cent, longevity was more than 30 minutes and the seed setting was very high. Seeds were viviparous, no

dormancy and good natural regeneration was found. Monoporate pollen was observed in both species.

10. Mode of pollination was anemophilous in both species. Although insect visit was observed in *O. travancorica* direct role in pollination could not be ascertained. Cross pollination was observed. Attempts for hybridization between the two species was not successful.

11. All the flowered clumps of *O. travancorica* dried after flowering and seed set, while flowered culms in *D. stocksii* dried after flowering without seed set. From fourth year onwards the new shoots originated from the rhizome were vegetative indicating that the species has the capacity to revert to vegetative phase.

12. Comparison of starch, reducing sugars, phenols, protein and crude fat indicated that there is higher utilization of starch, reducing sugars and crude fat in *O. travancorica.* Increase in total phenol was also found while increase in protein was seen in *D. stocksii.*

13. Analysis of amino acids in two species during flowering and post-flowering stages indicated that there is different in the profile. Most of the amino acid except asparagine was less in post-flowering phase in *O.*

travancorica. At the same time level of five amino acids increased in *D. stocksii.*

14. The difference in the profile of fatty acids and vitamins was also evident in two stages in both the species. There was difference between species also. Most of the fatty acids except three were used up by *O. travancorica* during seed formation. Vitamin K was increasing in D. stocksii. Among the water soluble vitamins the difference was found in the pattern of utilization of thiamine and pyridoxine.

15. From the observations, the sterility in *D. stocksii* could be attributed to the less quantity of pollen produced, viability of pollen, percentage of anthesis, short receptivity of stigma etc. Although there are some indications from the bio-chemical observations regarding the differential utilization of food reserves in two types of post-flowering behaviour, definite conclusions regarding different components such as amino acids, fatty acids and vitamins could not be drawn.

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APPENDIX- I

ANOVA of Starch

Source	Degrees of freedom	Mean sum of squares
Location	2	1.01 ns
Species	1	1242.91*
Location x Species	2	11.07 ns
Flowering stage within species	8	152.51**
Culm within flowering stage x Species	20	0.24 ns
Error	236	0.139
Total	269	

*** Significant at 1 % level; * significant at 5 % level; ns non significant*

APPENDIX II

ANOVA of Reducing Sugar

Source	Degrees of freedom	Mean sum of squares
Location	2	0.1620 ns
Species	1	80.1587 ns
Location x Species	2	0.0986 ns
Flowering stage within species	8	10.4120**
Culm within flowering stage x Species	20	0.0460**
Error	236	0.0097
Total	269	

*** Significant at 1 % level; ns non significant*

APPENDIX III

ANOVA of Phenol

Source	Degrees of freedom	Mean sum of squares
Location	2	0.014 ns
Species	1	1.302 ns
Location x Species	2	0.031 ns
Flowering stage within species	2	0.277**
Error	28	0.002
Total	35	

*** Significant at 1 % level; ns non significant*

APPENDIX IV

Results of ANOVA of Protein

Source	Degrees of freedom	Mean sum of squares
Location	2	0.74 ns
Species	1	272.82**
Location x Species	2	2.77 ns
Flowering stage within species	8	2.004**
Culm within flowering stage x Species	20	0.09 ns
Error	236	0.104
Total	269	

*** Significant at 1 % level; ns non significant*

APPENDIX V

ANOVA of Asparagine

Source	Degrees of freedom	Mean sum of squares
Location	2	26.818 ns
Species	1	20.210 ns
Location x Species	2	8.563 ns
Flowering stage within species	2	4.664**
Error	28	0.386
Total	35	

*** Significant at 1 % level; ns non significant*

APPENDIX VI

ANOVA of Threonine

Source	Degrees of freedom	Mean sum of squares
Location	2	0.308 ns
Species	1	0.024 ns
Location x Species	2	0.588 ns
Flowering stage within species	2	3.142**
Error	28	0.173
Total	35	

*** Significant at 1 % level; ns non significant*

APPENDIX VII

ANOVA of Serine

Source	Degrees of freedom	Mean sum of squares
Location	2	4.312 ns
Species	1	8.003 ns
Location x Species	2	2.446 ns
Flowering stage within species	2	6.022**
Error	28	0.420
Total	35	

*** Significant at 1 % level; ns non significant*

APPENDIX VIII

ANOVA of Glutamine

Source	Degrees of freedom	Mean sum of squares
Location	2	3.663 ns
Species	1	29.503*
Location x Species	2	9.030 ns
Flowering stage within species	2	1.435 ns
Error	28	1.370
Total	35	

** Significant at 5 % level; ns non significant*

APPENDIX IX

ANOVA of Proline

Source	Degrees of freedom	Mean sum of squares
Location	2	3.516*
Species	1	1.628 ns
Location x Species	2	1.202 ns
Flowering stage within species	2	0.176 ns
Error	28	0.213
Total	35	

** Significant at 5 % level; ns non significant*

APPENDIX X

ANOVA of Glycine

Source	Degrees of freedom	Mean sum of squares
Location	2	0.215 ns
Species	1	8.420 ns
Location x Species	2	0.327 ns
Flowering stage within species	2	5.540**
Error	28	0.065
Total	35	

*** Significant at 1 % level; ns non significant*

APPENDIX XI

ANOVA of Alanine

Source	Degrees of freedom	Mean sum of squares
Location	2	0.495 ns
Species	1	5.478 ns
Location x Species	2	4.083 ns
Flowering stage within species	2	5.359**
Error	28	0.071
Total	35	

*** Significant at 1 % level; ns non significant*

APPENDIX XII

ANOVA of Valine

Source	Degrees of freedom	Mean sum of squares
Location	2	3.955 ns
Species	1	34.327 ns
Location x Species	2	1.202 ns
Flowering stage within species	2	2.732**
Error	28	0.169
Total	35	

*** Significant at 1 % level; ns non significant*

APPENDIX XIII

ANOVA of Iso leucine

Source	Degrees of freedom	Mean sum of squares
Location	2	0.040 ns
Species	1	3.931*
Location x Species	2	2.079 ns
Flowering stage within species	2	0.215*
Error	28	0.064
Total	35	

** Significant at 5 % level; ns non significant*

APPENDIX XIV

ANOVA of Leucine

Source	Degrees of freedom	Mean sum of squares
Location	2	0.634 ns
Species	1	5.646 ns
Location x Species	2	2.327 ns
Flowering stage within species	2	4.172**
Error	28	0.113
Total	35	

*** Significant at 1 % level; ns non significant*

APPENDIX XV

ANOVA of Tyrosine

Source	Degrees of freedom	Mean sum of squares
Location	2	3.125 ns
Species	1	57.120 ns
Location x Species	2	6.482 ns
Flowering stage within species	2	23.411**
Error	28	0.666
Total	35	

*** Significant at 1 % level; ns non significant*

APPENDIX XVI

ANOVA of Phenylalanine

Source	Degrees of freedom	Mean sum of squares
Location	2	5.736 ns
Species	1	5.128 ns
Location x Species	2	1.936 ns
Flowering stage within species	2	22.415**
Error	28	0.442
Total	35	

*** Significant at 1 % level; ns non significant*

APPENDIX XVII

ANOVA of Histidine

Source	Degrees of freedom	Mean sum of squares
Location	2	0.116 ns
Species	1	10.472*
Location x Species	2	1.990 ns
Flowering stage within species	2	0.677*
Error	28	0.160
Total	35	

** Significant at 1 % level; ns non significant*

APPENDIX XVIII

ANOVA of Lysine

Source	Degrees of freedom	Mean sum of squares
Location	2	12.570 ns
Species	1	16.938 ns
Location x Species	2	27.252 ns
Flowering stage within species	2	16.115**
Error	28	0.779
Total	35	

*** Significant at 1 % level; ns non significant*

APPENDIX XIX

ANOVA of Arginine

Source	Degrees of freedom	Mean sum of squares
Location	2	0.885 ns
Species	1	49.515 ns
Location x Species	2	1.664 ns
Flowering stage within species	2	18.636**
Error	28	0.143
Total	35	

*** Significant at 1 % level; ns non significant*

APPENDIX XX

ANOVA of Tryptophan

Source	Degrees of freedom	Mean sum of squares
Location	2	2.364 ns
Species	1	0.249 ns
Location x Species	2	2.009 ns
Flowering stage within species	2	3.444**
Error	28	0.045
Total	35	

*** Significant at 1 % level; ns non significant*

APPENDIX XXI

ANOVA of Fat

Source	Degrees of freedom	Mean sum of squares
Location	2	0.010 ns
Species	1	0.650 ns
Location x Species	2	0.022 ns
Flowering stage within species	8	0.180**
Culm within flowering stage x Species	20	0.0006**
Error	236	0.0002
Total	269	

*** Significant at 1 % level; ns non significant*

APPENDIX XXII

ANOVA of C14 Myristic acid

Source	Degrees of freedom	Mean sum of squares
Location	2	20.789 ns
Species	1	1.567 ns
Location x Species	2	0.837 ns
Flowering stage within species	2	26.439**
Error	28	0.329
Total	35	

*** Significant at 1 % level; ns non significant*

APPENDIX XXIII

ANOVA of C15 Pentadecanoic acid

Source	Degrees of freedom	Mean sum of squares
Location	2	3.205 ns
Species	1	1.293 ns
Location x Species	2	5.997 ns
Flowering stage within species	2	9.738**
Error	28	0.752
Total	35	

*** Significant at 5 % level; ns non significant*

APPENDIX XXIV

ANOVA of C16 Palmitic acid

Source	Degrees of freedom	Mean sum of squares
Location	2	114.903 ns
Species	1	117.735 ns
Location x Species	2	366.447 ns
Flowering stage within species	2	17.815 ns
Error	28	6.146
Total	35	

ns non significant

APPENDIX XXV

ANOVA of C18 Stearic acid

Source	Degrees of freedom	Mean sum of squares
Location	2	7.365 ns
Species	1	68.118 ns
Location x Species	2	7.315 ns
Flowering stage within species	2	10.909**
Error	28	0.073
Total	35	

*** Significant at 5 % level; ns non significant*

APPENDIX XXVI

ANOVA of C20 Archidic acid

Source	Degrees of freedom	Mean sum of squares
Location	2	2.893*
Species	1	0.146 ns
Location x Species	2	1.007 ns
Flowering stage within species	2	0.080*
Error	28	0.021
Total	35	

** Significant at 5 % level; ns non significant*

APPENDIX XXVII

ANOVA of C24 Lignoceric acid

Source	Degrees of freedom	Mean sum of squares
Location	2	7.020 ns
Species	1	0.205 ns
Location x Species	2	0.532 ns
Flowering stage within species	2	4.174*
Error	28	1.072
Total	35	

** Significant at 5 % level; ns non significant*

APPENDIX XXVIII

ANOVA **of Vitamin A (IU/100g)**

Source	Degrees of freedom	Mean sum of squares
Location	2	1.661 ns
Species	1	7.778*
Location x Species	2	3.046 ns
Flowering stage within species	2	19.206**
Error	28	1.231
Total	35	

*** Significant at 1 % level; ns non significant*

APPENDIX XXIX

ANOVA of Vitamin D (IU/100g)

Source	Degrees of freedom	Mean sum of squares
Location	2	3397.511 ns
Species	1	40516.993*
Location x Species	2	30605.815*
Flowering stage within species	2	612.349**
Error	28	71.347
Total	35	

*** Significant at 1 % level; ns non significant*

APPENDIX XXX

ANOVA of Vitamin E (µg/100g)

Source	Degrees of freedom	Mean sum of squares
Location	2	1708.294 ns
Species	1	2875.223 ns
Location x Species	2	24.385 ns
Flowering stage within species	2	9280.601**
Error	28	104.896
Total	35	

*** Significant at 1 % level; ns non significant*

APPENDIX XXXI

ANOVA of Vitamin K (µg/100g)

Source	Degrees of freedom	Mean sum of squares
Location	2	345.651 ns
Species	1	3.349 ns
Location x Species	2	13.653 ns
Flowering stage within species	2	361.828**
Error	28	10.202
Total	35	

*** Significant at 1 % level; ns non significant*

APPENDIX XXXII

ANOVA of Nicotinic acid

Source	Degrees of freedom	Mean sum of squares
Location	2	0.012 ns
Species	1	0.0005 ns
Location x Species	2	0.019 ns
Flowering stage within species	2	0.016**
Error	28	0.0009
Total	35	

*** Significant at 1 % level; ns non significant*

APPENDIX XXXIII

ANOVA of Thiamine

Source	Degrees of freedom	Mean sum of squares
Location	2	34.807 ns
Species	1	74.976 ns
Location x Species	2	98.258 ns
Flowering stage within species	2	67.567**
Error	28	3.151
Total	35	

*** Significant at 1 % level; ns non significant*

APPENDIX XXXIV

ANOVA of Pyridoxine

Source	Degrees of freedom	Mean sum of squares
Location	2	33.261 ns
Species	1	328.133 ns
Location x Species	2	36.051 ns
Flowering stage within species	2	146.903**
Error	28	1.667
Total	35	

** Significant at 5 % level; ns non significant*

APPENDIX XXXV

ANOVA of Folic acid

Source	Degrees of freedom	Mean sum of squares
Location	2	5.985 ns
Species	1	0.134 ns
Location x Species	2	9.162 ns
Flowering stage within species	2	28.851**
Error	28	0.213
Total	35	

** Significant at 5 % level; ns non significant*

APPENDIX XXXVI

ANOVA of Riboflavin

Source	Degrees of freedom	Mean sum of squares
Location	2	0.294 ns
Species	1	140.318 ns
Location x Species	2	45.111 ns
Flowering stage within species	2	19.286**
Error	28	1.302
Total	35	

*** Significant at 1 % level; ns non significant*

APPENDIX XXXVII

ANOVA of Pantothenic acid

Source	Degrees of freedom	Mean sum of squares
Location	2	5.540 ns
Species	1	38.014*
Location x Species	2	6.406 ns
Flowering stage within species	2	1.989**
Error	28	0.212
Total	35	

*** Significant at 1 % level; ns non significant*

APPENDIX XXXVII

LIST OF PUBLICATIONS

1. **Beena. V.B**, Seethalakshmi. K.K and Raveendran.V.P. 2006. Flowering and Reproductive Biology of two endemic bamboo species of Western Ghats- *Dendrocalamus stocksii* and *Pseudoxytenanthera ritcheyi*. ***Journal of Bamboo and rattan.*** 6:1.

2. Seethalakshmi.K.K, Raveendran.V.P, Jijeesh.C.M and **Beena.V.B**. 2007. Recent Flowering of Different Bamboo species in Kerala. ***Evergreen*** 57 -58: 12-14.

3. Jijeesh.C.M, Seethalakshmi.K.K, **Beena.V.B** and Raveendran.V.P. 2009. Flowering and Reproductive Biology of an endemic bamboo-*Pseudoxytenanthera monadelpha* (Thw.) Soderstrom and Ellis. ***Phytomorpholgy***. 59: 35-39.

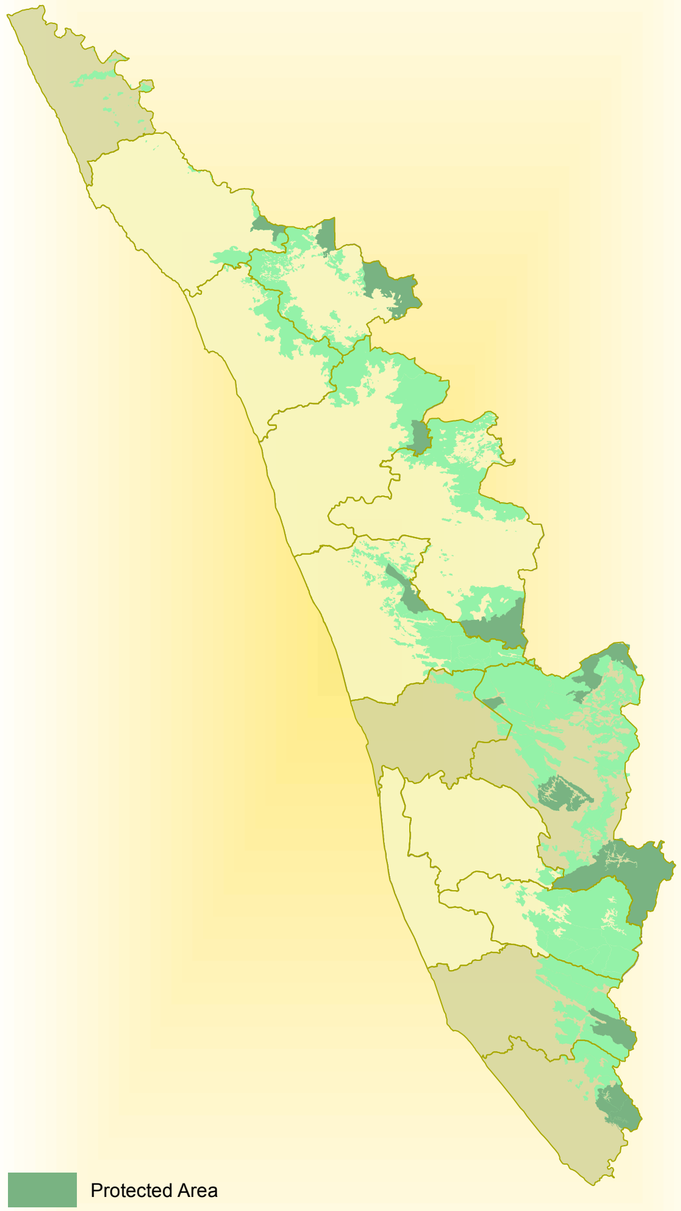
4. Seethalakshmi.K.K, Jijeesh. C.M, **Beena.V.B** and Raveendran.V.P. 2009. Flowering and regeneration of three endemic reed bamboos of Western Ghats- *Ochlandra travancorica, O.soderstromiana* and *O.spirostylis.* ***Bamboo Science and Culture.*** 22(1)32-39.

5 Seethalakshmi.K.K, Jijeesh. C.M, **Beena.V.B** and Raveendran.V.P. 2010. Flowering and post flowering reversion to vegetative phase of the giant bamboo- *Dendrocalamus giganteus* Wall. Ex Munro in Kerala. ***Journal of non-timber forest products*** 17 (1) 1-6.

6 Seethalakshmi.K.K, Jijeesh. C.M, **Beena.V.B** and Raveendran.V.P. 2010. Flowering and reproductive biology of selected bamboos of Kerala. In: National conference on "Developmental biology". NACON d D Bio 2010. Bangalore, September 15 to 17

7 **Beena, V.B.** and Seethalakshmi, K.K**.** 2010. Biochemical changes during and after flowering of Dendrocalamus stocksii and Ochlandra travacncorica. In: Proceedings of 22nd Kerla Science Congress (ed. EP. Yesodharan) Jointly organised by Kerala State Council for Science Technology and Environment, Kerala Forest Research Institute, Science and Technology Department, Govt. of Kerala and Department of Science and Technology & Department of Biotechnology, Govt of India, , 28-31January 2010, Peechi, Thrisur Kerala. 597-598

KERALA



Protected Area

Forest Area

Kilometers

0 60 120

KASARGODE

Chongla

poinachi

Periya

Cheruvathur

Kilometers

0 10 20

ERNAKULAM

Munnar

Neriyamangalam

IDUKKI

Kilometers

0 15 30

KOLLAM

Kulathupuzha

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TRIVANDRUM

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Dendrocalamus stocksii

Ochlandra travancorica

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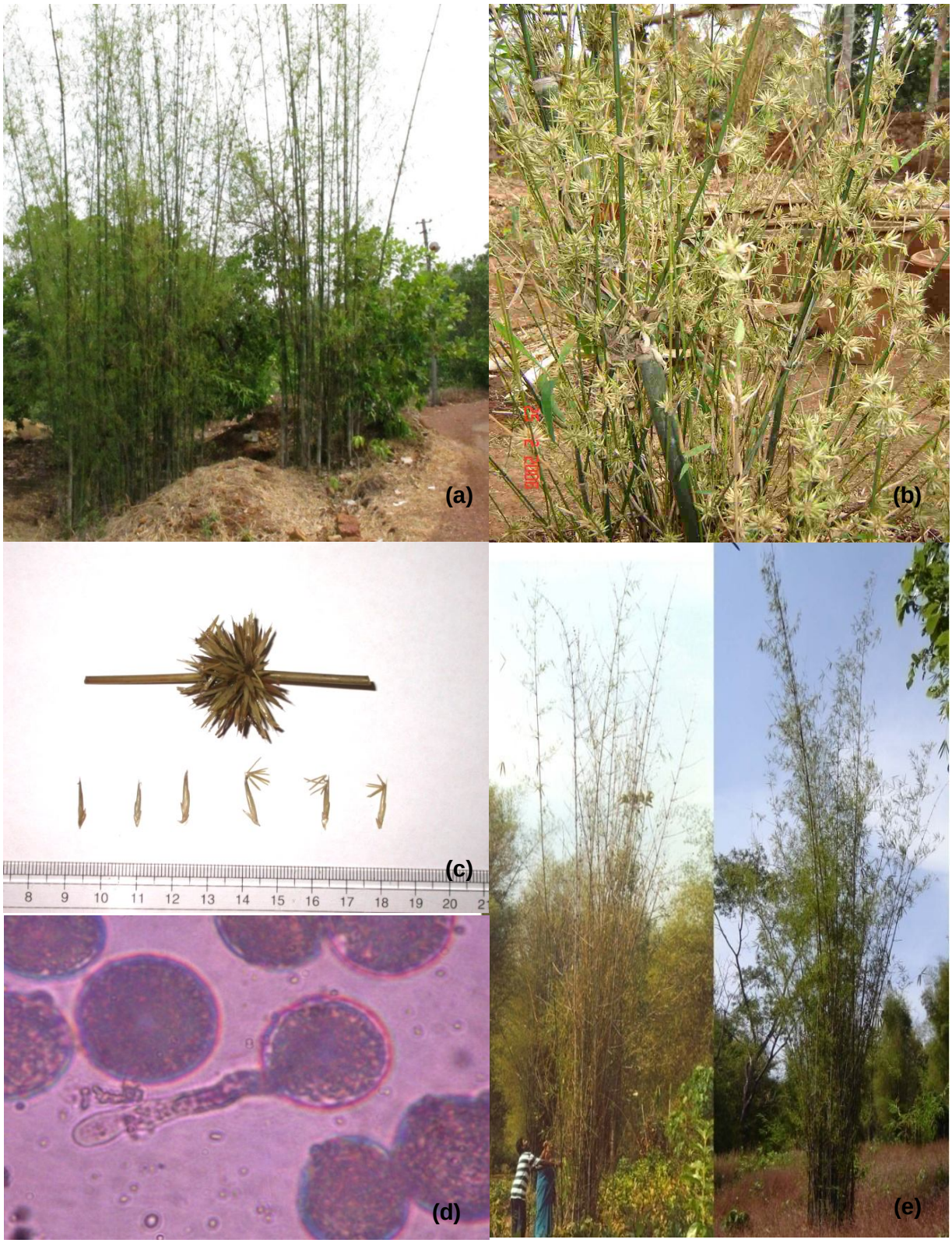
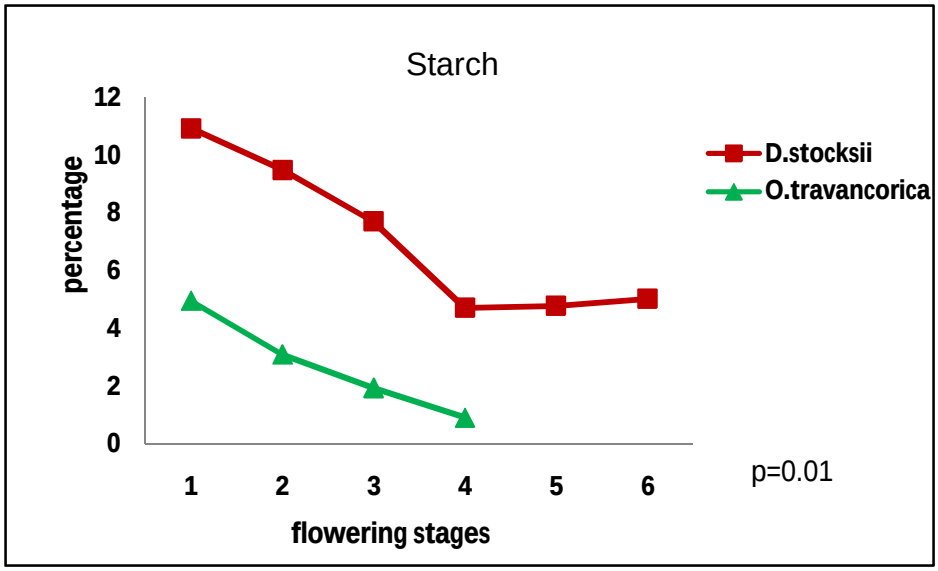
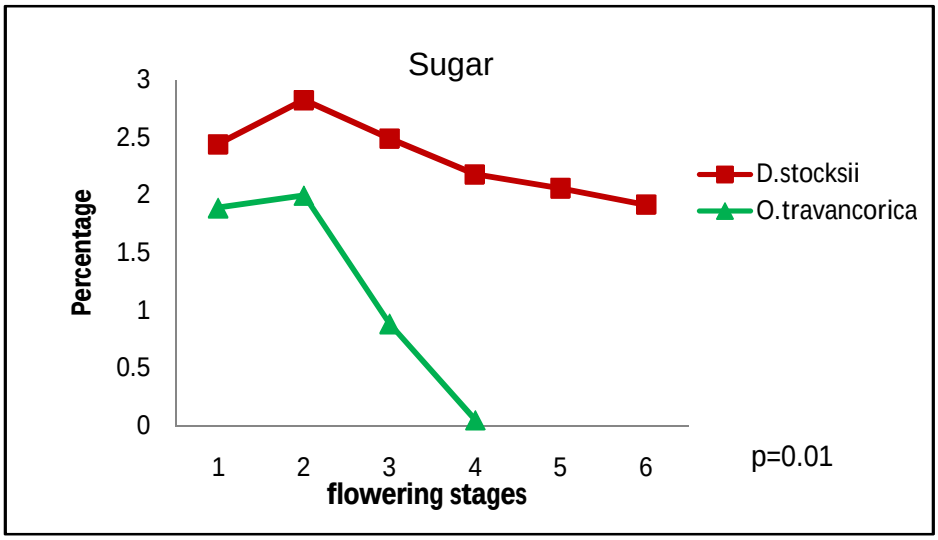


Figure 2 (a) Clumps of *D. stocksii*, (b) flowered culm cuttings in the nursery, (c) portion of inflorescence and florets, (d) germinated pollen grain *in vitro,* (e) flowered and rejuvenated clumps.

Figure 3 (a) Clump of *O. travancorica,* (b) portion of inflorescence showing flower with emerged out anthers, (c) flower with stigma, (d) insects visiting anthers (e) germinated pollen grains *in vitro.*





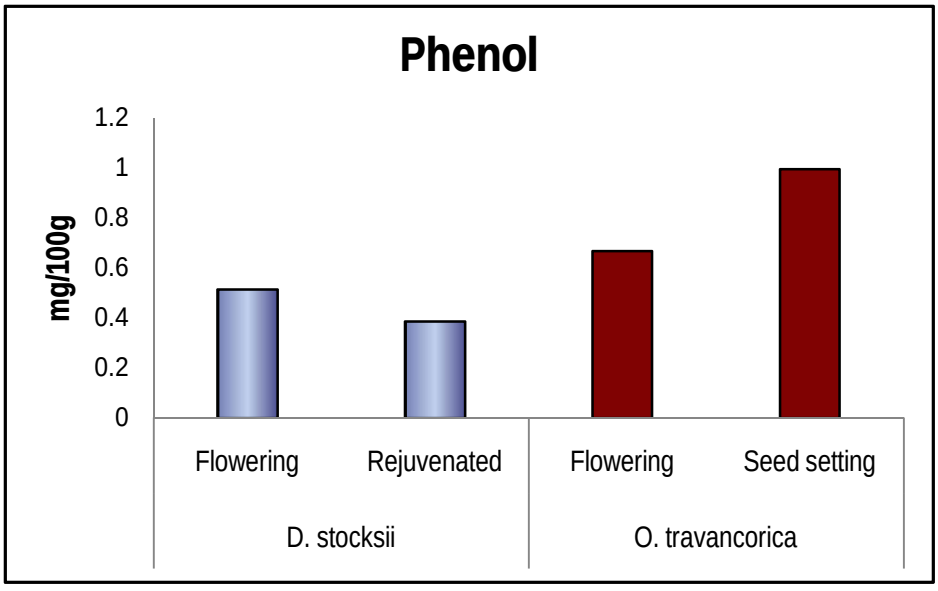
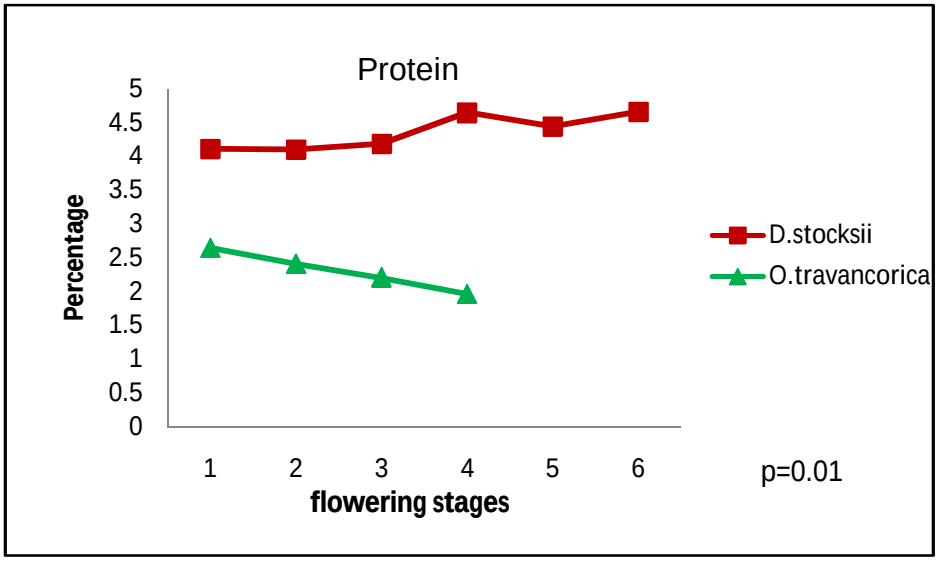
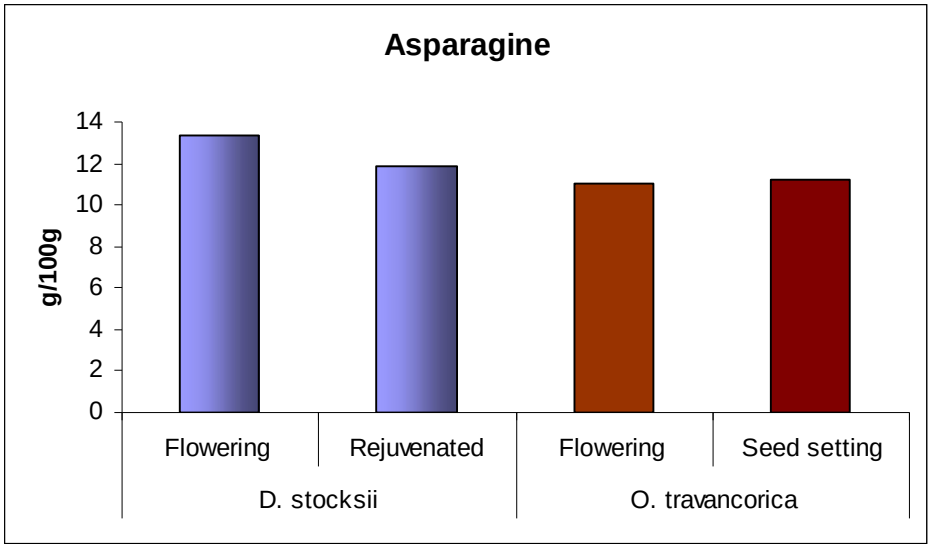


Figure 4.. Variation in starch, sugar and phenol during flowering and post flowering stages of *D. stocksii* and *O. travancorica*





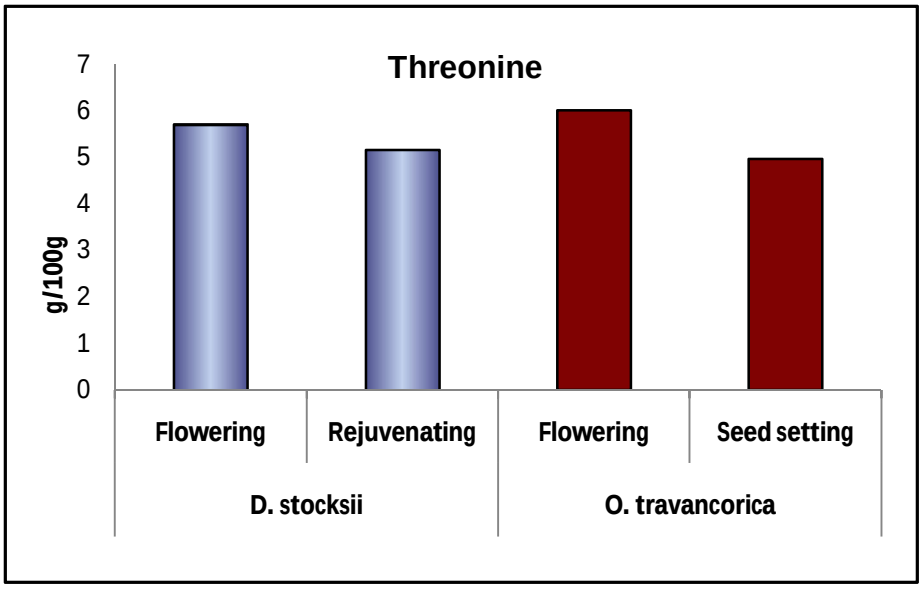
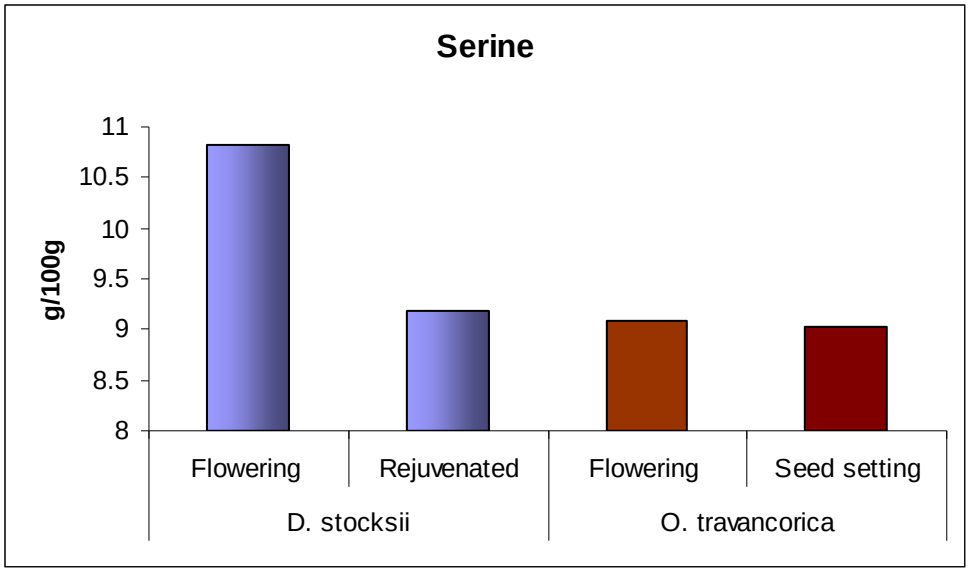
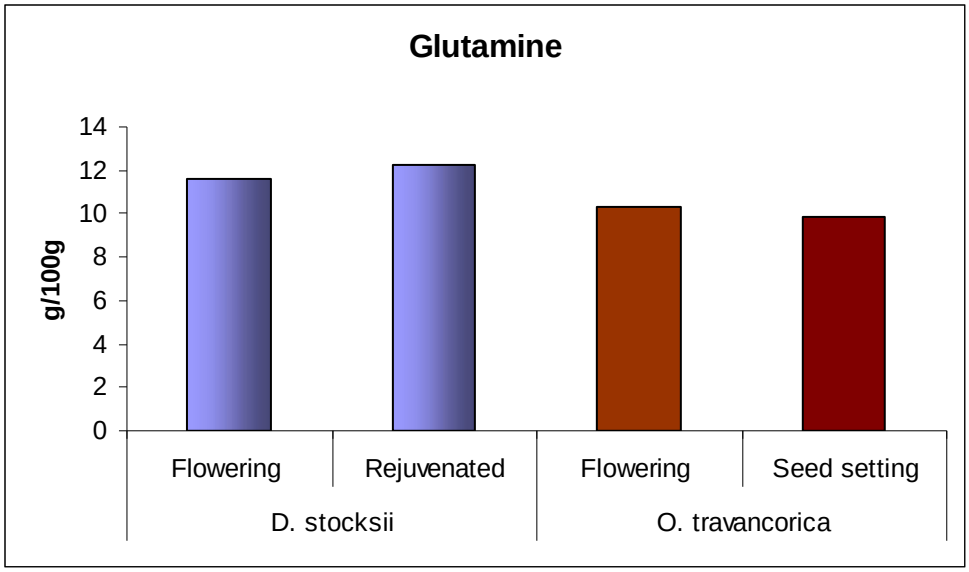


Figure 5. Variation in protein, asparagine and threonine during flowering and post flowering stages of *D. stocksii* and *O. travancorica*





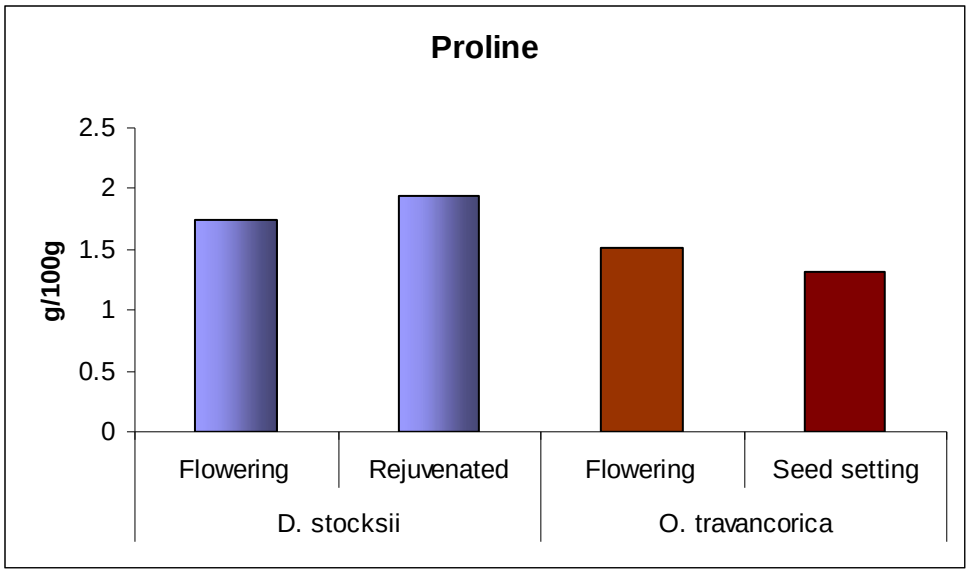


Figure 6. Variation in serine, glutamine and proline during flowering and post flowering stages of *D. stocksii* and *O. travancorica*

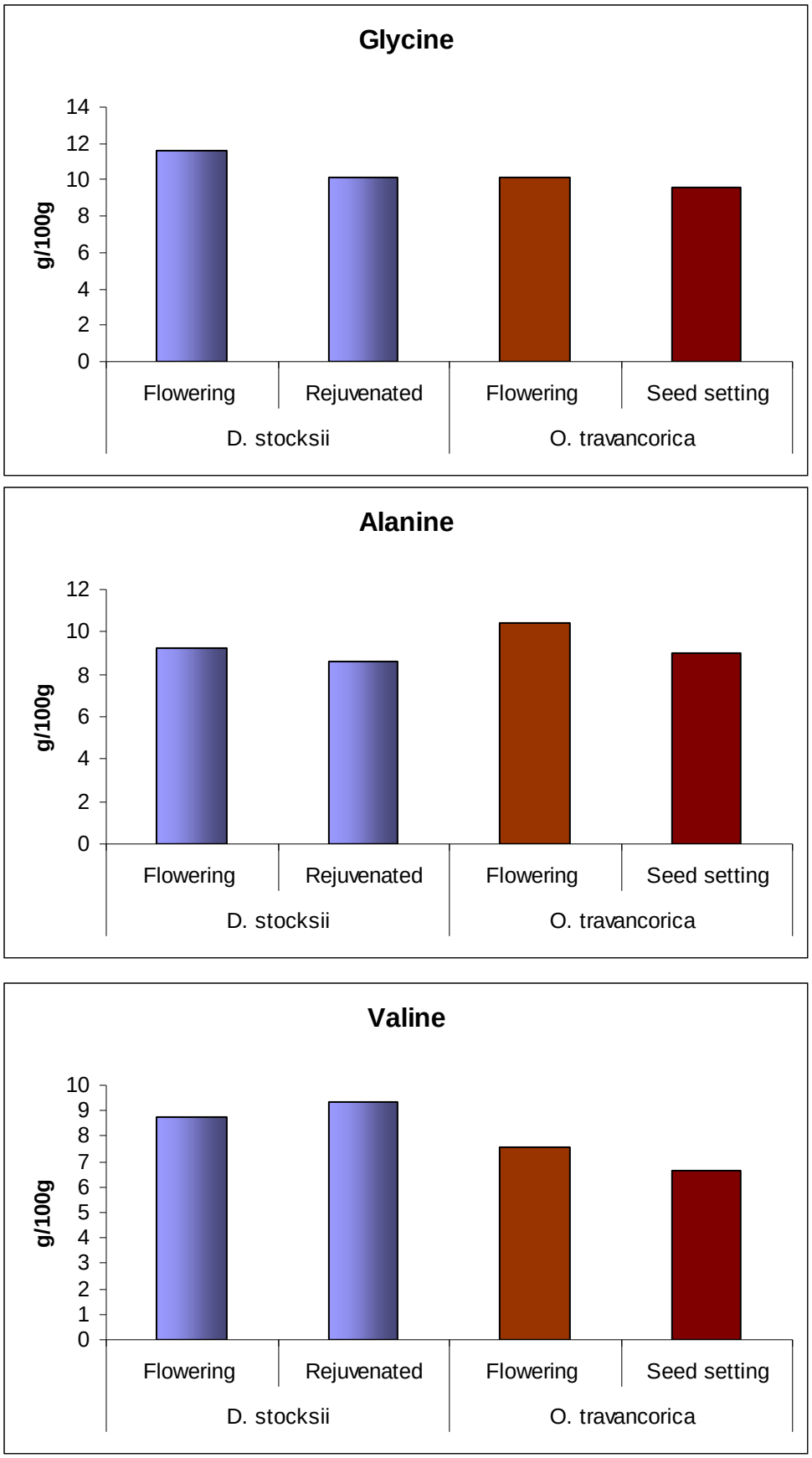
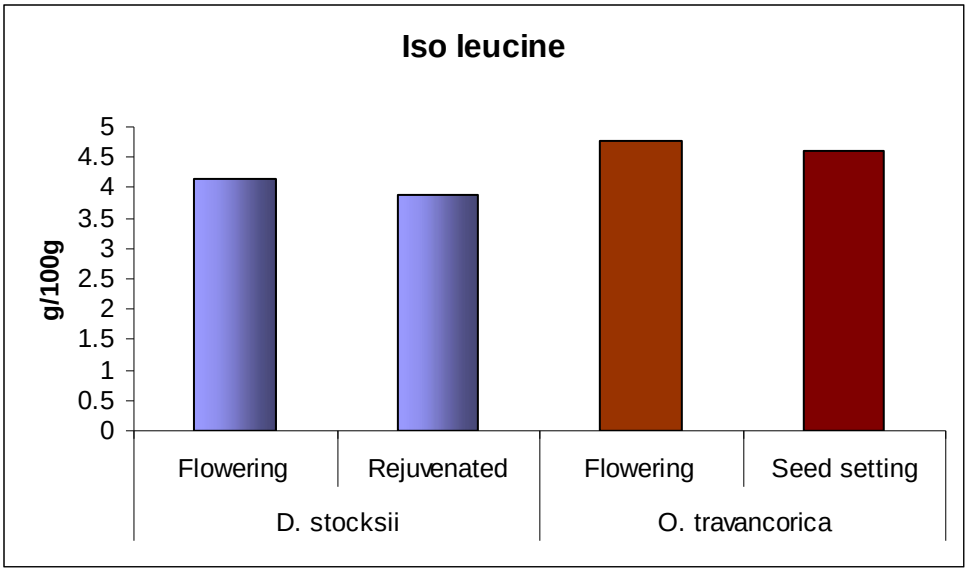
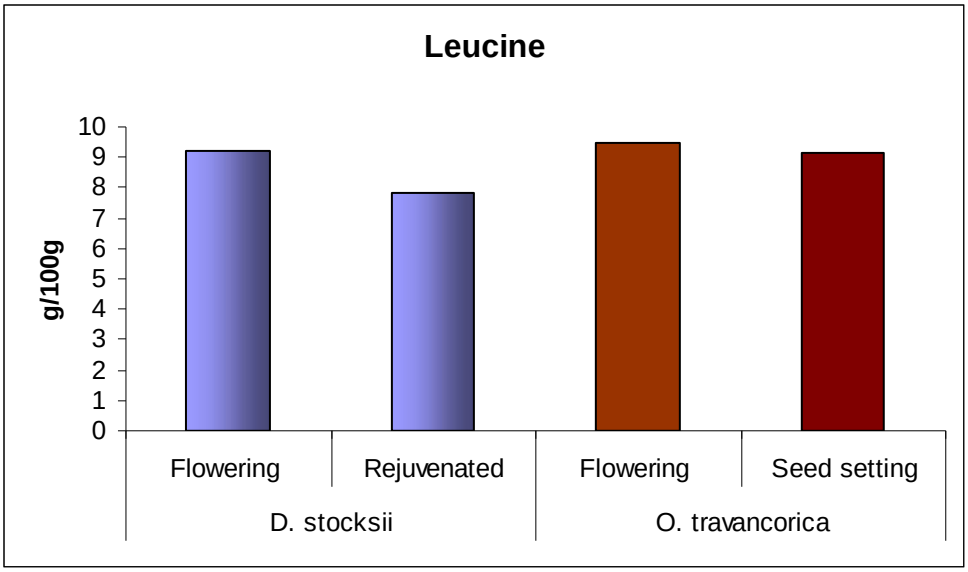


Figure 7. Variation in glycine, alanine and valine during flowering and post flowering stages of *D. stocksii* and *O. travancorica*





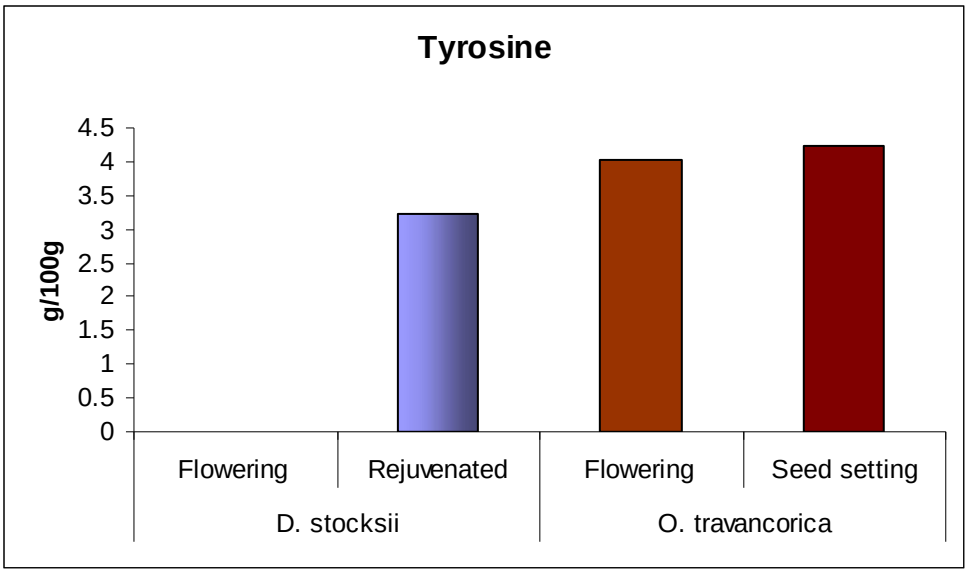
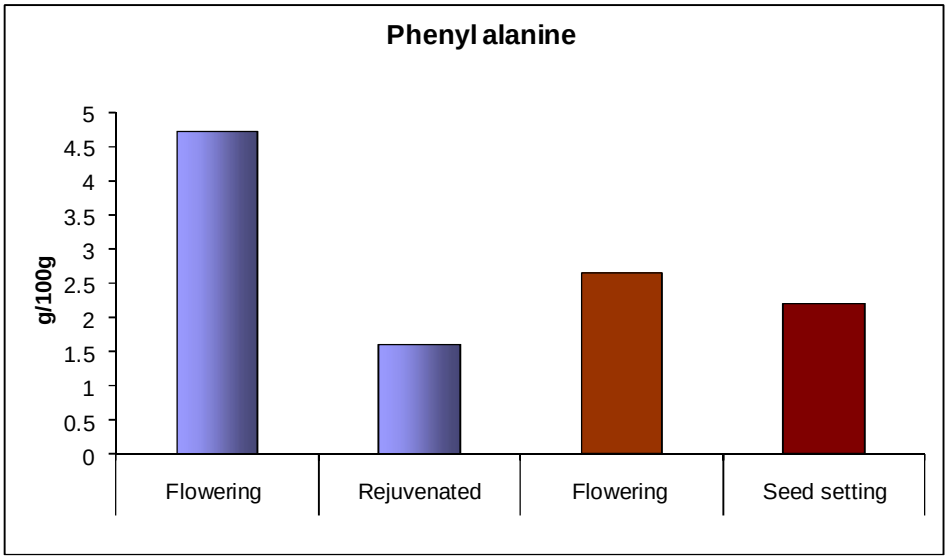
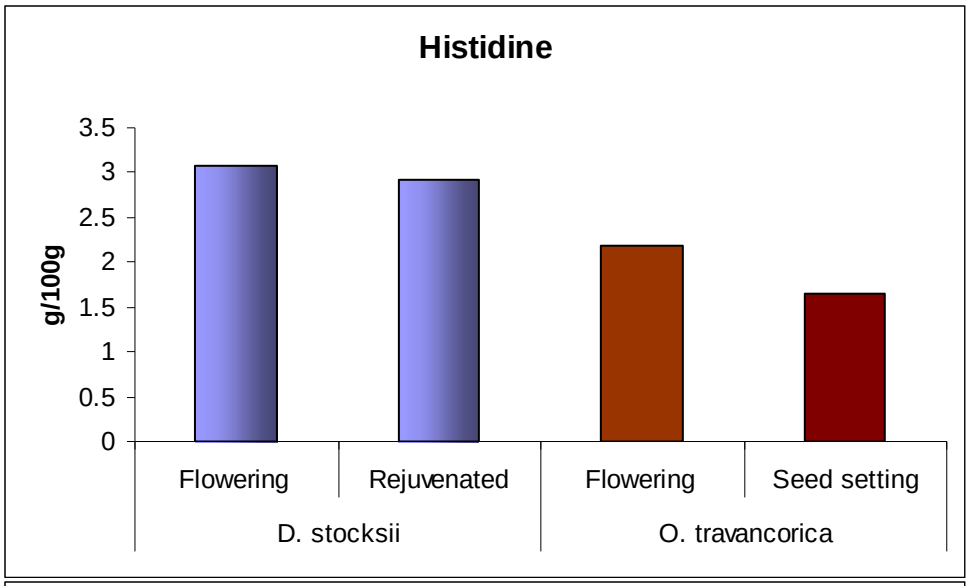


Figure 8. Variation in Iso leucine, leucine and tyrosine during flowering and post flowering stages of *D. stocksii* and *O. travancorica*





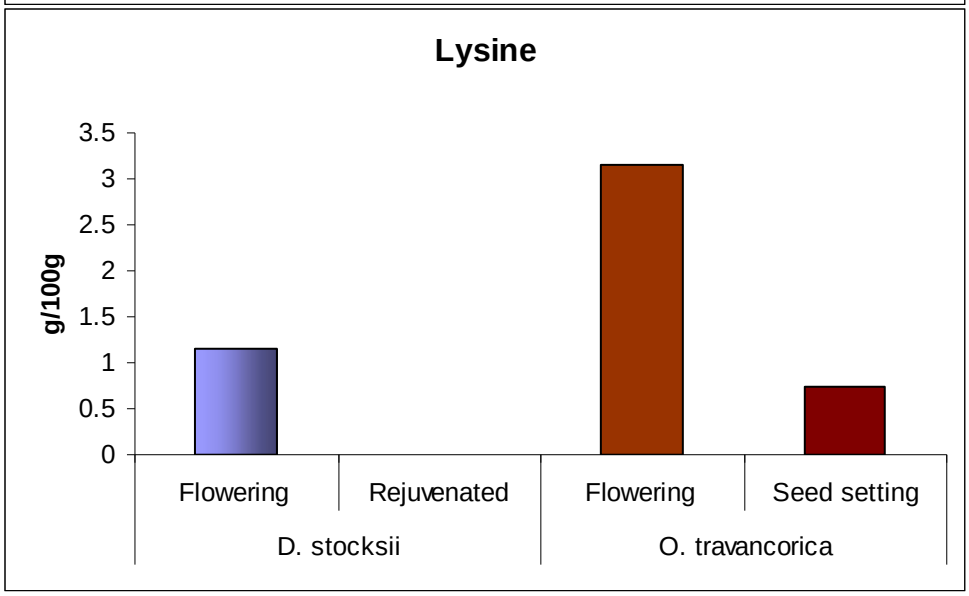


Figure 9. Variation in phenyl alanine, histidine and lysine during flowering and post flowering stages of *D. stocksii* and *O. travancorica*

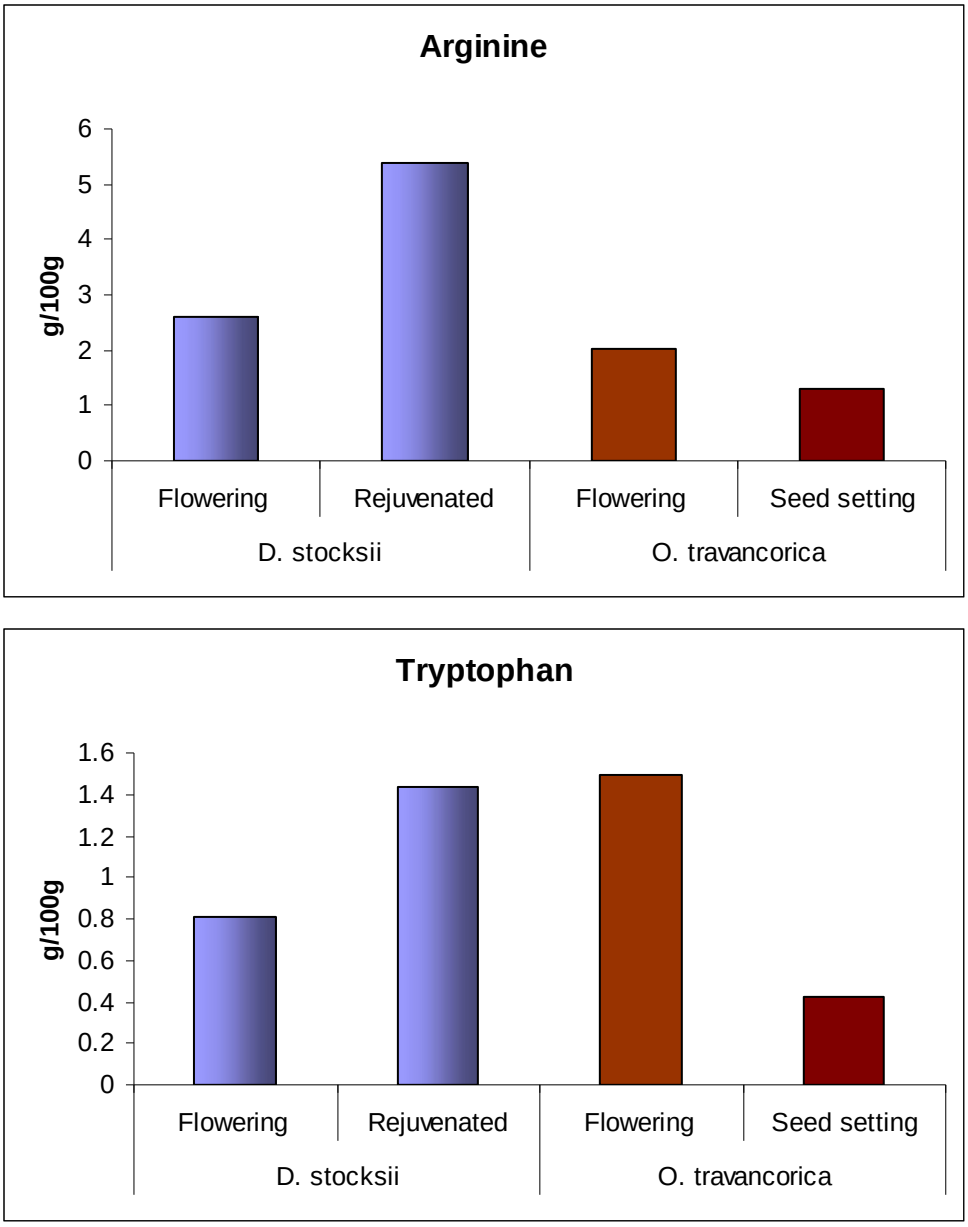
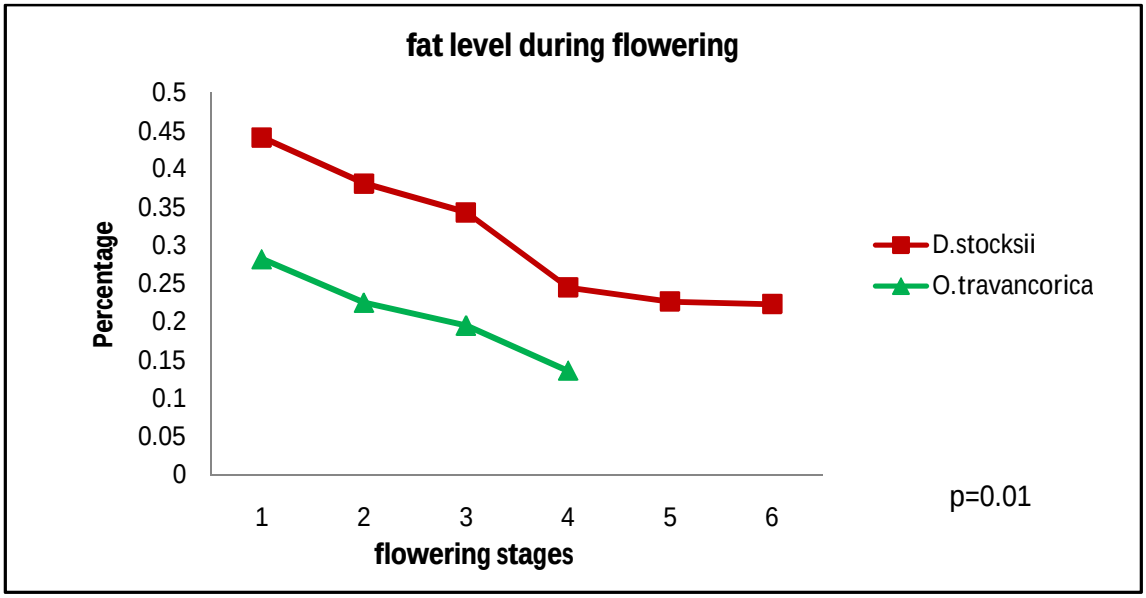
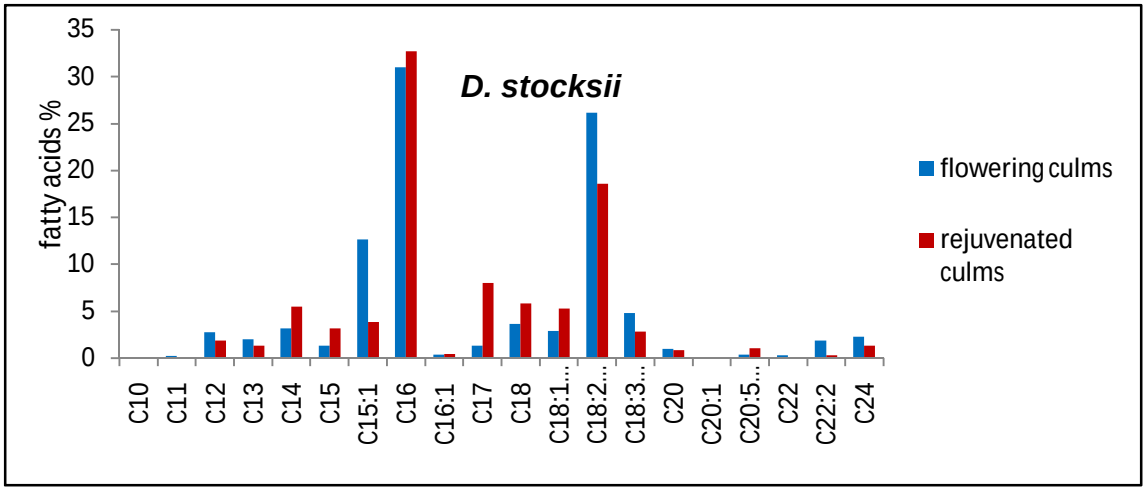


Figure 10. Variation in arginine and tryptophan during flowering and post flowering stages of *D. stocksii* and *O. travancorica*





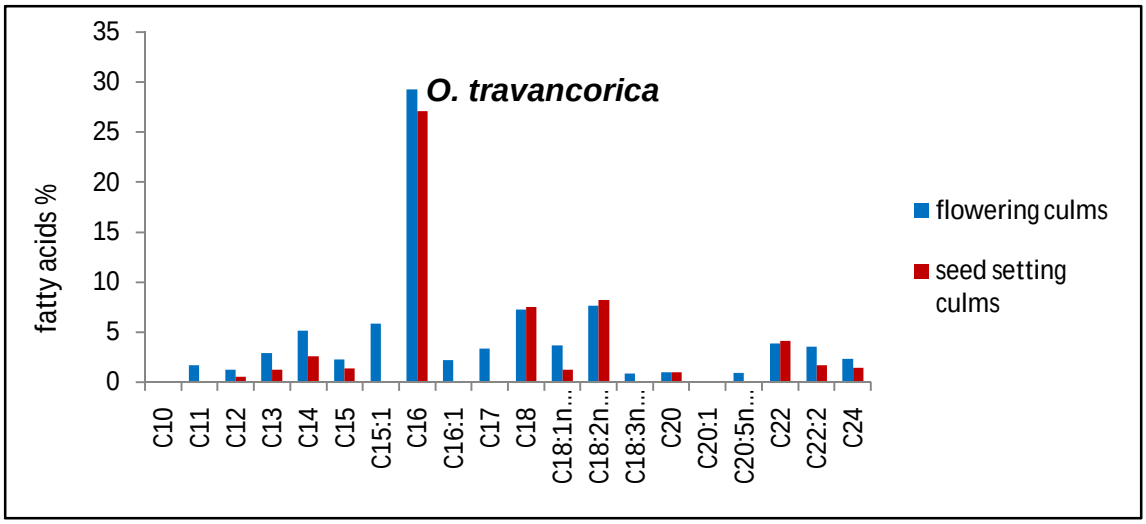
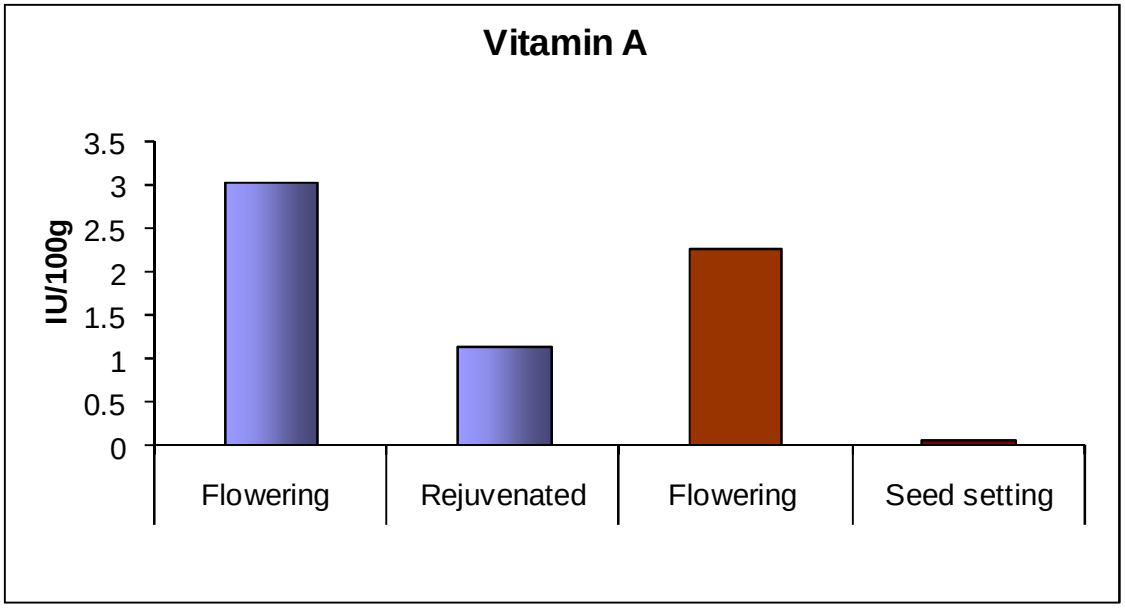


Figure 11. Variation in fat and fatty acids during flowering and post flowering stages of *D. stocksii* and *O. travancorica*



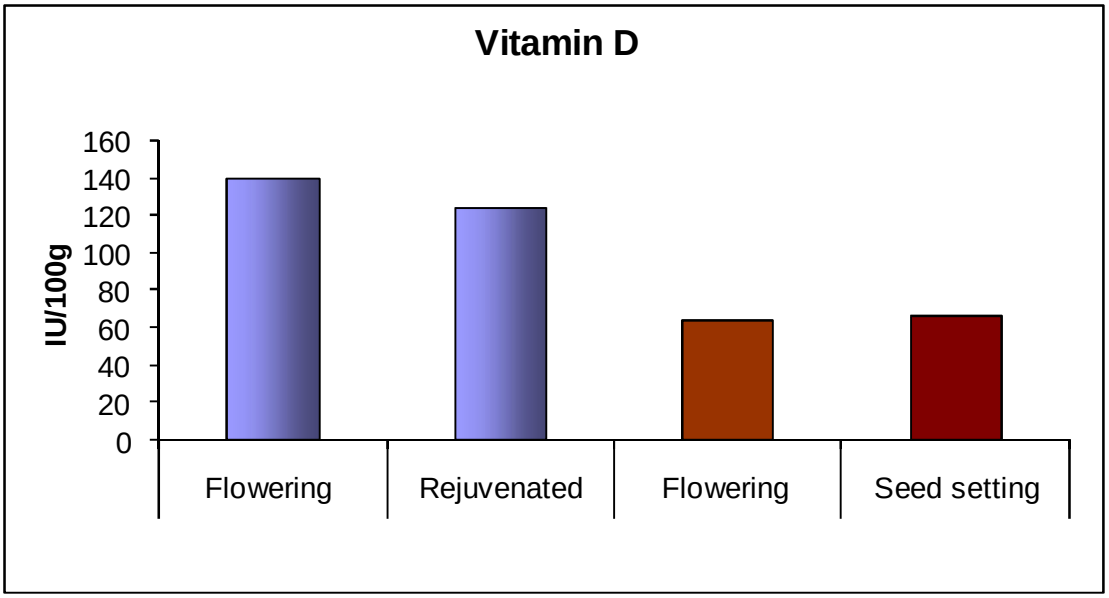
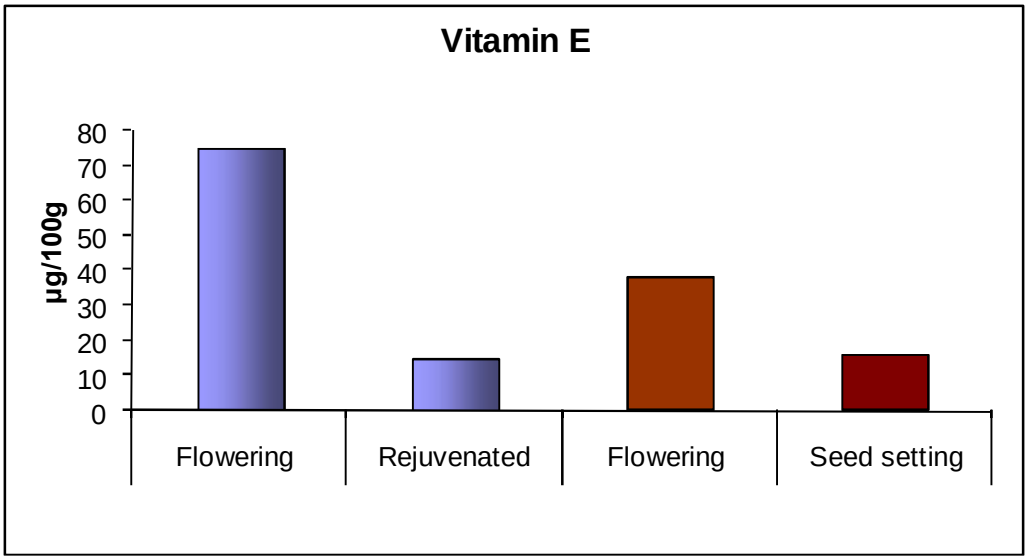


Figure 12. Variation in vitamin A and vitamin D during flowering and post flowering stages of *D. stocksii* and *O. travancorica*



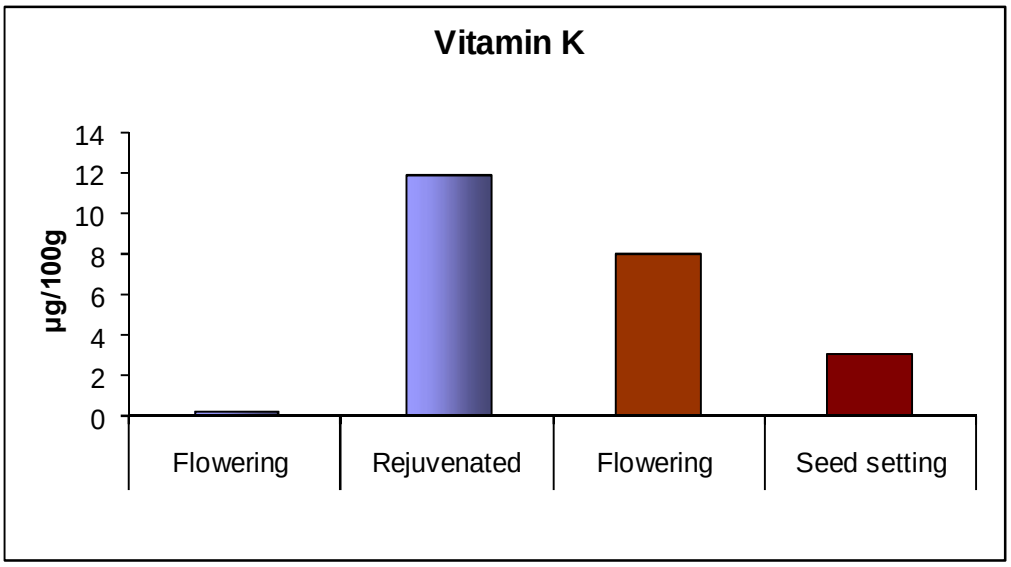


Figure 13. Variation in vitamin E and vitamin K during flowering and post flowering stages of *D. stocksii* and *O. travancorica*

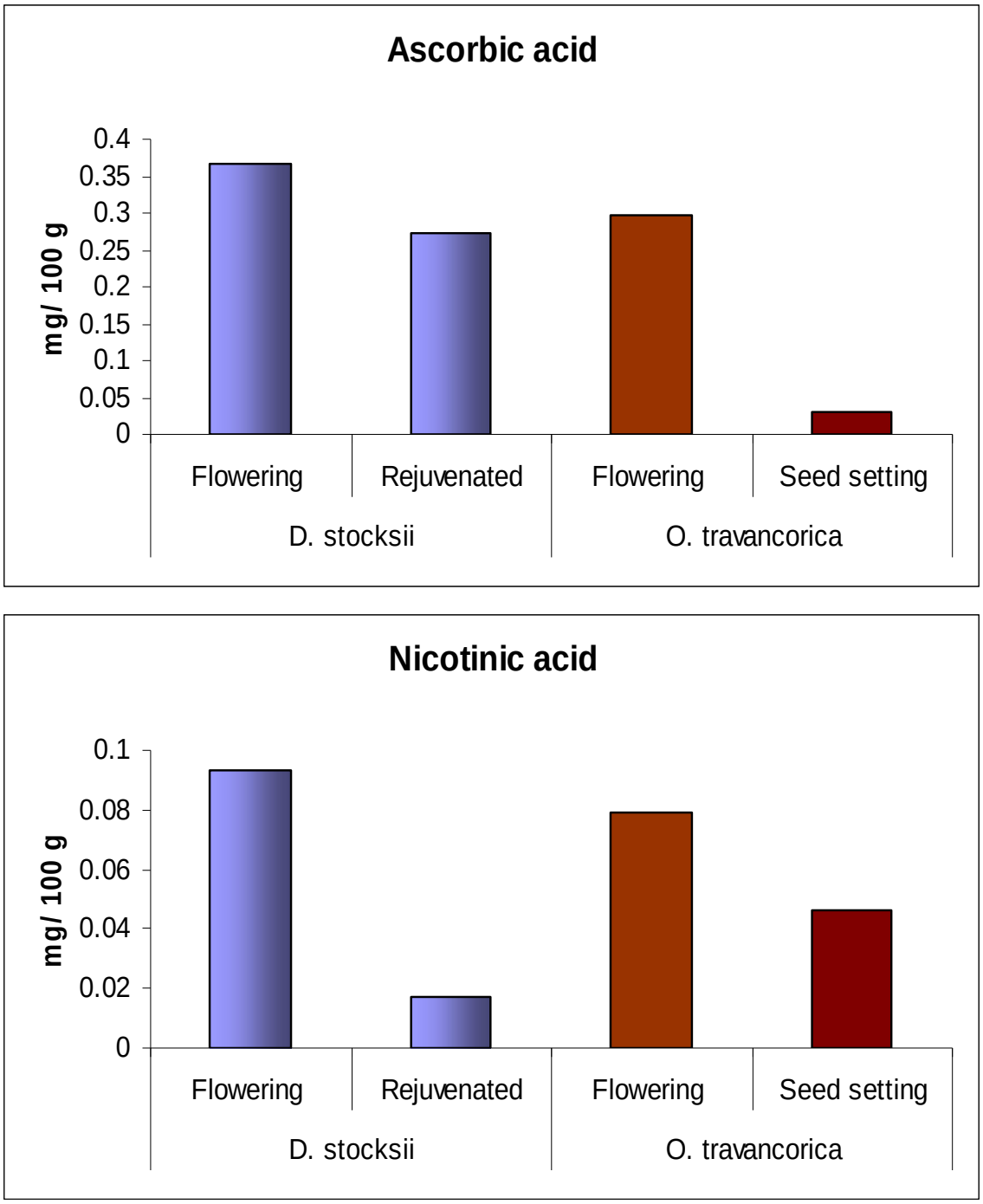


Figure 14. Variation in ascorbic acid and nicotinic acid during flowering and post flowering stages of *D. stocksii* and *O. travancorica*

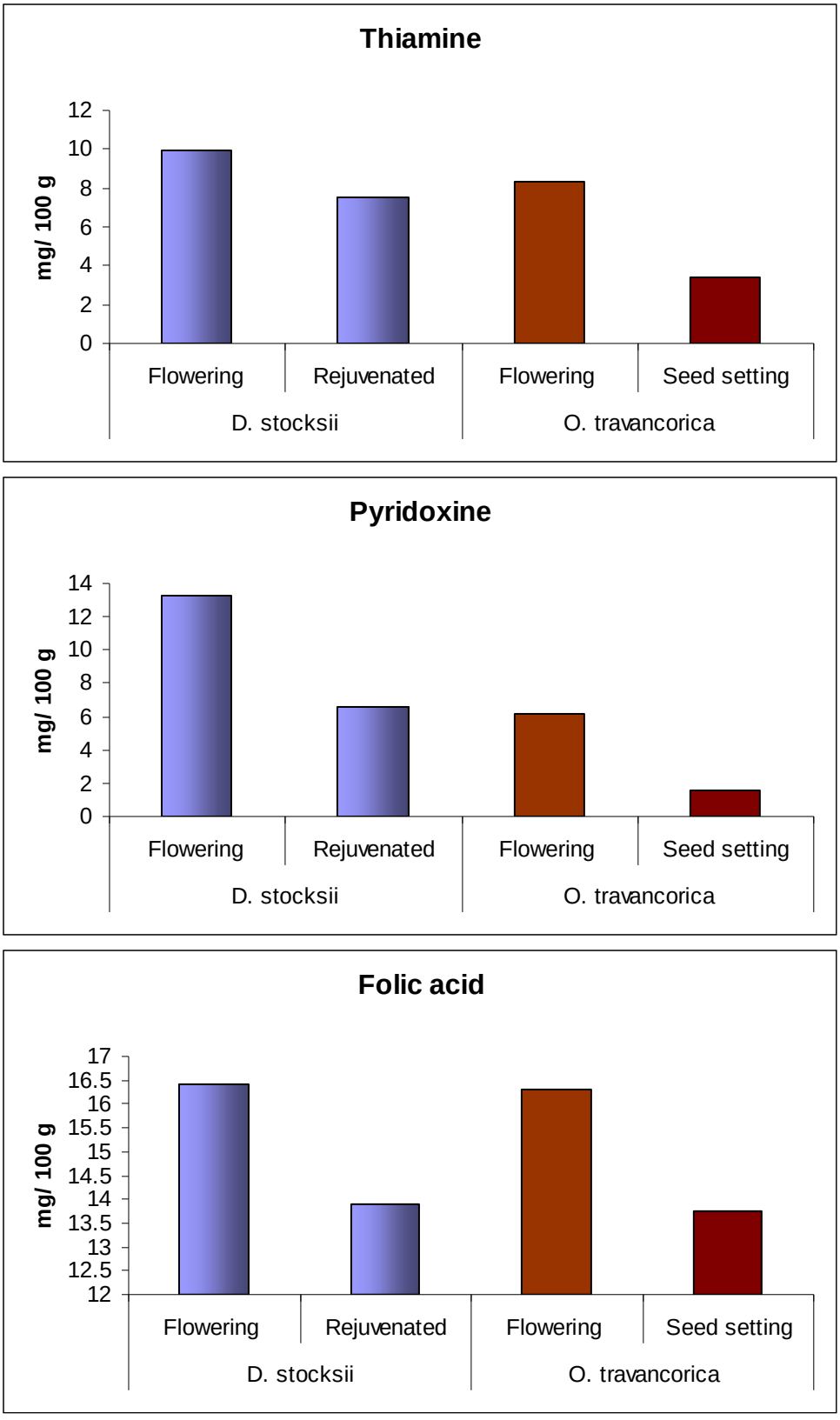


Figure 15. Variation in thiamine, pyridoxine and folic acid during flowering and post flowering stages of *D. stocksii* and *O. travancorica*

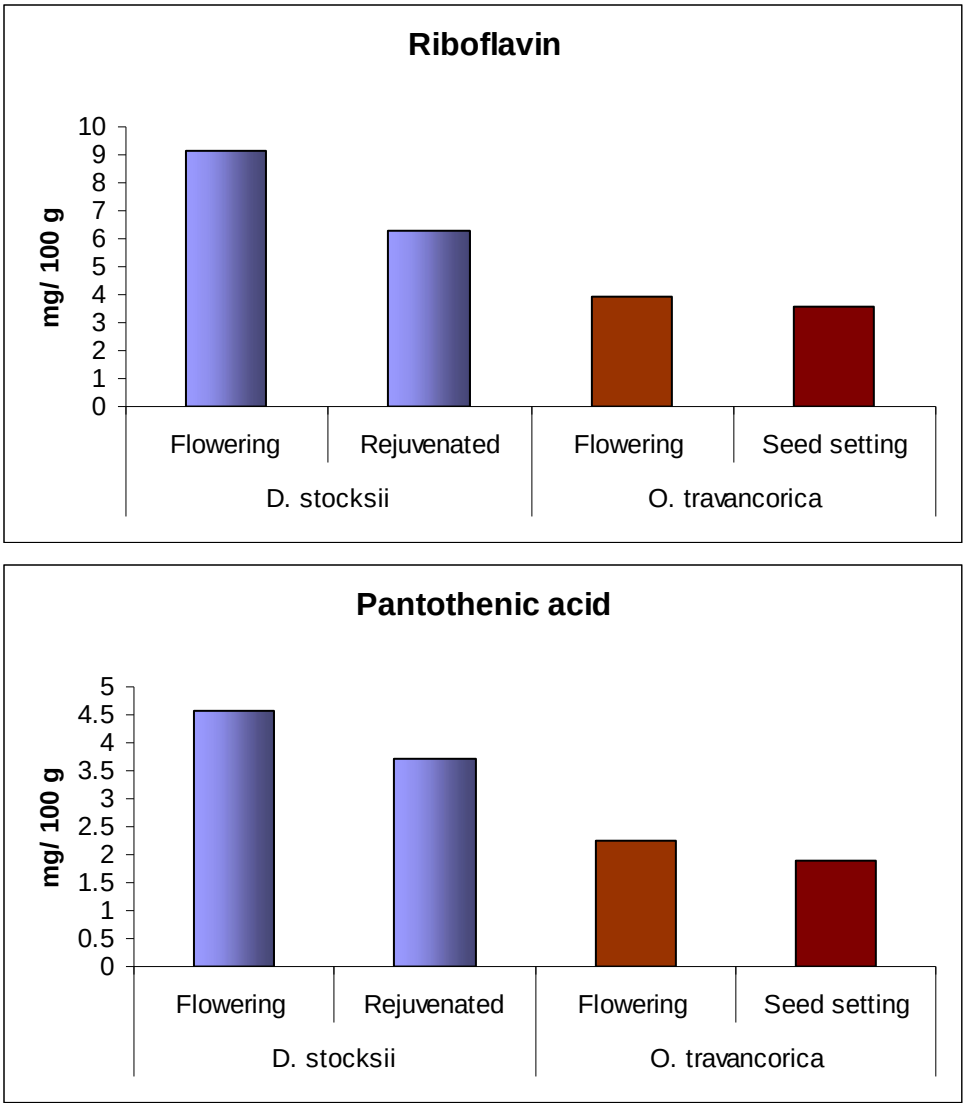


Figure 16. Variation in riboflavin and pantothenic acid during flowering and post flowering stages of *D. stocksii* and *O. travancorica*