REPRODUCTIVE BEHAVIOUR OF ADAPATHIYAN

(*Holostemma adakodien* Schult.)

By

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THESIS

Submitted in partial fulfilment of the
requirement for the degree

Master of Science in Horticulture

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1997

DECLARATION

I hereby declare that this thesis entitled "**Reproductive Behaviour of Adapathiyan** ***(Holostemma adakodien* Schult)** is a bonafide record of research work done by me during the course of research and this thesis has not previously formed the basis for award of any degree, diploma, associateship, fellowship or other similar title of any other University or Society.

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Certified that the thesis entitled **"Reproductive Behaviour of Adapathiyan *(Holostemma adakodien* Schult)** is a record of research work done independently by **Miss. S.MANJU**, under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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The award of ICAR Junior Fellowship is also acknowledged.

I also gladly remember the help of Miss. Deepa sukumar for the neat figures in the thesis. I have no words to express my deep sense of gratitude to Reji and my dearest friends Naija, Sreekala, Deepa, Anu, Duethi and Priya.

My sincere thanks to each and everyone of the labourers of College of Horticulture associated with my work. I duly acknowledge Mr. Murali and Mr. Asokan for the effort they put in, for the neat and prompt typing of this thesis.

Although last, but sincerely and cordially I extend my heartfelt thanks to my father, mother, sister and all other family memebrs. I have no words to elaborate their love, moral support and constant inspiration extended to me.

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To the

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Introduction

INTRODUCTION

Plant derived preparations based on the traditional pharmacopoeias form the therapeutic armour of majority of the world. An upsurge for natural products created a sort of green wave for phytoproducts across the world. In India, the classical medical systems and folk lore lean heavily on natural products . They together use about 500 higher plants . The forests of India, which harbour a rich and diverse group of medicinal flora satisfy the sole requirements of all these systems. This indiscriminate gathering of plant material from forests without considering the natural perpetuation necessitated the domestication of many economic and highly demanded medicinal crops. Adoption of commercial cultivation and the initiation of methodology for improvement of plant strains is a need of the hour.

Holostemma adakodien Schult. known in vernacular as adapathiyan or adakodien belongs to the family Asclepiadaceae ,the milk weed family. Adapathiyan roots find varied use in Ayurvedic system of medicine.This is the accepted source of Jivanti and reckoned as an important rasayana drug capable of maintaining youthful vigour and strength. The roots are reported to possess cooling, alterative,tonic, astringent and lactative properties (CSIR,1959). Roots made into a paste are applied to eyes in ophthalmia and also in orchitis, diabetes, gonorrhoea, coughs and stomach ache. It cures ulcers, biliousness, diseases of the blood, worms, itching, leucoderma and for vesicular calculi (Kirtikar and Basu, 1975). The medicinal properties are attributed to the sugars present in the roots. Ramaih *et al* (1981) isolated these sugars as α-amyrin , lupeol , and β-sitosterol .

Adapathiyan plants are distributed from Tropical Himalayan region to South India including Kerala. Its population is very much reduced within this range of distribution and hence considered as vulnerable and included in the Red List of the

medicinal plants of South India brought out by the Foundation for Revitilisation of Local Health Traditions. But the plant is domesticated at present owing to its wide use and highly remunerative prices. In commercial cultivation, availability of propagules is important. Though the crop can be vegetatively propagated by propagules like root and stem cuttings, seedlings are reported to be the best planting materials (Meera,1994). Similar to other Asclepiads, fruit set in adapathiyan also is very rare. This becomes a major constraint for the large scale cultivation of this species.

Systematic studies on reproductive behaviour have not been undertaken in this crop. Basic information on flowering behaviour, floral biology and pollination mechanism is essential to formulate suitable breeding programmes for crop improvement. This information is also required to unravel the reasons for low fruit set in adapathiyan.

Morphotypes based on pigmentation and leaf type such as purple cordate, purple elongate, green cordate and green elongate could be identified. Among these , purple pigmented types are said to be more productive and highly demanded for medicinal preparations. Evaluation of these morphotypes for morphological, yield and qualitative differences will provide a scientific basis for the relative preference. Hybridisation between these morphotypes give an indication of the crossability between them and gene action governing the inheritance of pigmentation and leaf shape. The evaluation of the seedling progenies can result in the isolation of ideal types.

In this context a detailed study in adapathiyan was undertaken with the following major objectives.

1) To understand the flowering behaviour , floral biology and pollination mechanism in adapathiyan and to unravel the reasons for low fruit set.

2) To evaluate the morphotypes for morphological and qualitative differences and attempt hybridisation between morphotypes and assess the extent of variability in the seedling progenies in the nursery stage to isolate the ideal types.

Review of Literature

REVIEW OF LITERATURE

Holostemma adakodien Schult. belongs to the family Asclepiadaceae. The family is denoted as milkweed family. It is a very large family of about 2850 species of perennial herbs and mostly climbing shrubs distributed through some 347 genera (Baumgardt, 1982). The members of the family are largely tropical with only a few genera in the temperate zones.

Flowers are generally regular, bisexual and pentamerous in terminal or axillary umbels or cymes. Calyx consists of five free sepals, and are imbricately arranged. Corolla is five lobed. Corona arises as out growth from petals or stamens. Stamens are five in number with filaments fused round the ovary. Anthers adhere to the stigma by broad connectives to form the gynostegium. Pollen are arranged in one or two glandular or waxy masses in each anther cell. Gynoecium consists of two free carpels with marginal placentation. The two styles cohere above and dilate to form gynostegium. Fruit consists of two follicles with light and winged seeds (Shukla and Shital, 1979 and Radford, 1986).

Holostemma adakodien comes under the tribe Asclepiadeae which include *Calotropis* also (Swarupanandan *et al.*, 1996) Synonyms of *Holostemma adakodien* are *H. rheedii* and *H. annulare*. *Holostemma adakodien*, known in vernacular as adakodien or adapathiyan is the accepted source of Jivanti, reckoned as an important rasayana drug capable of maintaining youthful vigour and strength. Roots are used as a remedy for various ailments like gonorrhoea, ophthalmia, diabetes, cough, orchitis and leucoderma (Kirtikar and Basu, 1975).

The genus is distributed in Tropical Himalayan region, Dehradun, Konkan, Kanyakumari and Kerala (Sivarajan and Balachandran, 1994). Its population is very much reduced within this range of distribution and it is classified as endangered. Main reason for the this reduced range of distribution is the low fruit set, which is the general feature

of all Asclepiads (Sreedevi, 1989) Detailed search into the flowering behaviour, floral biology and pollination mechanism is essential to account for the low fruit set percentage. Available reports pertaining to these aspects in Asclepiads and other crops are reviewed here.

2.1 Flowering :

2.1.1. Flowering pattern and floral biology:

Flowering season in adakodien was reported to be July to October in Vellanikkara condition by Meera (1994) whereas it is September to November in Peechi (Sasidharan and Sivarajan, 1996). Season starts from June - July in *Asclepias syriaca* (Wilson and Bertin, 1979). However two seasons were reported in another Asclepiad, *A. tuberosa* by Wyatt (1980) in May to July and later in September.

Flowers in adapathiyan occur in sublateral few flowered umbellate cymes, in the axils of leaves (Kirtikar and Basu, 1975). Inflorescence consists of about 5-10 flowers and the number of inflorescence per plant varied from 14-20 (Meera, 1994). Flowers are bracteate, pedicellate, complete, actinomorphic, bisexual, hypogynous, cyclic and pentamerous. Calyx is deeply five partite. Sepals are five mm long, broadly ovate, obtuse and veined. (Kirtikar and Basu, 1975). Corolla is gamopetalous subrotate and divided about two third of the way down. Petals are 1.3 cm long and 1 cm wide, ovate oblong, obtuse and overlap to the right (Sivarajan and Balachandran, 1994).

The asclepiadaceous flower is characterised by the presence of corona, an accessory structure to the petals. It consists of faucal annulus arising from the corolla tube (Vasishta, 1974). Corona in *Holostemma* arise from the staminal column (staminal corona) and consists of fleshy truncate ring of 2.5 mm height . Corona is primarily meant for secreting and storing nectar (Shukla and Misra, 1979)

Androecium consists of five stamens, epipetalous and inserted at the base of the petals and alternate with them. Filaments fuse together to form a ten winged column, surrounding the gynoecium. Stamens fuse with the stigmatic disc to form the gynostegium, a five angled disc. Anthers are large with stiff wings and with membraneous tips inflexed over the column. Pollen contents of each anther cell are granular or united into one or two pollen masses (Gamble, 1986). When granular, each granule formed of about four grains loosely united and contained in a spoon or trowel-like appendicle, attached by a caudicle to the pollen carrier on the style apex. In *Holostemma*, pollen are united into a waxy mass, opaque and without plucid margin, called as pollinium. Pollinia attached in pairs by caudicles to the dark coloured pollen carriers or translators, is the identifying feature of the tribe **Asclepiadeae**.

Gynoecium is bicarpellary and apocarpous. The ovaries and styles are separate except for the stigma that are fused to form a five angled disc with an anther adnate to each side. Each carpel is unilocular, with many ovules on marginal placenta.

2.1.2 Anthesis and anther dehiscence:

No reports are available about the time of anthesis and anther dehiscence in adokodien or in other Asclepiads. The reproductive span of a flower was reported to be 6.2 days in *Asclepias exaltata* (Wyatt and Shannon, 1986), 4-5 days in *Sarcostemma viminale* (Liede and Whitehead,1991) and 7 days in *Vincetoxicum nigrum* (Lumer and Yost,1995)

2.1.3 Stigma receptivity:

The characterstic angiosperm stigma was studied in detail by Heslop and Shivanna (1977) in about thousand species of plants. Sporophytic self incompatibility was reported to be associated with dry papillate stigma. Trinucleate pollen not readily germinated *in vitro* tend to be associated with dry stigma while wet stigma forms tend to have binucleate

pollen easily germinated in liquid or semi-solid media. In Asclepiads, it is reported that receptive side of the stigma is the lower lateral sides of the stigmatic head (Sreedevi, 1989).

2.2. Pollen studies:

The term Palynology refers to pollen and spore science. The importance of Palynology in plant taxonomy was emphasised by Wodehouse (1935) and Erdtman (1952). Bhojwani and Bhatnagar (1978) refered to the study of external morphological features of mature pollen grain as Palynology. Palynology deals with the pre-tetrad and post tetrad stages, the latter including pollen production, pollen morphology, anthesis, pollen dissemination, pollination, pollen germination and fertilisation (Srivastava, 1982). The storage and germination of pollen grains play an important role in assisted pollination and hybridisation programme.

2.2.1 Pollen morphology:

The analysis of pollen morphology has been used as an effective aid to throw light on taxonomy, phylogeny and evolution of angiosperms (Nair, 1970) In Orchidaceae, Ericaceae and Asclepiadaceae pollen is embedded in pollinium (Sparrow, 1948). Sreedevi and Namboodiri (1979) reported that anthers have near oval shape in *Asclepias* and *Dregea*. Pollen grains were also not of uniform shape or size, even in the normal meiosis, although all of them were viable. The presence of exineless pollen protoplasts at the time of germination and their normal development are also atypical features.

2.2.2. Pollen production:

The exact measurement of the amount of pollen produced per anther is essential to evaluate the worth of a particular plant as a pollinator. Stanley and Linskens (1974) computed the pollen output of some plants by emptying the thecae and suspending the

grains in a fixed portion of suspension. But in Asclepiads, since pollen grains are kept covered within the pollinium, it is difficult to estimate the number iof pollen per pollinium (Viswanathan and Lakshmanan, 1984).

2.2.3 Pollen germination:

Pollinium behave in the same way as pollen in germination. Pollen tube emerge from the convex side of the pollenium, through the germ pore. Some kind of polarity existing in the pollinia direct the pollen tubes towards the germ pore only. The direction of tube growth was not markedly affected even with new outlets opened (Galil and Zeroni, 1965). This was supported by the fact that germ pore areas have differentiated wall region for the emanation of pollen tubes (Jalaja and Namboodiri, 1975). Later Sreedevi and Namboodiri (1982) reported that polartiy of pollen tubes within the pollinium is governed by the permeability of the germ furrow region and not by the innate orientation of the pollen grains or chemotropic factors.

Nectar present in the stigmatic chamber was the germination medium for the pollen. Pollen did not germinate in dry stigmatic chambers. Nectar should contain 5-30 per cent sugar for pollen germination (Kevan *et al.*, 1989). In *Asclepias syriaca* the nectar was inhabited by an yeast, *Metschnikowia reukaufii.* which produces a substance that inhibits pollen germination. This might be an important limiting factor in fertilisation and perhaps fruit set in *Asclepias.* Yeast is transferred from flower to flower by pollinators like *Apis mellifera* and *Bombus* spp. (Eisikowitch, *et al*, 1990)

2.2.4 Pollen viability:

The extent of pollen viability is of vital importance in hybridisation work. Appearance of pollen alone, even at collection time is not always a good index of viability (Stanley and Linskens, 1974). They suggested various methods for testing the viability of pollen grain including both germination and nongermination assays.

2.2.4.1 Stain test :

Staining the pollen with the different chemicals or dyes has been adopted to assess the viability of the pollen grain. Stains which give colour to viable pollen is often used as indices of viability. Viswanathan and Lakshmanan (1984) reported that Alexander's stain can be used to determine the pollen viability in *Calotropis gigantea*. Fertile pollen grains were stained red and the sterile ones green. They obtained 78.9 per cent viability.

2.2.4.2 *In vitro* germination method :

An artificial medium supplemented with the various required nutrients especially sugars, have proved successful to germinate pollen grains in a large number of plants. Apart from osmotic role, the externally applied sugars in the medium definitely served as nutrient material for the growing tubes. Germination percentage in *C. gigantea* could not be calculated for the exact number of pollen tubes and the number of cells involved therein could not be ascertained (Viswanathan and Lakshmanan, 1984). A higher germination percentage of 96 per cent was obtained in *Calotropis*, when pollinia were cultured in Brewbaker and Kwack's medium (Brewbaker and Kwack, 1963). They gave the composition of the medium which proved its merit in a wide variety of pollen grains studied. Sreedevi and Namboodiri (1971) studied the effect of growth substances on pollen tube growth in *Calotropis*. None of the growth substances tested promoted pollen germination. Low concentration enhanced rate of tube growth and higher concentration inhibited .

2.2.4.3 Effect of boric acid:

It was Schumucker (1935) who observed that boron as borate was stimulant to pollen germination and tube growth in many species. It may be due to the fact that boron occurs in relatively high concentration (20-30 ppm) in pistillate tissue of the few species

studied. Thus 1-10 ppm of boric acid concentration in artificial medium stimulate pollen germination and tube growth. Thomson and Batjer (1950) reported that boron in low concentrations of 2.5 to 4.0 ppm has stimulative effects whereas at high concentrations, boron inhibited pollen germination and tube growth. Munzer (1960) found that 0.01 to 0.1 per cent of boric acid had a stimulating effect on pollen germination and tube growth in more than 60 angiosperm species. However, Parmer (1978) reported that sucrose media containing agar or boric acid had no special effect on pollen germination in phalsa when added with sucrose.

2.2.4.4 Effect of calcium :

The influence of calcium nitrate on pollen germination and tube growth was reported by various workers. Brink (1924) mentioned it as an inhibitor of pollen germination. Brewbaker and Kwack (1963) and Kwack (1965) have revealed the essential role of calcium in pollen germination and tube growth. They opined that growth was inhibited fully in the absence of calcium. However, ions like potassium, sodium or magnesium singly or together, would act to enhance calcium activity. The effects of increasing calcium were also apparent in the rigidity and straightness of pollen tubes, with little or no coiling observed at high levels of calcium. It is reported that improvement in germination and growth of pollen due to calcium relates primarily to the binding of calcium to pectate carboxyl groups along the pollen wall.

2.3 Pollination studies

Pollination is the transfer of pollen from one flower to the stigma of another flower or same flower.

2.3.1 Natural or open pollination

Rendle (1971) reported that in Asclepiadaceae, cross pollination is occuring through the agency of insects. An insect visiting the flower for nectar catches its leg in the slit between the cells of the adjacent anthers. Then the leg comes in contact with the notched base of the corpusculum and drags the latter off, bearing with it the pair of pollinia. The arms of the translator are hygroscopic and as they dry, bring the pollinia together, increasing the hold on the leg of the insect. The stigmatic surface lies beneath the anther slit, so that in visiting a flower in the female stage, the act of catching the leg in the slit will cause the pollinia to become attached to the receptive surface. The pollination in Asclepiads was reported to be a "lock and key mechanism" (Davis and Heywood, 1973). But the disadvantage of mechanical isolation are probably offset by the longivity of individual plants and by the large number of seeds produced. Kephart (1983) suggested that three species of *Asclepias* viz. *A syriaca, A. incarnata* and *A. verticillata* have predominantly allogamous breeding systems. No seed set was observed in these three species after artificial selfing. The reason attributed was either late pre fertilisation or early post fertilisation incompatibility.

Sreedevi (1989) made a detailed investigation on the pollination of Asclepiads and reported that Asclepiad flower is highly adapted for cross pollination and a complex system for insect pollination was observed. The complex translator ensures the transfer of entire pollen of an anther in one transmission. Because of the provision of a lateral stigmatic chamber, perfect insertion of pollinia is ensured and once it is pollinated, it cannot be removed even by other insct visitors.The notch of the corpusculum and hygroscopic nature of caudicle helps in its attachment to the leg of insect. Also shifting of the receptive surface to the lateral sides helps easy germination of pollinia.

2.3.2 Pollinating agents

Galil and Zeroni (1965) reported that honey bees were the pollinators in *Asclepias curassavica*. Honey bees also act as pollinator to *A. syriaca*, accounting for 26 to 49 per cent of all vectors (Wilson and Bertin, 1979). Wyatt (1978) reported that most of the members of this family share pollinators. Kephart (1983) studied pollinators in 3 species of *Asclepias*. They were *Bombus griseocollis* and *Apis mellifera* in *A. syriaca*, *Polistes* spp and *Sphex* spp in *A. verticillata* and *Sphex pennsylvanicus*, *B. griseocollis*, *A. mellifera* and *Xylocopa virginica* in *A. incarnata*. *Melissodes disponsa*, a bee was reported to be the pollinator in *A. quadrifolia* (Pleasants and Chaplin, 1983). Sreedevi (1989) observed that though the size and shape of the pollinia were species specific, any insect with appropriate size and behaviour could pollinate Asclepiad flower. *Apis* and *Helictus* are the frequent pollinators of Asclepiads, but flies and wasps are also efficient pollinators. The pollinator of *Sarcostemma viminale* was *Apis mellifera* (Liede and Whitehead, 1991).

Pollinators in *Calotropis* sp. were studied by many workers. Ramakrishna and Arekal (1979) reported the pollinators to be *Xylocopa dissimilis* and *X. collaris* in *C. gigantea* in Karnataka region. *X. pubescens* and *X. fenestrata* were reported *in C. procera* subsp. *hamiltonii* by Ali and Ali (1989).

2.3.3 Self pollination:

Cross pollination is the rule in Asclepiads. But Sreedevi (1989) reported that in addition to cross pollination and fertilisation, a considerable percentage of flowers were fertilised by *in situ* germination of pollinium in *Calotropis*, *Asclepias*, and *Daemia*.

2.3.4 Controlled pollination

Sparrow (1948) illustrated a method for hand pollination in *Asclepias syriaca*. The process consists of extraction of pollinia from the pockets and placement in the stigmatic cavities. After the pollinia are extracted from the pockets, they may be used directly or placed temporarily on a filter paper in a petri dish. The paired pollinia may or may not be separated in making a pollination. For successful pollination, it is essential that the more convex edge of a pollinium contact the stigmate surface hidden within the cleft. The complexity of the floral mechanism and the apparent difficulty of removing and inserting pollinia has been presumed to "render any Asclepiad the most forbidding subject for breeding experiements that can well be imagined in the flowering plants" (Woodson, 1962). Wyatt (1976) attempted artificial pollination in *A. tuberosa* and observed an average fruit set of 14.8 per cent in all crossing.

2.4. Compatibility studies

Self incompatibility, the inability of a plant with functional gametes to produce selfed seeds, occurs widely among flowering plants in at least 71 families (Brewbaker, 1959). Incompatibility results from the inhibition of pollen tube growth at one of the two major sites on the stigma at or soon after germination and in the pistil, during the first few hours of tube growth.

Moore (1946) and Sparrow and Pearson (1948) have reported a complete lack of self compatibility in *A. syriaca*. Sparrow (1948) confirmed the same result. Successful pollination with compatible pollen brings about certain obvious changes in the pollinated flowers viz. thickening and recurving of the pedicel, enlargement of the ovary and appearance of pubescence on the ovary. It was found that incompatible pollen is also capable of bringing about similar though somewhat weaker responses Woodson (1954) maintained the opinion that all species of *Asclepias* to be self incompatible and discounted reports of successful self pollination in *A. syriaca* and *A. incarnata*. Wyatt

(1976) reported self incompatibility in *A. syriaca*, though few individuals are self-compatible. Wilson *et al* (1979) reported self-incompatibility in *A. verticillata*. Later self-incompatibility was observed in most species of *Asclepias* (Wyatt, 1980). Kahn and Morse (1991) proposed post-fertilisation incompatibility mechanism for *A. syriaca*. Sage and Williams (1991) studied pollen pistil interaction in self incompatible *Asclepias exaltata*. There was no temporal, structural or histochemical differences between self and cross pollen tube growth. There were fewer free endosperm nuclei in selfed than in crossed ovules seven days after pollination. Integument of the selfed ovules ceased growth after 3-4 days of endosperm mitosis. This might be due to post zygotic mechanism or failure in gamete fusion.

2.5 Fruit set

Asclepiad fruit consists of two distinct follicles usually diverging from the base (Shukla and Shital, 1979). Occasionally one is abortive. Seeds are compressed or flat with a tuft of hairs (coma) at the hilum and endospermic. Embryo is large with flat cotyledons and short radicle.

Sparrow (1948) reported a low yield of matured fruits in *A. syriaca*.The reasons attributed were failure of insects to accomplish pollination and the failure of pollen tubes to penetrate the ovary. Only 64.5 per cent of the tagged flowers got pollinated in this crop and final fruit set was less than 10 per cent. Lack of pollination and pollination with incompatible pollen were the reasons for this low fruit set. When hand pollination was done in *A. syriaca*, only 10.35 per cent set was obtained

Hand pollination was tried in *A. tuberosa* by Wyatt (1976) for both crossing and selfing. Percentage of set were 22.8 and 18.0 respectively. Failures of pollen tubes to penetrate the ovary and effect fertilisation and similar failures at various stages of development were responsible for the physiological contribution to low fruit set. Natural fruit set was as low as one percent. Mechanical and physiological factors interact to result

in the observed low level of fruit set. Wilson *et al* (1979) reported a low percentage pod set in *A. verticillata*. Nectar production was maximum between 18.00 and 22.00 hours on the flower opening days. But insect visits were infrequent at these periods, which result in low set. Wyatt (1980) observed serious limitation leading to low fruit set in *A. tuberosa* to be competition among ovaries within the umbels. A low success percentage of 0.17 was reported for artificial pollinations. For a given number of flowers, large plants set fewer fruits than smaller plants.

Sreedevi (1989) listed reason for low fruit set in Asclepiads. The most important reason is the low degree of removal and insertion of pollinia into the stigmatic chamber. This is supported by field observations that considerable number of pollinia are seen misplaced on petals and nectaries. Cabin *et al* (1991) recorded a low fruit set percentage of 0.7 in *A. quadrifolia*, which might be due to pollen and resource limitation. Formicidae were observed actively removing pollinia from the flower which also contributed to low fruit set in *Gomphocarpus physocarpus* (Forster, 1994). The natural level of fruit set in *A. curassavica* is estimated to be 5 to10per cent. Seed set of 11.1 pre cent was reported in another Asclepiad, *Vincetoxicum nigrum* by Lumer and Yost (1995).

Relatively higher percentage of fruit set was observed in high density populations. The calorie reward per flower is low and pollinator has to visit at least 20 flowers to satisfy its energy requirements. Thus they are attracted to areas of dense plant population and result in higher fruit set (Henrich and Raven, 1972). Also large inflorescence had greater pod set because of greater insect visiting rates (Wilson and Price, 1977).

2.6 Chemical composition of roots

CSIR (1959) published the economic importance of *Holostemma adakodien* with the uses and chemical composition. Analysis of the root of powder revealed moisture (10.08%), protein (4.07%), sugar (24.0%), starch (32.54%), fibre (12.2%) and ash (3.07%) The ash contained calcium and phosphourus (2.50%) The medicinal properties are

attributed to the sugars present in the roots. Ramiah *et al* (1981) isolated and identified different sugars such as α - amyrin, lupeol and β - sitosterol and six amino acids such as alanine, aspartic acid, glycine, valine, serine and threonine from the root extracts and considered these chemicals as components of *Holostemma.*

Samuel *et al.*(1993) studied the influence of harvesting stage ranging from 8-11 months on the chemical components of *Holostemma.* The percentage of carbohydrate showed a decreasing trend as the age of the plant increased and the content was lowest (56.6%) in 11 months old crop.The protien (10.0%) and alkaloid (1.40%) contents were highest in 11 months old crop compared to 8 months (3.94 and 1.1%) and 9 months old crop (8.95% and 1.25%). Meera (1994) studied the influence of planting materials and stage of harvest on yield components of adakodien. Both these factors significantly influenced the soluble carbohydrate content. Maximum content was recorded by seedling (4.92%) followed by root stumps (4.86%), vine-cuttings 2 nodes (4.81%) vine cuttings 3 nodes (3.97%) and root cuttings (3.82%) which was the lowest. Comparing between stage of harvest the maximum content was obtained from 18 months old roots (7.48%) and minimum from 9 months old roots (2.36%). No significant difference was observed between planting materials and stage of harvest for the number of free amino acids, suggesting that the amino acid pattern in a species may be genetically controlled. The total amino acid content was found to be unaffected by the type of planting materials, but it showed an increase with increase in age of the plants. The content was maximum (0.14%) in 18 months old roots and minimum in 9 months (0.02%).

Materials and Methods

MATERIALS AND METHODS

The study entitled "Reproductive behaviour of adapathiyan (*Holostemma adakodien* Schult) was carried out at the Department of Plantation Crops and Spices, College of Horticulture, Vellanikkara, during the period from June 1996 to July 1997. The four morphotypes identified for the study included those with purple cordate leaved, green cordate leaved, purple elongate leaved and green elongate leaved (Plate 1 to 5). The investigation on flowering and floral characters, pollination, fruit set and development, evaluation of morphotypes and seedling variability were done on the plants maintained in the fields of Department of Plantation Crops and Spices.

3.1 Flowering and floral characters

3.1.1 Pattern of flowering :

Pattern of flowering in Holostemma plants was studied by observing 50 branches selected at random on each morphotype. Observations on season of flowering, position of inflorescence, number of inflorescence per plant and number of flowers per inflorescence were recorded in all the four morphotypes selected.

3.1.2 Flower bud development :

Periodic examination of the shoots tagged were made during the flowering season to find out the exact time of visual emergence of flower buds. Tagging of buds were done soon after the emergence of buds as purple bulging in axils of branches. Observations were made on the developmental stages at two days interval. The developing buds were examined and photographs were made at different stages.

Plate 1. Purple elongate morphotype.

Plate 2. Purple cordate morphotype.

Plate 3. Green elongate morphotype.

Plate 4. Green cordate morphotype.

Plate 5. Different leaf forms of adapathiyan.

1. Purple elongate
2. Purple cordate
3. Green elongate
4. Green cordate


1
2
3
4

3.1.3 Floral biology :

Studies on various aspects of floral biology viz. anthesis, anther dehiscence, stigma receptivity and pollen characters were carried out on the flowers of all morphotypes. The flowers were described and drawings were made.

3.3.1 Anthesis :

Preliminary observations showed that flower opens in the morning hours. In order to know the exact time of anthesis, 25 buds were tagged on the four morphotypes and observations were made at half hourly intervals from 08.00 hours to 10.30 hours.The maturity of the buds was determined from the colour and size of the buds.

3.1.3.2 Anther dehiscence :

The period of anther dehiscence was studied by tagging 25 buds of uniform size with light purple colour. Observations were made twice daily in the morning and evening, examining the pollinia for dehiscence using a hand lens. Later observations were repeated on mature buds at hourly intervals from 09.00 onwards to 13.00 hours up to fifth day after anthesis.

3.1.3.3. Stigma receptivity:

The receptivity of stigma was judged visually by the appearance of the lateral sides of the stigma. This was further confirmed by controlled pollination and observing the fruit set. Mature buds were emasculated and covered with polythene cover. They were later pollinated with pollinia from opened buds of male parent and *in vitro* germinated pollinia on Brewbaker and Kwack's medium. Pollination was done at six hourly intervals

starting from one day prior to anthesis and continued till one day after anthesis. Twenty buds were utilised for these studies at different stages.

3.2 Pollen studies

Pollen studies with respect to pollen morphology, size, fertility and germination were taken up. The pollinia for the studies were collected between 09.00 and 11.00 hours from unopened buds and opened flowers, on their first, second, third fourth and fifth day of opening. Detailed procedures for studying each aspect are given below:

3.2.1 Pollen morphology:

Twenty five freshly opened flowers were collected from the field. The diameter of the pollen grains was measured using an ocular micrometer. The diameter of fifty normal sized, well stained and well shaped pollen grains was recorded and the average was worked out.

3.2. 2 Estimation of pollen production:

Estimation of number of pollen produced per pollinia was tried using haemocytometer. Freshly opened flowers were collected and pollinia was taken out. Two hundred such pollinia were kept in Brewbaker and Kwack's medium for two hours for dehiscence. After dehiscence, pollinia were crushed in 0.5 ml of water containing 1 per cent extran and stirred thoroughly to obtain even dispersion of pollen grains in the suspension. A drop of the suspension drawn in a fine pipette was transferred to each of the two counting chambers of a haemocytometer. The counting chambers are 0.1 mm in depth so that the volume over one mm^2 is 0.1 mm^3 . On this basis, the number of pollen grains per pollinia was estimated using the formula:

$$N = \frac{a \times v \times 10^4}{n}$$

N = Number of pollen grains per pollinia

a = Mean number of pollen grains counted per corner square

v = Volume of suspension made with pollinia

n = Number of pollinia with which the suspension was made up

3.2.3 Pollen fertility:

3.2.3.1 Stain test :

Twenty five freshly opened flowers were selected from all the morphotypes. Pollen viability was tested using Alexander's stain. The fertile pollens were stained purple and the sterile ones green and the percentage viability was worked out. The pollen grains could not be liberated by dissecting the fresh pollinia (Viswanathan and Lakshmanan, 1984) Therefore pollinia were dipped in Brewbaker and Kwack's medium for two hours to initiate first pollen tube and then crushed carefully to liberate the pollen grains for staining.

3.2.3.2 *In vitro* pollen germination:

Pollen germination in water, water + sucrose and Brewbaker and Kwack's medium was noted (Viswanathan and Lakshmanan, 1984) The Brewbaker & Kwack's medium contains sucrose 10 per cent, boric acid 100 ppm, calcium nitrate ($CaNO_3$. $4H_2O$) 300 ppm, magnesium sulphate ($MgSO_4$.$7H_2O$) 200 ppm and potassium nitrate 100 ppm. The pollinia from freshly opened flowers were dissected out and kept in a drop of medium on a clean glass slide. This was kept in a dessica- - ollen germination was ascertained by examining pollen tube growth 24 hours after inoculation.

3.3 Pollination studies:

3.3.1. Pollinating agents :

To study the agents of pollination, the plants were closely observed during flowering season. The insects which visit the flower during anthesis were collected using an insect net. They were killed and body parts were examined for any deposit of pollinia.

3.3.2. Mode of pollination :

To ascertain the precise mode of pollination, fruit set by four modes of pollination was studied. For each study, 50 flowers were utilised, randomly distributed in the morphotypes to be studied. Observation on fruit set was recorded five days after pollination.

a) Natural / open pollination :

To know the extent of open pollination, individual flower buds were selected and tagged before anthesis. These were later examined for fruit set and extent of fruit set was worked out.

b) Self pollination :

The extent of self pollination was studied on flowers selected randomly. Individual flowers were covered with the polythene covers one day prior to anthesis to prevent any chance of cross pollination from outside. The covers were removed four days after flower opening and fruit set was recorded. Selfing was done artificially also using intact pollinia from the same plant, and also pollinia germinated in artificial medium. Pollinia were collected from flowers on the first day of opening.

c) Natural cross pollination :

For knowing the extent of natural cross pollination, individual flowers were emasculated one day prior to anthesis and left for natural pollinating agents. The subsequent fruit set was determined.

d) Artificial cross pollination :

Flowers were emasculated and covered with polythene cover one day before anthesis. These were hand pollinated on the next day by inserting the pollinia from desired male parent directly into the stigmatic cavities after lifting the membraneous covering on the stigma. Another method was also tried by carrying out pollination with germinated pollinia. For this purpose, the pollinia from desired male parent were collected on the day of anthesis and kept for *in vitro* germination in Brewbaker and Kwack's medium. These pollinia were utilised for pollination on the next day. In both these methods, pollinated flowers were kept covered for four days and tagged. Fruit set was observed later.

Eight combinations of crossing between the morphotypes were done as detailed below:

1. Purple cordate leaved	x	Green cordate leaved
2. Purple elongate leaved	x	Green elongate leaved
3. Purple cordate leaved	x	Green elongate leaved
4. Purple elongate leaved	x	Green cordate leaved
5. Green cordate leaved	x	Purple cordate leaved
6. Green elongate leaved	x	Purple elongate leaved
7. Green cordate leaved	x	Purple elongate leaved
8. Green elongate leaved	x	Purple cordate leaved

3.4 Fruit set and maturity:

3.4.1 Days to fruit set:

To assess the percentage of natural fruit set, 25 flower buds were tagged on each morphotype at fully developed bud stage. Observations were made daily for a period of one week. These observations were utilised to find out the days to fruit set.

3.4.2 Days to fruit maturity :

Twenty five fruits were tagged on the day of shedding of flower parts to determine the days taken to reach maturity. Fruit maturity was assessed by the change in colour from green to light straw colour.

3.5. Seed:

3.5.1 Number of seeds:

Twenty straw coloured fruits were collected and seeds were extracted. Number of seeds per fruit was counted and the mean number was worked out.

3.5.2 Hundred seed weight:

Hundred seed weight was found out by weighing 100 fully developed brown coloured seeds from all the morphotypes studied.

3.5.3. Seed germination:

Seed germination was tested using 100 seeds. They were soaked for 24 hours in water and placed on a moist filter paper in a petri dish. Germination count was taken every day from third day onwards. These observations were subsequently confirmed by

sowing 100 seeds in moist sand in a tray which were pre-soaked overnight in water. Seeds germinated each day were counted.

3.6. Biometric characters:

3.6.1 Collar girth:

Collar girth was measured by taking the diameter with a twine. The corresponding length was obtained from a scale and expressed in centimetre. Five replications of each morphotype were observed.

3.6.2 Internodal length:

Length of five lower most internodes of a single plant was measured with the help of a scale. Five replications were measured and the average was expressed in centimetre.

3.6.3 Number of branches:

Number of branches in each of the five replications were counted.

3.7 Yield of roots:

The four morphotypes were uprooted two years after planting and the fresh root yield was recorded in gram per plant.

3.8 Biochemical studies:

Root samples of the four morphotypes were analysed to assess the chemical components. The methodology followed for each analysis is given below:

3.8.1 Insoluble sugars:

Content of insoluble sugars was determined by anthrone method using dried root powder. (Malik and Singh, 1980) The content was expressed in gram per 100 gram of the sample.

3.8.2. Soluble sugars:

Phenol sulphuric acid method was followed to estimate the soluble sugars present in dry root powder (Sadasivam and Manikam, 1992) Golden brown colour developed by the sample was measured at 490 nm wave length and the content was recorded in per cent.

3.8.3 Protein:

Protein estimation was done by Lowrey's method (Sadasivam and Manikam, 1992) and the content was expressed in milligram per 100 milligram of the fresh root sample.

3.8.4. Total free amino acid:

Total amino acid content in fresh root sample was estimated using ninhydrin reagent, which develop a purple coloured product with amino acids which is colorimetrically measured at 570 nm (Sadasivam and Manikam, 1992).

3.9 Seedling variability:

Seeds were extracted from each fruit, obtained by controlled pollination and open pollination. Seeds were pre-soaked in water and sown on a raised bed made ofsand and soil. Germination count was taken at three days interval. Seedlings at 3 to 4 leaf stage

were transplanted to polythene bags filled with potting mixture. Two months after transplanting, biometric observations of all the hybrid and open pollinated seedlings were taken to assess the variability in terms of pigmentation, leaf shape, leaf size, collar girth, number of branches and internodal length as detailed earlier. Leaf size was worked out by multiplying the product of length and breadth with a constant 0.995 (Meera, 1994).

3.10 Statistical analysis:

The data collected on different characters were analysed by applying the technique of analysis of variance (ANOVA) for CRD following Panse and Sukhatme (1978).

Results

RESULTS

Breeding works are the major pavements to upgrade the yield and quality of any crop plant. To formulate scientific breeding programme, knowledge about floral biology and reproductive behaviour is unavoidable. Therefore, an attempt was made to study the reproductive biology and mode of pollination in adapathiyan and also to outline the reasons for low fruit set. Evaluation of morphotypes and crossability between them was also undertaken. The results of the studies on various aspects of reproductive biology , morphology, yield and quality are presented as mean of the population and as variation among four morphotypes.

4.1 Flowering and floral characters

4.1.1 Pattern of flowering

Visual emergence of flower buds commenced in June in all the morphotypes except green cordate in which flower buds appeared in July (Table 1). Flower opening was found to progress steadily thereafter . The peak flowering period was also late in green cordate types (September - October) while in other three , peak was noticed in August - September.

Flowers appear in cymose inflorescence in the leaf axils (Plate 5) and hang downwards (Plate 6). Flower opening in an inflorescence started from periphery and progressed towards the centre .The data furnished in Table 2 showed that the number of inflorescence per plant ranges from 10 - 64 with a mean of 33.75. Two to twenty flowers were observed per inflorescence and mean was 8.0. Total number of flowers per plant varied from 39.00 in green cordate type to 878.04 in green elongate type with a mean of 336.39. Number of days for complete opening of an inflorescence varied from 4 to 15 with an average of 8.8. Inflorescence characteristics of the morphotypes presented in Table 3 showed that the highest number of inflorescence per plant was recorded in green

Table 1.Pattern of flowering in four morphotypes of adapathiyan

Morphotype	Month of visual emergence of bud	Period of maximum flowering	End of flowering Season
Purple elongate	June	August-September	October
Green elongate	June	August-September	October
Purple cordate	June	August	September
Green cordate	July	September-October	November

Table 2.Observation on flowering and fruit set of adapathiyan

Sl.No	Character	Range	Mean
1.	Number of inforescence per plant	10-64	33.75
2.	Number of flowers per inflorescence	2-20	8.00
3.	Number of flowers per plant	39.00-878.04	336.09
4.	Number of days for complete opening of inflorescence	4-15	8.80
5.	Days to fruit set	4-5	4.05
6.	Days to fruit maturity	102-158	139.05
7.	Fruit set (%)	5-17	12.84
8.	Number of seeds per fruit	170-447	286.35
9.	Hundred seed weight (g)	0.60-0.79	0.70
10.	Seed germination (%) (Top of paper method)	85.2-93.3	89.25.

Table 3. Inflorescence characteristics of different morphotypes of adapathiyan

Morphotypes	Mean number of days for complete opening of flowers in an inflorescence	Mean number of inflorescence per plant	Mean number of flowers per inflorescence	Mean number of flowers per plant
Purple elongate	9.0[a]	47.4[b]	6.2[b]	293.88[a]
Green elongate	7.4[a]	54.2[b]	16.2[c]	878.04[a]
Purple cordate	11.6[a]	20.4[a]	6.6[b]	134.64[a]
Green cordate	7.2[a]	13.0[a]	3.0[a]	39.00[a]

Treatment means in a column with same letter do not differ significantly

Plate 6. Cymose inflorescence of adapathiyan.

Plate 7. Overall view of a plant in flowering stage.

elongate type (54.2) and the lowest in green cordate type (13.0).Elongate types and cordate types were statistically on par. Purple elongate and purple cordate types were on par with respect to mean number of flowers per inflorescence (6.2 and 6.6 respectively). However green and purple types and also among themselves. All the morphotypes were statistically on par in respect of mean number of days for complete opening of flowers in an inflorescence with a range of 7.2 (green cordate) to 11.6 (purple cordate) . In green types, flower opening of an inflorescence was faster (7.4 and 7.2 days) when compared to purple types (9.0 and 11.6 days).

4.1.2 Flower bud development

After visual emergence, flower buds passed through a series of morphological changes to reach the anthesis stage. The whole period of flower bud development was divided into twelve approximate stages (Plate 8). The chronological order of these stages was studied and data are presented (Table 4)

Stage 1: Tiny and compact with very small pedicel covered with sepals. Colour of compact mass was purple. Bracts were clear.

Stage 2: Bud appeared fully, covered with sepals . The bud became light purplish cream.

Stage 3: Bud size increased, covered with green sepals . equal in length to that of the bud. Bracts started yellowing .

Stage 4: The head of the bud was globular and the colour of bud was cream tinged with purple . Bracts dropped down.

Stage 5: The length of globular head and basal part was nearly equal . The pedicel became prominent.

Stage 6: The calyx tip became more pointed and fleshy. The girth of bud increased.

Stage 7: Calyx restricted to the bottom portion, making the bud fully exposed.

Stage 8: Pedicel length increased further. Demarcation of petals became distinct.

Table 4. Duration of different stages of flower development

Morphotype	Duration (days)											
	Stage 1	2	3	4	5	6	7	8	9	10	11	12
Purple elongate	0	2	4	7	10	13	16	19	22	24	26	27
Green elongate	0	3	7	10	13	16	18	20	21	24	25	26
Purple cordate	0	4	6	9	12	15	18	19	22	25	27	28
Green elongate	0	3	7	9	12	15	17	19	21	22	24	25

Stage 9: Purplish tinge to the flower bud increased. The petals assumed a nearly triangular shape.

Stage 10: Basal portion became more swollen and the tip portion flattened slightly.

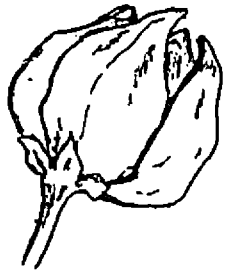
Stage 11: The tip became more flat and the basal part of petals start separating.

Stage 12: Anthesis occurs, with stigma exposed first.

In general, time lapse between two successive stages was more in the early stage compared to later stages. But the size increase was significant during later stages. The number of days required to reach the anthesis stage ranged from 25 in green cordate to 28 in purple cordate.

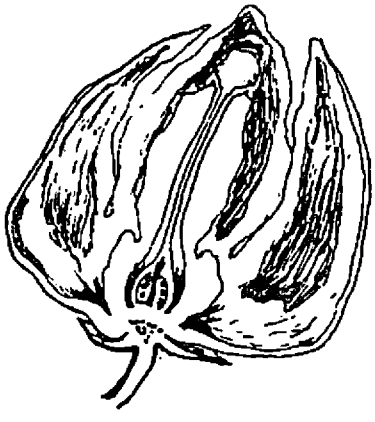
4.1.3 Floral biology

Adapathiyan flowers are bisexual and complete. Flowers are produced axillary on current season shoots in cymose inflorescence (Figure 1). Flowers are purplish crimson inside and pale pink outside. Flower colour of purple types is deep pink when compared to green types. They are bracteate, actinomorphic, regular, hypogynous and pentamerous. Pedicels are 2.5 to 5.0 cm. long. Calyx is deeply five partite with broadly ovate and obtuse lobes. Corolla is gamopetalous, deeply lobed, subrotate and overlap to the right. Corona arises from the base of the staminal column and is cupular, entire and uniseriate. Stamens are five in number and seen adnate to the base of the corolla tube and filaments cohere in five winged stigmatic head to form the gynostegium. Pollen grains at maturity are seen as pendulous mass called pollinium (Plates 9 and 11). Each stamen bear two pollinia, united by means of caudicles to form a translator. Pistil is bicarpellary, apocarpous and enclosed by the staminal tube. Two styles are free but united at the stigmatic head. Fruit consists of two distinct follicles diverging from the base.



Open flower

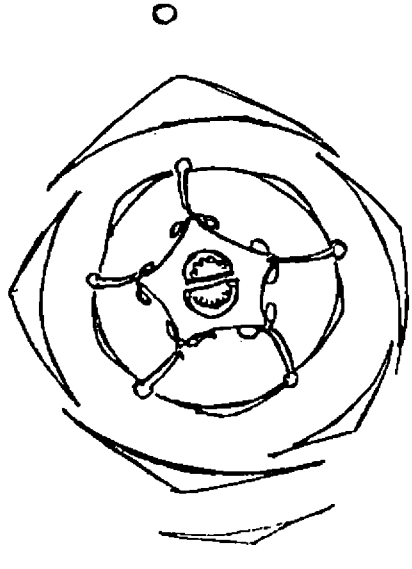
Gynostegium



L.S. of flower

Pollinia

T.S. of ovary



Floral diagram

$\updownarrow \oplus K_5 \overline{C_{(5)} A_{(5)}} G_{\underline{2}}$

Floral formula

Figure 1 Floral biology of adapathiyan

Plate 8. Stages of flower bud development.

4.1.3.1 Anthesis

Period of anthesis of the morphotypes taken at half hourly intervals are presented in Table 5. Anthesis started from 08.30 hours onwards in all the three types except green cordate and a fairly high anthesis (40%) was observed in purple elongate at this time. Maximum anthesis occured between 09.00 and 09.30 hours in all the morphotypes. During this period 54 per cent of the flowers had opened. The anthesis was continued up to 10.00 hours and the mean number of flowers opened from 09.30 to 10.00 hrs. The data also revealed that 12 per cent of the flowers opened during the period from 08.00 - 09.00 hrs. Thus within the time interval of 09.00 to 10 .00 hours 81 per cent of total flowers opened denoting this time interval to be the peak period of anthesis. After 10.00 hours, an insignificant per cent (6) flower buds opened. In purple elongate type anthesis was completed by 10.00 hours whereas in the other three types it was extended up to 10.30 hours.

4.1.3.2 Anther dehiscence

The results on anther dehiscence are presented in Table 6. Anther dehiscence occurred in flowers on the fourth day of opening. At this time flowers were in the wilting stage with petals almost closed. The colour of stigma changed to deep yellow with dry appearance without nectar. But nectar was found at the bottom part of the gynostegium. Such flowers are less visited by insect pollinators. Anther dehiscence started from 09.00 hours onwards on the fourth day and maximum dehiscence (92%) was noted from 11.00 to 13.00 hours. Not much difference in dehiscence per cent was observed thereafter. But no fruit set could be obtained by controlled pollination on fourth day.

4.1.3.3 Stigma receptivity

Shiny surface, light cream colour and nectariferous surface indicate receptive stigma. Lateral sides of the stigma were found to be receptive (Plate 10) which is covered

Table 5.Period of Anthesis of morphotypes of adapathiyan

Time hour	Number of flowers observed	Number of flowers opened				% of Total				Mean
		PE	GE	PC	GC	PE	GE	PC	GC	
08.00-08.30	25	0	0	0	0	0	0	0	0	0
08.30-09.00	25	10	2	1	0	40	8	4	0	13
09.00-09.30	25	11	17	12	14	44	68	48	56	54
9.30-10.00	25	4	5	9	9	16	20	36	36	27
10.00-10.30	25	0	1	3	2	0	4	12	8	6

Table 6. Period of anther dehisence in adapathiyan

Time	Number of flowers observed	Number dehisced	Dehiscence(%)
On the day of anthesis	25	0	0
Second day of anthesis	25	0	0
Third day of anthesis	25	0	0
Fourth day (09.00 to 11.00)	25	2	8
Fourth day (11.00 to 13.00)	25	23	92

Plate 9. Pollinia with translator and caudicles.

Plate 10. Lateral receptive side of the stigma

by tight membrane (Plate 11). After the first day of flower opening, stigma gradually lost the fresh colour, appeared dried and turned light brown. Hand pollination at different intervals starting from 24.00 hrs before anthesis to 48.00 hrs after anthesis showed that the maximum fruit set of 10 per cent was recorded when pollination was done at anthesis. Five per cent fruit set was obtained on pollinating the flowers 02.00 hrs after anthesis (Table 7). Maximum fruit set of 10 per cent was obtained when the flowers were pollinated between 09.00 and 11.00 hours indicating maximum receptivity. Thus the study indicated that the stigma receptivity was very short and the receptivity lasted for only for two hours after anthesis.

4.2. Pollen studies

4.2.1 Morphology

Pollen grains were agglutinated in clavate shaped, slightly curved pollinia. The two pollinia in one pair are attached by caudicles to the dark coloured pollen carriers or translators. Pollinia has one straight and one convex side. Line of dehiscence was seen on the convex side of the pollinia from where pollen tubes emerged after germination (Plate 12).

Pollen grains were more or less circular or oval in shape without exine. Examination of pollen grains under microscope showed that the individual pollen had a diameter of 59.4 μ with a range of 50 - 75 μ. Number of pollen grains observed per field was very low in the range of 2 to 3 (Plate 13).

4.2.2 Pollen production

Two hundred dehisced pollinia were crushed in water containing extran. But the suspension was very turbid and an even dispersion could not be obtained. When further diluted, no pollen grains could be observed in the microscopic field. This may be due to the sticky nature of pollen grains inside the pollinia. Stickiness was found to be

Table 7.Period of stigma receptivity in adapathiyan

Pollination time	Number of flowers pollinated	Number of fruits set	Percentage fruit set (%)
24 h before anthesis	20	0	0
18 h before anthesis	20	0	0
2 h before anthesis	20	0	0
At the time of anthesis	20	2	10
2 h after anthesis	20	1	5
6 h after anthesis	20	0	0
24 h after anthesis	20	0	0
48 h after anthesis	20	0	0

Plate 11. Stigma with membrane and translators inside the gynostegium.

Plate 12. Pollen germination from the convex side of the pollinia.

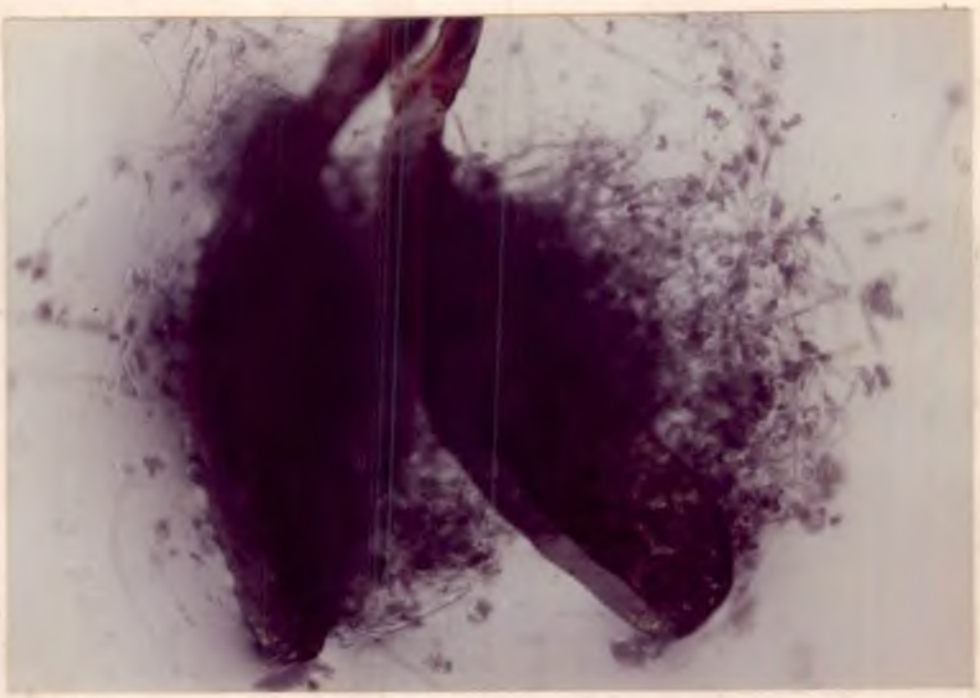
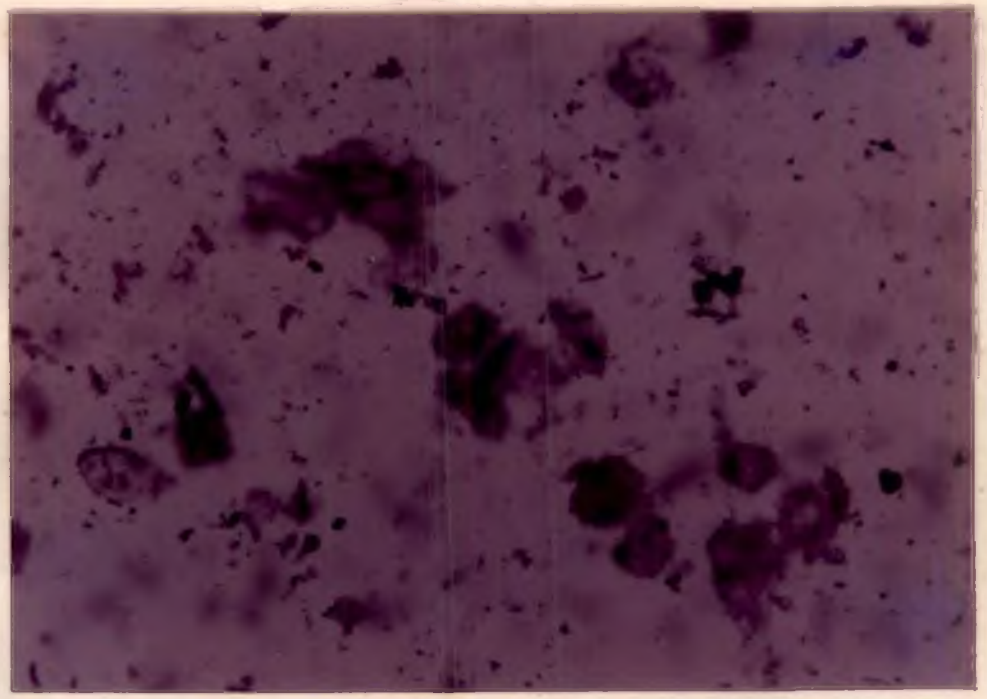


Plate 13 Pollen grains



accelerated when suspension was prepared. Due to the peculiar nature of pollinial wall, liberation of pollen from pollinia also was difficult.

4.2.3 Fertility

Pollen fertility was tried by germination and non germination assays.

Pollen fertility was assessed using stain test in pollinia collected from first, second, third, fourth and fifth day after opening of flowers. In first and second day of opening of flowers, higher pollen fertility values were observed (95.0% and 94.4% respectively). Fertility decreased gradually thereafter and on the fifth day only 57.1 per cent fertility was recorded (Table 8). In all the above cases, proper staining was obtained only after dehiscing the pollinia by keeping in distilled water for one to two hours. Variation in pollen fertility in morphotypes was studied (Table 9) and the data indicated that the fertility ranged from 90 per cent (green cordate) to 100 per cent (green elongate).

Observations on pollen germination during germination test at different intervals could not be recorded since the separation of pollen grains was difficult due to the sticky nature. It was also noted that teased out pollen grains from the pollinia could not be germinated. So, *in vitro* germination of pollinia was tried in distilled water, distilled water + sucrose and Brewbaker and Kwack's medium and germination was classified as sparse, medium and profuse. Line of dehiscence was noted in all pollinia when placed in distilled water and distilled water +sucrose. Pollen tube emanation was observed from the convex side of the pollinia. Irrespective of the season of flowering, germination and pollen tube growth of pollinia placed in Brewbaker and Kwack's media continued to be high whereas the results were not consistent in the case of pollinia placed in other media and sometimes they failed to germinate. When pollinia were placed in Brewbaker and Kwack's medium with caudicle and without caudicle, no variation in germination was noted. When pollinia were provided with transverse and vertical cuts, pollen tubes were seen emerging from the transversely cut region whereas no such emergence was noticed in vertical cuts. Pollinia collected from flowers of first to fifth day

Table 8.Pollen fertility as influenced by days after anthesis in adapathiyan

Days of flower opening	Number of pollen grains observed	Number of fertile pollen grains	Percentage pollen fertility(%)
Day of anthesis	20	19	95.0
Second day of anthesis	18	17	94.4
Third day of anthesis	15	13	86.7
Fourth day of anthesis	7	5	71.4
Fifth day of anthesis	7	4	57.1

Table 9. Pollen fertility of morphotypes of adapathiyan

Morphotype	Number of pollen grains observed	Number of fertile pollen grains	Percentage pollen fertility(%)
Purple elongate	21	20	95.24
Green elongate	23	23	100.00
Purple cordate	12	11	91.67
Green cordate	10	9	90.00

of opening were germinated *in vitro* . No difference in germination was noted among pollinia collected after first, second and third day of flower opening. But germination of pollinia collected from fourth and fifth day after the opening of flowers was sparse.

Among the morphotypes, green elongate type alone gave profuse germination while purple types recorded medium germination. Sparse germination was recorded in green cordate type alone.

4.3 Pollination studies

4.3.1 Pollinating agents

Carpenter bees of the order *Xylocopidae* and genus *Xylocopa* were found to visit the flowers. Numerous pollinia attached to the insect leg confirmed the entomophilous nature (Plate 14). Insect activity was found from flower opening till evening but maximum insect activity was found just after flower opening. When the insect visits the flower, translator of pollinia get attached to the leg of the insect and is removed from the flower as the insect moves away. During its visit on another flower these pollinia get inserted into the stigmatic cavity, helping pollination. Apart from theoretical situation, many pollinia were seen misplaced on the petals, stigmatic tips and nectaries. This misplacement may be explained by the fact that receptive side of the stigma is covered with a transparent and tight membrane. It is difficult for the insect to remove this membrane and place the pollinia into the stigmatic cavity. Since the flowers hang downwards, it is difficult for the insect to rest on the flower (Plate 15). When the insect tries to get a tight grip on the flower, injury to the flower was seen. The latex from the injuries coagulated there which prevented pollinial germination.

Plate 14. Pollinator of adapathiyan (Carpenter bee) with pollinia attached to the leg.

Plate 15. Pollinator resting on a flower.

4.3.2 Mode of pollination

Data on the percentage of fruit set under different modes of pollination are presented in Table 10.It is evident that only cross pollination occurs in adapathiyan. Open pollination and natural crosspollination showed very small difference in percentage of fruit set (12.7 and 11.6% respectively). Artificial crossing gave lower set (6.4%) than natural crossing (11.6%). Artificial crossing with intact pollinia and *in vitro* germinated pollinia were attempted. Cross pollination with *in vitro* germinated pollinia alone gave fruit set. Crossing with intact pollinia recorded no fruit set though low pollen germination and slight enlargement of ovaries were observed. Artificial selfing with *in vitro* germinated and intact pollinia and natural selfing resulted in no fruit set. But slight initial enlargement of ovaries was noted. This shows the possibility of self incompatibility mechanism operating in adapathiyan.

Fruit set observed in different crosses are presented in Table 11. The results showed that all the cross combination set fruit indicating that all the morphotypes are cross compatible. Higher set was observed in the cross GE x PE (13.30%) followed by its reciprocal cross PE x GE (9.09%). Least set was observed in the cross PE x GC (2.85%) and its reciprocal GC x PE also recorded less set of 3.3%.

Fruit set under different modes of pollination showed slight variation among morphotypes (Table 10). Maximum fruit set in open pollination was recorded in green cordate type (14.2), closely followed by purple elongate type (13.84) and minimum in green elongate type (10.8). Green cordate types recorded the highest set of 12.8 per cent in natural crossing also and the least in purple cordate (10.0). In artificial crossing with *in vitro* germinated pollinia, green elongate types recorded the highest fruit set (8.8) while green cordate showed the least (4.4).

Table 10 .Fruit set under different modes of pollination

Treatments	Number of flower observed	Number of fruits set				Fruit set(%)				Mean (%)
		PE	GE	PC	GC	PE	GE	PC	GC	
Open pollination	25	3.5	2.7	2.9	3.6	13.8	10.8	11.9	14.2	12.7
Natural self pollination	25	0	0	0	0	0	0	0	0	0
Artificial self pollination	25	0	0	0	0	0	0	0	0	0
Natural cross pollination	25	2.9	3.0	2.5	3.2	11.6	12.0	10.0	12.8	11.7
Artificial crossing with germinated pollina	25	1.7	2.2	1.4	1.1	6.9	8.8	5.6	4.4	6.4
Artificial crossing with intact pollinia	25	0	0	0	0	0	0	0	0	0

Table 11. Cross compatibility studies in morphotypes of adapathiyan

Sl. No.	Crosses	Number of flowers pollinated	Number of fruits set	Fruit set (%)
1.	GE x PE	30	4	13.30
2.	GE x PC	41	2	4.87
3.	PE x GE	33	3	9.09
4.	PE x GC	35	1	2.85
5.	PC x GE	30	2	6.67
6.	PC x GC	44	2	4.54
7.	GC x PE	33	1	3.30
8.	GC x PC	37	2	5.40

4.4 Fruit set and maturity

Fruit is a follicle (Plate 16). After anthesis flowers take 4 to 5 days to set fruits. Days to fruit maturity in adapathiyan ranged from 102.00 to 158.00 days with a mean of 139.05 (Table 2). Among the morphotypes , there was no significant difference in days to fruit set (Table 11).But the purple and green types differed significantly with respect to days to fruit maturity. Purple elongate types took maximum days to mature (150.4) and green elongate types the minimum (123.4).Out of the total fruits produced , 5.1 per cent fruits contained both the follicles fully developed.

4.5 Seed

Seeds are compressed or flat with large embryo and flat cotelydons (Plate 17). Number of seeds per fruit ranged from 170 to 447 in adapathiyan (Table 2). Hundred seed weight varied from 0.60 to 0.79 g with an average of 0.70 g. Seed germination in adapathiyan recorded a mean value of 89.25 per cent within the range of 85.2 to 93.3 (Table 2), as indicated in germination tests.

Among the seed characters studied, the morphotypes were statistically different in respect of seeds per fruit alone (Table 13). The highest number of seeds were observed in purple elongate type (418.4) while green types recorded with not much difference values (292.4 in green elongate type and 241.6 in green cordate type). The least value was observed in purple cordate type (193.0). All the morphotypes were on per with respect to 100 seed weight and seed germination percentage. The highest 100 seed weight was observed in green elongate type(0.70 g) and the lowest in purple cordate (0.68 g). But maximum germination percentage in the nursery was recorded in green cordate types (46.25) and minimum in green elongate types (18.91).

Table 12.Variation in fruit set and fruit maturity period of different morphotypes of adapathiyan

Morphotype	Days to fruit set	Days to fruit maturity
Purple elongate	4.2[a]	150.4[b]
Green elongate	4.2[a]	123.4[b]
Purple cordate	4.0[a]	148.6[b]
Green cordate	3.8[a]	133.8[b]

Treatment means in a column with same letter do not differ significantly.

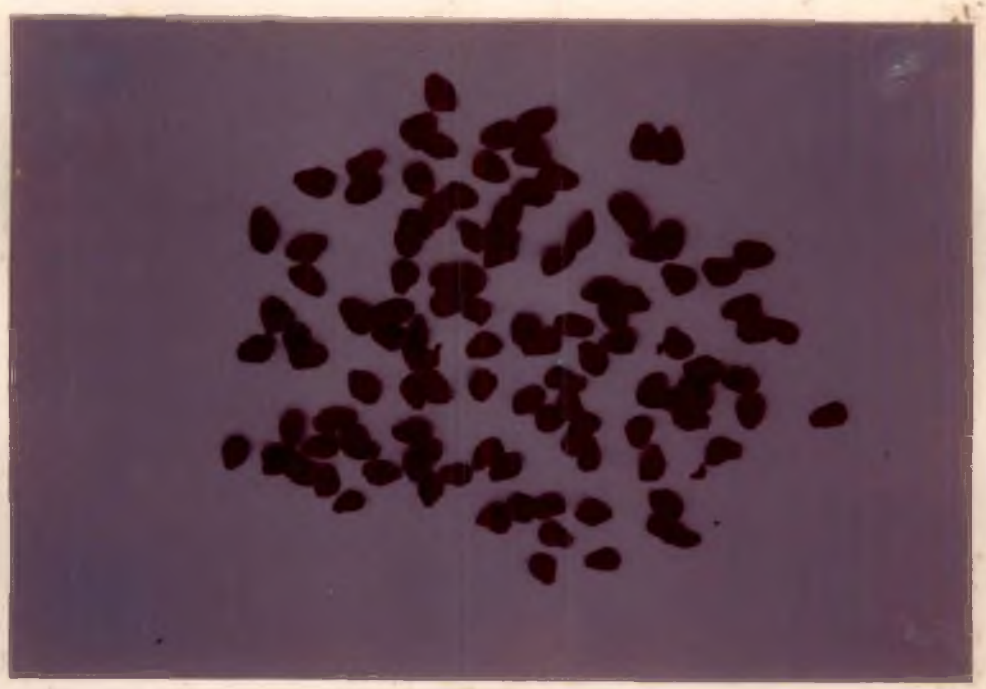
Table 13. Variation in seed characters of morphotypes of adapathiyan

Morphotype	Number of seeds per fruit	100 seed weight (g)	Seed germination (%)
Purple elongate	418.4[d]	0. 691[a]	35.19[a]
Green elongate	292.4[c]	0.703[a]	18.19[a]
Purple cordate	193.0[a]	0.689[a]	37.62[a]
Green cordate	241.6[b]	0.701[a]	46.25[a]

Treatment means in a column with same letter do not differ significantly

Plate 16. Fruit of adapathiyan.

Plate 17. Dry seeds of adapathiyan.



4.6 Biometric characters

Variation of morphotypes with respect to the characters like internodal length, collar girth and number of branches was studied (Table 14). The morphotypes were on par with respect to biometric characters. Length of internode was maximum in purple elongate type (7.64cm) and minimum in purple cordate (3.02cm) while collar girth recorded the highest value in purple cordate type (2.4cm) and the least in purple elongate types (1.8cm). Branching was the highest in purple elongate (2.8) followed by green elongate (2.6) and the least in purple cordate and green cordate types (2.2).

4.7 Yield

When root yield of morphotypes were compared, the highest yield was recorded in purple cordate type (65.5g plant^{-1}) and the least in green elongate type (42.6g plant^{-1}) as shown in Table 14.

4.8 Biochemical characters

The results are presented in Table 15. In adapathiyan , soluble and insoluble sugar contents in roots were fairly high with a mean of 6.06 and 34.59 per cent respectively. Root amino acid content recorded a percentage of 0.66. Protein content in roots and leaves was observed to be 0.63 and 2.09 per cent respectively.

Morphotypes were statistically on par with respect to soluble and insoluble sugar and protein content in leaves and roots. The highest per cent of soluble sugars was observed in purple elongate (8.52%) and that of insoluble sugar in green elongate (40.70%). The lowest per cent was recorded in green elongate (4.76%) followed by

Table 14. Biometric characters and yield of roots of four morphotypes of adapathiyan

Morphotypes	Internodal length (cm)	Collar girth (cm)	Number of branches	Yield of roots(g plant^{-1})
Purple elongate	7.64[a]	1.8[a]	2.8[a]	49.3[a]
Green elongate	7.32[a]	2.3[a]	2.6[a]	42.6[a]
Purple cordate	3.02[a]	2.4[a]	2.2[a]	65.6[a]
Green cordate	5.86[a]	2.0[a]	2.2[a]	60.6[a]
Mean	5.96	2.1	2.5	54.5

Treatment means in a column with same letter do not differ significantly

Table 15. Biochemical variation in four morphotypes of adapathiyan

	Root					Leaf
Morphotype	Soluble sugar (%)	Insoluble sugar (%)	Total sugar (%)	Amino acid (%)	Protein (%)	Protein (%)
Purple elongate	8.52[a]	25.54[a]	46.63[a]	0.21[a]	0.63[a]	1.94[a]
Green elongate	4.76[a]	34.27[a]	42.71[a]	0.88[a]	0.55[a]	2.23[a]
Purple cordate	4.85[a]	37.86[a]	39.03[a]	0.83[c]	0.76[a]	1.91[a]
Green cordate	5.93[a]	40.70[a]	34.06[a]	0.71[b]	0.58[a]	2.27[a]
Mean	6.02	34.59	40.60	0.66	0.63	2.09

Treatment means in a column with same letter do not differ significantly

purple elongate (25.54%). Soluble sugars present in the roots are medicinally important (8.52%). The results of this study indicated that purple elongate type contained the highest percentage of soluble sugars , followed by green cordate (5.93%) and purple cordate (4.85%). A generalisation on the superior medicinal property of purple types could not be made but the purple elongate type was found to be qualitatively superior as evinced by the data. Total sugar content was maximum in purple elongate (46.63) and minimum in green cordate (34.06). Root protein content was maximum in purple cordate (0.76%) which also contained the least leaf protein content (1.91%). Green elongate type recorded the least root protein content of 0.55 per cent and green cordate type recorded the highest leaf protein (2.27%). In all the morphotypes, leaf protein content was more than twice the root protein content. Total amino acid content in roots showed significant difference in the range of 0.21 to 0.88 per cent in the four types. Maximum content recorded in green elongate type (0.88%) was on par with that in purple cordate types (0.83%).

4.9 Seedling variability

In the hybrid and open pollinated progenies, leaf pigmentation and shape followed no definite Mendelian ratio confirming the polygenic nature of the characters (Table 16). Quantitative estimation of seedling variability was carried out and results are summarised in Table 17.

Leaf area, collar girth, number of branches and internodal length showed significant difference in the seedlings. H_3, H_4, and P_2 showed similar leaf area pattern, but differed significantly from H_5, H_6, and H_7. P_2 recorded the least range of 6.6 to 28.3cm^2 in leaf area while the highest range was observed in H_2 (5.9 - 71.4 cm^2). H_2, H_5, H_6,H_7,P_1 and P_4 recorded higher mean leaf area when compared to the overall mean of 31.44 cm^2.

Table 16. Inheritance of leaf shape and pigmentation in seedlings:

Hybrid or open pollinated progeny	Leaf shape		Pigmentation	
	%C	%E	%G	%P
H_1 - PE x GE	50	50	13	87
H_2 - PE x GC	75	25	51	49
H_3 - PC x GE	92	8	16	84
H_4 - PC x GC	88	12	49	51
H_5 - GC x PE	39	61	38	62
H_6 - GC x PC	33	67	11	89
H_7 - GE x PE	26	74	10	90
H_8 - GE x PC	52	48	52	48
P_1 - OP with parent PE	62	38	22	78
P_2 - OP with parent GE	100	0	25	75
P_3 - OP with parent PC	20	80	50	50
P_4 - OP with unknown parent	46	54	44	56
Mean	56.93	43.08	31.75	68.25

Table 17. Variability in biometric characters of seedlings.

Hybrid or open pollinated progeny	Leaf area (cm^2)		Collar girth (cm)		Number of branches		Internodal length (cm)	
	Range	Mean	Range	Mean	Range	Mean	Range	Mean
H_1-PE × GE	9.8 - 56.2	31.2^{ab}	0.5-1.3	0.9^{ab}	0-1	0.2^{a}	1.1-2.8	1.8^{a}
H_2-PE × GC	5.9-71.4	43.1^{c}	0.5-1.9	1.2^{c}	0-2	0.7^{b}	1.1-3.4	2.1^{ab}
H_3-PC × GE	10.6-43.7	23.1^{a}	0.7-1.1	0.9^{ab}	0-2	0.2^{a}	1.1-2.1	1.5^{a}
H_4-PC × GC	5.3-41.6	25.7^{a}	0.7-1.3	1.1^{bc}	0-1	0.1^{a}	1.4-4.1	2.3^{b}
H_5-GC × PE	7.6-64.1	33.4^{bc}	0.5-1.0	0.9^{a}	0-1	0.1^{a}	1.1-3.8	2.0^{ab}
H_6-GC × PC	26.8-43.4	35.3^{bc}	1.0-1.3	1.1^{bc}	0-2	0.4^{ab}	1.2-1.6	1.4^{a}
H_7-GE × PE	10.5-64.1	39.0^{C}	0.5-1.7	1.0^{b}	0-1	0.1^{a}	1.2-5.4	2.1^{ab}
H_8-GE × PC	6.3-54.8	28.0^{ab}	0.5-1.2	0.9^{b}	0.0	0.0	1.1-2.2	2.0^{ab}
P_1-OP with parent PE	11.2-64.9	36.6^{bc}	0.6-1.3	1.1^{bc}	0-2	0.8^{b}	0.9-2.5	1.7^{a}
P_2- OP with parent GE	6.6-28.3	19.3^{a}	1.0-1.1	1.0^{ab}	0-1	0.1^{a}	1.1-2.2	1.6^{a}
P_3 - OP with parent PC	20.3-37.3	30.3^{ab}	0.8-1	0.9^{ab}	0-1	0.0	1.6-5.1	2.7^{b}
P_4 - OP with unknown parent	3.7-68.6	32.5^{b}	0.5-0.8	1.0^{ab}	0-1	0.0	1.1-8.9	2.1^{ab}
Range	19.3-43.1		0.9-1.2		0.0-0.8		1.4-2.7	
Mean	31.4		1.0		0.2		1.9	

Treatment means in a column with same letter do not differ significantly.

With respect to collar girth, H_2 alone formed one group which differed significantly from H_1,H_3,H_5, P_2, P_3 and P_4. Widest range for collar girth was observed in H_2 (0.5 - 1.9) while the narrowest range in P_2 (1.0-1.1). Overall range observed was 0.86 - 1.16 cm. H_2, H_4, H_6, H_7, P_1 and P_2 were superior in collar girth when compared to the overall mean of 1.0.

Number of branches followed a very small range of 0.0 to 0.83, with a mean of 0.24. Except H_2, H_6 and P_1 all others recorded lower mean values when compared to the overall mean of 0.24. H_1, H_3, H_4, H_5, H_7 and P_2 were statistically on per, but varied significantly from P_1 and H_2 while H_6 showed an intermediate pattern.

H_1, H_3, H_6, $P_1$and P_2 formed one group while P_3 and H_4 formed a second group and H_2, H_5, H_7, H_8 and P_4 formed an intermediate group which showed similarity to both the other groups statistically with respect to internodal length. H_2, H_4, H_5, H_7, H_8, P_3 and P_4 showed superior values when compared to the overall mean of 1.93. Overall range recorded was narrow (1.42 to 2.68) while the widest range was recorded in P_4 (1.1- 8.9). In none of the characters, hybrids or open pollinated progenies formed separate groups.

Discussion

DISCUSSION

The results of the present investigation on reproductive behaviour of adapathiyan (*Holostemma adakodien* Schult.) are discussed in this chapter.

5.1 Flowering and floral characters

Holostemma is a profuse flowering plant. Flowering was found to follow an almost similar pattern in the morphotypes studied. Flowering occur on current season shoots. Occurrence of vegetative flush also is noted in leaf axils with flower buds.

Visual emergence of flower buds commenced in June in all the morphotypes except green cordate in which flower buds appeared in July. Peak flowering period was also late in green cordate type (September-October) while it was August-September in other three types. The flowering season ended in October in elongate types but in November in green cordate type and in September in purple cordate type. These observations are in concurrence with the report of Meera(1994). According to Sasidharan and Sivarajan(1996) the flowering season is September-November. The slight variation observed in the present study might be due to the difference in morphotypes and maturity of the plants.

Flowers appear in axillary cymes and hang downwards. Flowers opening started from the periphery of an inflorescence and progressed towards the centre. The number of inflorescence per plant varied from 10-64 and the morphotypes showed significant difference. Elongate types were different from cordate types. Number of flowers per inflorescence varied from 2-20 with a mean of 8.0. Purple types were on par but green types differed significantly. Total number of flowers per plant recorded an average of 336.39 with a range of 39.0 to 878.04. Number of days for complete opening of an inflorescence varied from 4-15 with an average of 8.8. Completion of flower opening in an inflorescence was the fastest in green cordate type (11.6) This might be due to the fact that green cordate types produce the least number of flowers per inflorescence (3.0). But a

corresponding higher value for number of flowers per inflorescence was not observed in purple cordate type (6.6). The highest value was observed in green elongate type (16.2) where flower opening was completed in 7.4 days.

In adapathiyan, the flower bud development from bud emergence to anthesis was found to follow twelve arbitrary stages. This period of development was found to take maximum span in purple cordate types (28 days) and minimum in green cordate types (25 days). This is in conformation with the observation of Meera (1994).

Adapathiyan flowers are bisexual, complete bracteate, pedicellate, actinomorphic, hypogynous, cyclic and pentamerous. Sepals and petals are five each in number and are in imbricate aestivation. Corona arises from the base of the staminal column. Stamens are attached to the base of the corolla tube and filaments fuse with the stigmatic head to form the gynostegium. Pollen grains agglutinate to form two pollinia in each stamen. Pistil is bicarpellary, apocarpous and each locule with many ovules on marginal placenta. Sivarajan and Balachandran (1994) and Kirtikar and Basu (1975) made these observations. Holostemma flower represents a typical asclepiadaceous flower due to the presence of corona, pollinia and gynostegium (Vasishta, 1974).

Anthesis started from 08.30 hours onwards and extended up to 10.30 hours. Green cordate type showed late anthesis where flowers opened from 09.00 to 09.30 hours. But the peak period (09.00 to 09.30) coincided with that of other morphotypes (09.00 to 09.30). In general, 81 per cent flowers opened between 09.00 and 10.00 hours. Flowers remained open for four more days.

Anther dehiscence was observed on fourth day of flower opening with maximum between 11.00 and 13.00 hours and thereafter no increase in dehiscence was noted. During this period, *in situ* germination was noticed though very rare. *In situ* germination was reported in other Asclepiads by Sreedevi (1989). But self pollination does not occur in Holostemma since the peak stigma receptivity coincides with anthesis and the receptivity is lost immediately. On fourth day, flowers were in the wilting stage

and stigma appeared dry and deep yellow. Nectar was not observed on the lateral sides of the stigma. But nectar was present at the bottom portion of the gynostegium which could be detected only when flower is cut transversely. On the fifth day, flower would close and petals lose their turgidity. Pollinia also lost their natural shiny light yellow colour.

Stigma receptivity is indicated by shiny appearance, light cream colour and presence of nectar. Maximum stigma receptivity coincided with maximum flower opening period from 09.00 to 11.00 hours as proved by controlled pollination. The colour of stigma changed from second day of flower opening onwards and became deep yellow when flower wilted. The receptive side of the stigma was shifted to lateral sides, which was reported by Sreedevi (1989) in other Asclepiads. Lateral sides of the stigma were tightly covered with a transparent membranous covering. Stigma receptivity is for a very short period and flowers exhibit protogynous nature. These factors contribute to the low seed set observed in adapathiyan

5.2 Pollen studies

Pollen grains are agglutinated in the form of pollinia which are clavate shaped, yellow, flat structures. Pollen grains are more or less circular or oval in shape without exine. Similar observations were reported by Sreedevi and Namboodiri(1979) in *Asclepias* and *Dregea*. Pollen diameter varied from 50-75μ with a mean of 59.4 μ.

Assessment of pollen production was not successful since no pollen grains were observed in the haemocytometer under microscopic field. This may be due to the fact that pollen grains were agglutinated inside the pollinia and the peculiar structure of the pollinial wall did not liberate the pollen from the pollinia. It was also not possible to tease out the pollinia and remove the pollinial wall. Even with 200 pollinia, proper dispersion could not be obtained. The suspension appeared turbid and sticky. The suspension media containing water and extran could not remove this stickiness but turbid appearance was enhanced.

For the same reasons, pollen fertility could be assessed by stain test and *in vitro* germination was not successful. Viswanathan and Lakshmanan, 1984 reported that the exact number of pollen tubes and cells involved therein could not be ascertained. It was also reported that teased out pollen grains from the pollinia of *Calotropis gigantea* could not be germinated well on any media.

Thus pollen fertility was assessed by Alexander's stain test in each day of flower opening. Pollen fertility recorded very close values of 95 and 94 per cent on first and second day. Thereafter it showed a progressive decrease with to 57.1 per cent on fifth day. In *Calotropis gigantea,* 78.9 per cent fertility was recorded by Viswanathan and Lakshmanan (1984). Morphotypes also showed variation in pollen fertility ranging from 90 per cent (green cordate type) to 100 per cent (green elongate type).

Pollinia were placed for *in vitro* germination in distilled water alone, distilled water + sucrose and Brewbaker and Kwack's medium and germination was grouped as sparse, medium and profuse. Though line of dehiscence could be seen in all the media, germination and pollen tube growth showed consistent result in Brewbaker and Kwack's medium alone. Pollinia with and without caudicle did not show any variation in germination indicating the role of caudicle as a means for transfer of pollinia by insects. Transverse cut on the pollinia resulted in tube emergence from the cut region. This conforms the report of Sreedevei and Namboodiri (1982) that polarity of pollen tubes within the pollinium was governed by permeability of the germ furrow region alone. When flowers on each day of opening were kept for *in vitro* germination of pollinia, sparse germination was noted in flowers collected after four or five days of opening. Among the morphotypes, green elongate type alone gave profuse germination while green cordate recorded sparse germination and the other two types recorded medium germination. In the artificial medium, sucrose provide the energy need of the pollen grains and control osmotic concentration. Brewbaker and Kwack's medium contains, considerable amount of calcium, boron, magnesium and potassium are there. Calcium is very essential for pollen germination without which growth may be fully inhibited. Ions like K, Na or Mg singly or together could act to enhance calcium activity (Brewbaker and

Kwack, 1963). Boric acid is a stimulant for pollen germination. Gauch and Dugger (1953) accounted the effect of boron for the formation of an ionisable sucrose- borate complex which moved through the cells more rapidly than non-ionisable sucrose molecules. Since Brewbaker and Kwack's medium contained all these elements in comparatively higher proportions it was found most suitable for pollen germination in adapathiyan also. This medium was reported to be suitable for another Asclepiad, *Calotropis gigantea* . (Viswanathan and Lakshmanan, 1984).

5.3 Pollination studies

Pollination in adapathiyan is entomophilous, as in other Asclepiads as reported by Rendle (1975). Presence of nectariferous gynostegium is in favour of insect pollination. Carpenter bees (*Xylocopa spp*) were found to be the pollinating agents in adapathiyan. *Xylocopa spp* were the pollinating agents in *Calotropis gigantea* as reported by Ramakrishna and Arekal (1979) and Ali and Ali (1989). Position of the pollinia enclosed within the gynostegium, downward position of the flower and tight membrane covering the lateral receptive side of the stigma may totally block other modes of pollination. The translator of the pollinia is an adaptation for insect pollination. When the insects visit one flower, translator get attached to its leg and is removed from the flower as the insect moves away. During its visit on another flower, these pollinia may be inserted into the stigmatic cavity. Many-a-times pollinia were seen misplaced on the petals, stigmatic tips and nectaries. When the insect tries to get a good grip on the downward positioned flower, injury to the flower is probable. The exuded latex getting coagulated at the stigmatic sides may hinder further pollinial germination.

Pollination studies with different modes of pollination revealed that adapathiyan flower was solely cross pollinated. The highest percentage of fruit set was observed under open pollination (12.7) followed by natural crossing (11.6) and artificial crossing with *in vitro* germinated pollinia (6.4). *In situ* germination was seen very rarely but is reported in other Asclepiads by Sreedevi (1989) In adapathiyan this was noted only on fourth day of anthesis, when stigma was non-receptive climating the chance for self pollination. No fruit

set was observed when selfing was done artificially and naturally. Chance for natural selfing is prevented by the innate protogynous nature, the restricted span of stigma receptivity and wide time lapse between two phases. The flowers subjected to artificial selfing showed slight ovary enlargement before the flowers dropped which is similar to the observation in *Asclepias syriaca* (Sparrow, 1948). This hints the involvement of self-incompatibility, probably post fertilisation incompatibility as a hindrance for artificial self-pollination. This can also be considered as another adaptation for cross pollination in adapathiyan.. A similar case of post fertilisation incompatibility mechanism was proposed in *Asclepias syriaca* (Kahn and Morse, 1991). In this behaviour also adapathiyan forms single group with most species of *Asclepias* which exhibits self-incompatibility (Wyatt, 1980).

Compatibility studies indicated that all the morphotypes were cross compatible. The highest fruit set was recorded in the cross GE x PE (13.30) and the lowest in PE x GC (2.85). It was seen that when morphotypes with profuse pollen germination (green elongate) was used as male parent, higher set was recorded as the crosses PE x GE (9.09) and PC x GE (6.67). Green cordate type which recorded sparse pollen germination when used as male parent, produced least set in PE x GC (2.85) and a lesser value in PC x GC (4.54).

Fruit set could not be improved by artificial crossing. When crossing was done with intact pollinia, no set was obtained. Such pollinia dehisced on the stigma, but growth of tubes was sparse. To rectify this problem, artificial cross pollination done with *in vitro* germinated pollinia gave fruit set, but was low when compared to open pollination. During artificial crossing, the membrane covering the receptive surface of the stigma had to be removed which is likely to cause injury to the flower and later may coagulate on the receptive side of the stigma. This may hinder further pollen tube growth. This may be the reason for the difference in fruit set between natural and artificial pollination. There is every chance for pre- zygotic or post fertilisation barriers operating at various stages of development which also contribute to the low fruit set. In the case of open pollination also the same problems arise. Though the pollinial mechanism and translator are adaptations

for highly efficient insect pollination and pollen dispersal, misplacement of pollinia is most common probably due to the lateral shifting of receptive surface of stigma which is hidden by the membrane. It is to be presumed that pollination is only accidental during the act of nectar foraging. Thus pollen limitation during pollination also led to decreased fruit set as suggested by Cabin *et al* (1991). Sparrow (1948) also suggested that lack of pollination caused low fruit set. Lower set on artificial crossing than natural open pollination was also reported in *Asclepias tuberosa* where fruit set was 0.17 per cent (Wyatt, 1980). Sparrow (1948) reported a similar case where artificial crossing could not improve seed set (10.35%) over natural set (10.0%) in *Asclepias syriaca.*

5.4. Fruit set and maturity

In adapathiyan, flowers generally take 4-5 days for fruit set. Not much variation was observed among the morphotypes for the days to fruit set and it ranged from 3.8 to 4.2. Fruits take 102-158 days to attain maturity. Purple and green types differed significantly with respect to days to fruit maturity, but they were homogenous among themselves. During the fruit development stages, the size increase of the ovary was most rapid immediately after the fruit set. Out of the two ovaries, one usually abort as reported by Vasista (1974). In adapathiyan, 5.1 per cent of total fruits produced contained both the carpels.

In concurrence with the finding of Sreedevi (1989) that fruit set in Asclepiads to be low (5-10%), in adapathiyan also fruit set is low (5-17%). Though the members of the Asclepiadaceae family of which adapathiyan also forms a part, were well adapted for cross pollination by insects, certain lacunae during pollination result in low fruit set. Pollen removal from the flower is not low, but its insertion into receptive stigmatic surface is very low. Ants rob many pollinia. Pollen being highly fertile, pre-zygotic and post fertilisation barriers operating may also result in low fruit set, similar to *Asclepias exaltata* (Sage and Williams, 1991).

5.5 Seed

Each fruit carries on an average 170-447 seeds on an average. Among the morphotypes, purple cordate type produced the lowest number of seeds per fruit. Hundred seed weight varied from 0.60 to 0.79 g with an average of 0.70 g. A high seed germination percent was noted (89.25) when germination tests were carried out in petridish but the same was decreased under field conditions. Different morphotypes were on par with respect to seed germination percentage. The highest percentage was recorded in green cordate (46.25) and the lowest in green elongate type (18.19).The production of large number of seeds, efficient means of distribution and high seed germination percentage are of great significance in propagation. The causes of low germination under field conditions have to be investigated and methods are to be standarised to get higher germination percentage. Thus inefficiency in pollination mechanism may be overcome. But since the crop is endangered and highly demanded commercial cultivation has to be accelerated which naturally needs improvement in fruit set.

5.6 Biometric characters and yield

Biometric characters like internodal length, collar girth and number of branches were reported to have significant positive correlation with root yield (Meera, 1994). From the observation, purple elongate type recorded maximum value for internodal length (7.64 cm) and number of branches (2.8) .Collar girth recorded maximum value in purple cordate type (2.4cm). But it recorded least value for number of branches (2.2) and internodal length (3.02cm), and found to be most yielding (65.6 g plant^{-1}). Thus purple cordate type with highest root may be used for cultivation large scale.

5.7 Chemical components

Among the chemical components, sugar content in the roots contribute to the medicinal properties of adapathiyan (CSIR, 1959). These are amino sugars like α-amyrin, lupeol and β-sitosterol. Soluble sugar recorded highest percentage in purple elongate type

(8.52) and the lowest in green elongate type (4.76). Purple cordate (4.85) and green cordate (5.93) recorded intermediate values. Since soluble sugars are of medicinal value, it can be confirmed that purple elongate types are medicinally important but a generalisation on purple is not possible. Insoluble sugars and total sugar recorded the highest values in green elongate (40.70%) and green cordate (46.63%) while the corresponding lowest figures were recorded in purple elongate which were. 25.54 and 34.06 per cent respectively. Amino acid content was the highest in green elongate type (0.882%) and the least in purple elongate type (0.205%). Leaves contained more protein than roots in all the morphotypes which was almost three times the content present in roots. This suggests and supports the use of adapathiyan leaves as vegetable.

5.8. Seedling variabiliy

The hybrid and open pollinated progenies when considered as a whole, predominant leaf shape and colour were found to be cordate and purple respectively. The progenies of the four morphotypes and its reciprocals did not follow a Mendelian ratio with regard to leaf shape and pigmentation. This throws light into the fact that leaf shape and pigmentation are controlled by polygenes.

Biometric observations on seedling progenies showed that hybrid and open pollinated progenies did not form separate groups. In the case of leaf area, highest value was recorded by P_1 (36.58cm^2) and P_4 recorded higher value (32.47) compared to overall mean (31.4). P_1, P_2 and P_4 recorded equal or higher values when compared with overall mean collar girth (1.0cm). Among the hybrid progenies, five out of eight recorded lower values for collar girth when compared with overall mean. P_3 and H_3 (PCXGE) which have common female parent (purple cordate) recorded same value (0.9cm) formed one group. This shows that they are not at all different. With respect to number of branches also, highest value was recorded for P_1 (0.8). Four out of eight hybrids showed lower values than overall mean (0.2). P_1 and P_2 (PE x GC) with common female parent (purple elongate) recorded closer values (0.8 and 0.7) and formed one group. Also P_2 and H_7 (GE x PE) with green elongate type as female parent also recorded equal values (0.1) and

formed one group showing their complete homogeneity. Maximum value for internodal length was recorded in P_3 (2.7cm) which was much higher than general mean (1.9). P_1 and H_1 (PE x GE) formed one group with respect to internodal length and recorded closer values of 1.7cm and 1.8cm respectively. They share purple elongate type as a common female parent. Another similarity observed was between P_3 and H_4 (PC x GC) which form one group denoting their comparative performance. These observations suggest that open pollinated progenies are not at all inferior to hybrid progenies and many-a-times behave on par with hybrids or even superior. Out of the four characters, maximum values for two characters viz. internodal length and number of branches were recorded in open pollinated progenies. Exclusion of self- pollination through inherent factors and exclusive cross pollination favour the exploitation of heterosis even in open pollinated progenies. The superior performance of open pollinated progenies and hybrids involving purple elongate and purple cordate types as one of the parents suggest the collection of seeds from such parents. This increased vigour and growth suggest a possible high yield in the progenies. This is supported by the fact that purple types are more productive. Artificial crossing could not improve the seed set. In that aspect also open pollination is a better choice.

Adapathiyan plant flowers profusely with a protracted flowering season. The protogynous nature and short span of stigma receptivity hinder natural self pollination. Artificial selfing also was found unfruitful denoting the operation of self incompatibility mechanism in this crop but is found to be cross combatible. Cross pollination by entomophily is the mode of pollination for which the flower is highly adapted. Fruit set in general was low and artificial crossing could not improve the fruit set over open pollination. Though pollen fertility is high, the low fruit set can be attributed to the injuries caused to the flower during pollination which may promote abscission, inhibition of pollen tube growth on stigmatic surface due to pre zygotic and post fertilisation barriers which may be operating in adapathiyan. So methods to overcome these barriers is worth investigating. Being cross pollinated, open pollinated progenies exhibit heterosis and are not inferior to hybrid progenies. The superior performance of progenies involving purple cordate and purple elongate types as one of the parents highlights its utility as a seed parent.

Summary

SUMMARY

The present investigations on reproductive behaviour of adapathiyan (*Holostemma adakodien* Schult) was undertaken on the adapathiyan plants maintained by the Department of Plantation Crops and Spices, College of Horticulture, Vellanikkara, during the period from 1995-1996. The study was aimed at understanding the flowering behaviour, floral biology and pollination mechanism in adapathiyan and to unravel the reasons for low fruit set. It is also intended to evaluate the morphotypes for morphological and qualitative differences and attempt hybridisation between morphotypes and assess the extent of variability in the seedling progenies in the nursery stage to isolate the ideal types.

The results are summarised below:

The plants of adapathiyan showed a protracted period of flowering extending from June to November. In all the types except purple cordate, flowering season extended from June to October. In purple cordate type, it was extended from June to September. Peak period of flowering was observed from August to September in all the three types except green cordate wherein peak was observed during September-October.

The flower bud development in adapathiyan could be divided into twelve arbitrary stages. A period of 25-28 days was needed from visual flower bud emergence to flower opening.

Holostemma bears bisexual flowers. Inflorescence is axillary umbellate cymes. Flowers open from the periphery of an inflorescence towards the centre. The plant flowers profusely and bear upto 270 flowers per plant. Number of days for complete opening of an inflorescence varied from 4 to 15. Morphotypes did not differ significanltly with regard to this character and it ranged from 7.4 in green elongate to 11.6 in purple cordate. Number of inflorescence per plant observed was on par in cordate and elongate types.

Number of flowers per inflorescence varied significantly among the morphotypes with a maximum of 6.2 in green elongate type.

Flowers of adapathiyan are complete, regular, bisexual, actinomorphic and pentamerous. Calyx consists of five sepals and petals are five in number, united at the base. Androecium consists of five pairs of pollinia arranged on the lateral sides of the stigma. Gynoecium consists of two ovaries which are free and styles unite at the stigmatic head. Receptivity of stigma is shifted to lateral sides. Stamens fuse with the stigmate disc to form the gynostegium. Corona, a characterstic feature of asclepiadaceous flower is staminal in adapathiyan.

Anthesis started in adapathiyan from 08.30 hours onwards and extended upto 10.00 hours with the peak between 09.00 and 09.30 hours. In purple elongate types, a considerable percentage of anthesis occurred at 08.30 hours.

Anther dehiscence was observed in fourth day opened flower which was extended to fifth day opened flowers also, if it remained on the plant. The exact time of dehiscence was observed to be between 11.00 and 13.00 hours. At this stage, flowers were in the wilting stage and stigma seemed non-receptive.

Stigma receptivity was maximum on the first day opened flowers coinciding with the anthesis time. The stigma was pale cream, shiny and nectariferous at the receptive stage. Pollen grains were more or less circular in shape without exine having a diameter of 50-75 µ with a mean of 59.4 µ. Pollen production per pollinia could not be estimated since proper dispersion of pollen grains in water containing extran could not be obtained. Pollen fertility was assessed by germination and non-germination assays. Nongermination assay tried with Alexander's stain indicated that pollen fertility was very high in the range of 90-100 per cent among the morphotypes though number of pollen grains observed in each field were very low. As the days after anthesis increased, fertility decreased from 95.0 to 57.1. *In vitro* germination was tried on different media. Brewbaker and Kwack's

medium was proved to be the best medium which permitted profuse growth of pollen tubes. In distilled water and distilled water + sucrose, few tubes emerged and the results were not consistent.

Pollination was strictly entomophilous with carpenter bees (*Xylocopa* spp.) as the major pollinators. Flowers are suitably designed for insect pollination with pollinial mechanism. Other agents of pollination are prohibited since it is very difficult to remove the pollinia and its insertion into another flower. Receptive surface of the stigma is shifted to the lateral sides which is hidden by a thin membrane. When pollination by different modes were attempted, open pollination gave the maximum fruit set of 12.6 per cent while artificial pollination with *in vitro* germinated pollinia could produce only 6.4 per cent set. Natural selfing was totally inhibited by the protogynous nature and wide time gap between stigma receptivity and anther dehiscence. Artificial selfing also could not give any fruit set suggesting the existence of self incompatibility mechanism either in pre or post fertilisation stage.Crossing studies indicated that all the morphotypes were cross compatible.

Flowers took 3.8 to 4.2 days to set the fruits. Fruits attained maturity in 102-158 days. Fruit maturity period was maximum in purple elongate type (150.4 days) and minimum in green elongate type (123.4days). In general, fruit set percentage in adapathiyan varied from 5 to 17 with a mean of 12.84 per cent. Low insertion of pollinia by insects, pollinia being eaten away by ants, failure of pollen tubes to penetrate the ovary and effect fertilisation and similar failures at various stages of development are the reasons attributed for the low fruit set.

Seeds are flat and compressed with a tuft of hairs at the hilum region. Seeds per fruit varied from 170 to 447. The morphotypes sh significant different and ranged from 418.4 seeds in purple elongate type to 193.0 ... purple cordate type. Hundred seed weight exhibited a narrow range from 0.689 g to 0.703 g in the four morphotypes. Seed

germination percentage recorded was 89.25 per cent in germination test and 18.19 -46.25 per cent under field conditions.

Yield and biometric characters directly correlated to yield were studied in the morphotypes. Maximum yield was reported in purple cordate type (65.6 g) and minimum in green elongate type (42.6 g). Purple elongate types recorded highest values in terms of internodal length (7.64 cm) and number of branches (2.8). Collar girth recorded highest value in purple cordate type (2.4 cm). This observation support the fact that purple types are more productive.

Biochemical analysis of adapathiyan roots revealed that soluble sugars content was highest in purple elongate (8.52). Soluble sugars present in the roots contribute to the medicinal value in adapathiyan (CSIR, 1959). Amino acid content in roots recorded the highest value in green elongate type (0.882%) while root protein content showed the highest value in purple cordate (0.76%) and leaf protein content in green cordate (2.27%).

Seedling progenies obtained by hybridisation and open pollination were studied to assess the variability. Leaf shape and leaf pigmentation did not follow a Mendelian pattern of inheritance in both hybrid and open pollinated progenies suggesting the polygenic nature of those characters. In general, cordate leaf shape and purple leaf colour dominated over elongate leaf shape and green colour. In none of the biometric characters, hybrid and open-pollinated progenies formed separate groups. Being cross pollinated, open pollinated progenies are not much different from hybrid progenies. Two out of four characters viz. number of branches and internodal length recorded maximum values in open pollinated progenies of purple cordate and purple elongate types. Observations on all the characters indicated better performance of hybrid and open pollinated progenies with parents purple cordate or purple elongate type as one of the parents. This suggests that the collection of seeds from parents like purple cordate or purple elongate type be sufficient to exploit hybrid vigour in adapathiyan.

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Appendices

APPENDIX-1

Analysis of variance for flower and seed characters

Source	d.f.		Mean Squares		F value
	1	2	1	2	
Number of inflorescence per plant	3	16	2022.18	59.70	33.872*
Number of flowers per inflorescence	3	16	162.40	2.68	60.71*
Number of flowers per plant	3	16	730803.52	19031.53	38.4
Days to flower opening	3	16	20.67	7.08	2.92
Days to fruit set	3	16	0.18	0.15	1.22
Days to fruit maturity	3	16	820.85	63.78	12.87
Number of seeds per fruit	3	16	46984.32	421.48	111.48*
% of seed germination	3	16	234.75	461.80	0.508

* - Significant at 5% level

1. - Between morphotypes
2. - Within morphotypes

APPENDIX -2.

Analysis of variance for biometric characters and yield

Source	d.f.		Mean Squares		F value
	1	2	1	2	
Internodal length	3	16	22.27	25.73	0.865
Collar girth	3	16	0.38	0.25	1.54
Number of branches	3	16	0.45	0.73	0.62
Yield	3	16	548.45	19.18	28.60

1 - Between morphotypes
2 - Within morphotypes

APPENDIX -3.

Analysis of variance for biochemical characters

Source	d.f.		Mean Squares		F value
	1	2	1	2	
Soluble sugar	3	16	383.91	228.63	1.68
Insoluble sugar	3	16	216.74	28.68	7.55
Total sugar	3	16	617.64	284.31	9.23
Amino Acid	3	16	0.48	0.01	81.08*
Root protein	3	16	0.04	0.05	0.97
Leaf protein	3	16	0.18	0.23	0.79

* - Significant at 5% level

1 - Between morphotypes
2 - Within morphotypes

APPENDIX - 4

Analysis of variance for seedling characters

Source	d.f.		Mean squares		F value
	1	2	1	2	
Leaf area	11	294	958.57	201.09	4.77*
Collar girth	11	294	0.184	0.046	4.01*
Number of branches	11	294	1.96	0.46	4.30*
Internodal length	11	294	1.50	0.91	1.64*

* - Significant at 5% level

1 - Between morphotypes
2 - Within morphotypes

REPRODUCTIVE BEHAVIOUR OF ADAPATHIYAN

(*Holostemma adakodien* Schult.)

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ABSTRACT OF THE THESIS

Submitted in partial fulfilment of the
requirement for the degree

Master of Science in Horticulture

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1997

ABSTRACT

The present investigations were carried out in the Department of Plantation Crops and Spices, College of Horticulture, during the year 1995-1996. The study was undertaken with the objective of understanding the reproductive behaviour and pollination mechanism in adapathiyan and to unravel the reasons for low fruit set. It was also envisaged to evaluate the morphotypes for morphological and qualitative differences, to attempt hybridisation between morphotypes and to assess the seedling variability to isolate the ideal types.

Flowering pattern of morphotypes were almost similar. A protracted flowering period of 4-5 months starting from June-November was observed in all the types except green cordate in which peak season was noted in September- October while in others it was August-September. Flower bud development in adapathiyan could be divided into 12 arbitrary stages which needed 25 to 28 days from visual flower bud emergence to flower opening.

Adapathiyan is a profuse flowering plant bearing cymose inflorescence in the leaf axils with 2-20 flowers per inflorescence and 10-64 inflorescence per plant. Flowers are bisexual, regular, complete and actinomorphic, typical of milkweed family . The peak anthesis time was between 09.00 and 09.30 hours. Anther dehiscence was observed in flowers on fourth day of opening at a time interval of 11.00 to 13.00 hours. Stigma remained receptive at the anthesis time on the first day of flower opening.

Pollen grains were more or less oval in shape with 50-75 µ diameter. Pollen production per pollinia could not be estimated as a proper suspenion of pollen grains in water containing extran could not be obtained. Pollen fertility assessed by Alexander's stain test indicated a high range of 90-100 per cent. *In vitro* pollen germination in Brewbaker and Kwack's medium gave only approximate idea about pollen fertility.

Adapathiyan plants are cross pollinated by insects. *Xylocopa* spp (carpenter bees) were identified to be the pollinating agents. Among the different modes of pollination tried, open pollination gave the highest fruit set (12.6%) followed by natural cross pollination (11.7%) and artificial crossing with germinated pollinia (6.4%). The low fruit set may be due to the injury caused to the flower during pollination or self incompability operating at pre and post fertilisation stages. Natural and artificial self pollination did not result in any fruit set denoting the existence of self incompatibility mechanism but all the morphotypes were cross compatible.

Fruits required a period of 102-158 days to complete maturity. Purple elongate types took 150.4 days to complete maturity while green elongate type took 123.4 days. Each fruit contained numerous seeds in the range of 170-447. Hundred seed weight varied from 0.60-0.79 g. Seed germination percentage was recorded in the range of 18.00-46.25.

Morphotypes when compared with respect to biometric characters which showed high positive correlation with yield purple elongate types recorded maximum values for internodal length (7.64 cm) and number of branches (2.8). Highest value for collar girth was observed in purple cordate (2.4 cm). These observations suggest purple elongate and purple cordate to be high yielding. But maximum root yield was observed in purple cordate (65.6 g per plant) and green cordate (60.6 g per plant) while purple elongate recorded 49.3 g per plant root yield. Biochemical studies on the morphotypes figured maximum value for soluble sugars (8.52%) in purple elongate and minimum in green elngate type (4.76%). Since the medicinal properties of adapathiyan are attributed to the soluble sugars, purple elongate types can be considered as medicinally important. Amino acid content in roots recorded highest value of 0.882 per cent in green elongate type whereas root protein was maximum in purple cordate (0.76%) and leaf protein in green cordate (2.27%).

Variability studies in seedlings of hybrid and open pollinated progenies showed that purple pigmentation and cordate leaf shape dominated in the progenies. Mendelian

ratio was not followed with respect to these characters in the progenies. Evaluation of seedling progenies with respect to biometric characters showed that the hybrid and open pollinated progenies did not form separate groups statistically. Thus it is made clear that self pollination being prohibited inherently, open polliniated progenies are as good as artificial hybrid progenies and purple elongate and purple cordate are good seed parents.