PRE-ZYGOTIC INCOMPATIBILITY AND EVALUATION OF OPEN POLLINATED AND HYBRID PROGENIES OF *Holostemma adakodien* Schult.

By

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THESIS

Submitted in partial fulfilment of the requirement for the degree of

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DECLARATION

I hereby declare that this thesis entitled **'Pre-zygotic incompatibility and evaluation of open pollinated and hybrid progenies of *Holostemma adakodien* Schult.'** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, fellowship or other similar title, of any other University or Society.

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Introduction

INTRODUCTION

Holostemma adakodien a member of Asclepiadaceae family, is an endangered species known for its medicinal use in ophthalmic diseases and as a general tonic. Use of *Holostemma adakodien* in the traditional system of medicine is reported from all parts of India. In each language it has a specific name, indicating the wide spread recognition of the medicinal properties of the plant. It is called 'Jivanti' in Sanskrit, 'Chirvel' in Hindi' and 'Atapatiyan', 'Atapotiyan' or 'Atakotiyan' in Malayalam (Warrier *et al.*, 1995).

The plant is extensive, laticiferous twining shrub with opposite leaves and flowers appearing in axillary umbellate cymes. Flowers are characterised by the presence of corona, gynostegium and bag like pollinia enclosing pollen grains. Two such pollinial bags from two adjacent half anthers are attached to a common corpusculum by two caudicles. Gynoecium consists of two free carpels with marginal placentation. Fruit consists of two follicles with light and winged seeds.

The roots of Holostemma are sweet, refrigerant, ophthalmic, emollient, alterant, tonic, stimulant, aphrodisiac, expectorant and galactogogue. They are useful in opthalmology, orchitis, cough, burning sensation, fever and to cure tridosha (Warrier *et al.*, 1995). The sugars and aminoacids present in the root tubers are responsible for the medicinal properties (Ramiah *et al.*, 1981).

Indiscriminate gathering of plant material from forests without considering the natural perpetuation necessitated the domestication of many economic and highly demanded medicinal species. Being highly remunerative, this is one among the few medicinal plants gaining the status of a commercial crop in Kerala. Naturally, adapathiyan is cross pollinated by insects but fruit set is very rare. Protogynous nature of the flowers, position of pollinia enclosed within the gynostegium, downward position of the flower and tight membrane covering the lateral receptive side of the stigma totally block other modes of pollination. Studies conducted by Manju (1997) revealed that even with assisted pollination, the fruit set could not be increased. In spite of high pollen fertility, low fruit set may be due to the incompatibility mechanism which is reported in other asclepiads and studies are essential to confirm the incompatibility reaction operating in adapathiyan and attempts are necessary to break this barrier. Self incompatibility was reported in most species of asclepiads by various workers. Studies on pollen pistil interaction will help to understand the physiological factors leading to low fruit set.

To sustain the interest generated in the cultivation of the crop, efforts are needed to maintain high level of productivity, either through crop improvement or management. Evaluation of germplasm accessions, open pollinated and hybrid progenies already available will help to locate high yielding types for commercial cultivation in Kerala. Seedling was rated as the best material for commercial cultivation among the different propagules evaluted (Meera, 1994). Selection parameters if found viable can be used to screen ideal types in the seedling stage and enable to make best use of the variability emerging out of cross pollination.

In this context, the present study in adapathiyan was undertaken with the following major objectives.

1. To study reproductive mechanism and the incompatibility reaction in different accessions and to find out the effect of different methods to overcome the incompatibility barrier.
2. To understand the physiological hindrances to successful fruit set by studying the pollen pistil interaction.
3. To characterise and evaluate germplasm accessions and hybrid progenies for exploiting the variability and to select ideal types.
4. To formulate selection parameters for screening superior types in the seedling stage based on evaluation of open pollinated progenies.

Review of Literature

REVIEW OF LITERATURE

The family Asclepiadaceae includes 320 genera and 1800 species. In India, 234 species belonging to more than 35 genera are found. Asclepiadaceae belongs to the order Gentianales. The family is subdivided into 2 sub families, Periplocoideae and Cynanchoideae. The latter is subdivided into 4 tribes and Asclepiadeae is one of the tribe (Vasishta, 1974). *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.*

Naturally, adapathiyan is cross pollinated by insects but fruit set is very rare. Even with assisted pollination, the fruit set cannot be increased, though a high pollen fertility was observed (Manju, 1997). Low fruit set may be due to incompatibility barriers and an attempt is needed to break this barrier. Evaluation of open pollinated and hybrid progenies already available will help to locate high yielding types for commercial cultivation. 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) and June-November by Manju (1997). Season starts from June-July in *Asclepias syriaca* (Wilson *et al.*, 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 2-20 flowers and the number of inflorescence per plant varied from 10-64 (Manju, 1997). Flowers are bracteate, pedicellate, complete, actinomorphic, bisexual, hypogynous, cyclic and pentamerous. Calyx is deeply five partite. Sepals are 5mm 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* arises 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). In *Holostemma*, pollen are united into a waxy mass, opaque and without placid 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

Reproductive span of a flower was reported to be 6.2 days in *Asclepias exaltata* (Wyatt and Shannon, 1986) and 4-5 days in *Sarcostemma viminale* (Liede and Whitehead, 1991). According to Manju (1997), anthesis in adapathiyan occurs during 9.00-11.00 a.m. Anther dehiscence started from 9.00 hours onwards on the fourth day and maximum dehiscence (92%) was noted from 11.00 to 13.00 hours (Manju, 1997).

2.1.3 Stigma receptivity

In Asclepiads, it is reported that receptive side of the stigma is the lower lateral sides of the stigmatic head (Sreedevi, 1989). Stigma receptivity is indicated by shiny appearance, light cream colour and presence of nectar. In adapathiyan maximum receptivity coincided with maximum flower opening period from 9.00 to 11.00 hours and flowers exhibit protogynous nature (Manju, 1997).

2.1.4 Pollen morphology

In Orchidaceae, Ericaeae 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 typical features. In *Holostemma adakodien* (Manju, 1997) observed that pollen grains were more or less circular or oval in shape without exine.

2.1.5 Pollen production

In Asclepiads, since pollen grains are kept covered within the pollinium, it is difficult to estimate the number of pollen per pollinium (Viswanathan and Lakshmanan, 1984). In adapathiyan, Manju (1997) found that due to the peculiar nature of pollinial wall, liberation of pollen from pollinia was difficult.

2.1.6 Pollen germination

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). Brewbaker and Kwack's medium was found to be the best for *in vitro* germination of pollinia in adapathiyan. No difference was noted among germination of 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 (Manju, 1997).

2.1.7 Pollen viability

Staining the pollen with 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 Alexanders 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. They also observed that the pollen grains could not be liberated by dissecting the fresh pollinia. Therefore to take up germination studies in adapathiyan, pollinia were dipped in Brewbaker and Kwack's medium for two hours to initiate first pollen tube. It was then crushed carefully to liberate the pollen grains for staining and 95 per cent fertility was reported (Manju, 1997).

2.2 Pollination studies

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

2.2.1 Natural or open pollination

Rendle (1971) reported that in Asclepiadaceae, cross pollination is occurring through the agency of insects. An insect visiting the flower for nector 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 (Manju, 1997). Kephart (1983) suggested that two species of *Asclepias* viz. *A. syriaca* and *A. verticillata* have predominantly allogamous breeding systems. No seedset was observed in these two 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 insect visitors. The notch of the corpusculum and hygroscopic nature of caudicle helps in its attachment to the leg of insect.

Manju (1997) reported that only cross pollination occurs in adapathiyan. Artificial selfing with *in vitro* germinated and intact pollinia and natural selfing resulted in no fruit set.

2.2.2 Pollinating agents

Galil and Zeroni (1965) reported that honey bees were the pollinators in *Asclepias curassovica*. Honey bees also act as pollinator to *A. syriaca,* accounting for 26 to 49 per cent of all vectors (Wilson *et al.*, 1979). Wyatt (1978) reported that most of the members of this family share pollinators. Kephart (1983) studied pollinators in three 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. incurnata. 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.

According to Manju (1997), pollinating agent in adapathiyan is carpenter bees of the order *Xylocopidae* and genus *xylocopa.* Insect activity was found from flower opening till evening but maximum insect activity was found just after flower opening.

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. putiescens* and *X. fenestrata* were reported in *C. procera* spp. *hamiltonii* by Ali and Ali (1989).

2.2.3 Self pollination

Though cross pollination is the general rule in Asclepiads, 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.

No fruit set was observed in Adapathiyan when selfing was done artificially and naturally (Manju, 1997). Chance for natural selfing is prevented by the innate protogynous nature, the restricted span of stigma receptivity and wide time lapse between two phases. Manju (1997) also reported the chance for pre-zygotic or post-fertilisation barriers operating in adapathiyan.

2.2.4 Fruit set

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

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.

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. Failure of pollen tube to penetrate the ovary and effect fertilisation and similar failure at various stages of development were responsible for the physiological contribution to low 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.

Sreedevi (1989) listed reasons 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 was supported by field observations that considerable number of pollinia were 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 five to ten per cent. Seedset of 11.1 per 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 requirement. Thus they are attracted to areas of dense plant population and result in higher fruit set (Henrich and Raven, 1972).

In adapathiyan, fruit set is low (5-17%), pollen removal from the flower is not low, but its insertion into receptive stigmatic surface is very low. Pollen being highly fertile, pre-zygotic and post-fertilisation barriers operating may be responsible for low fruit set (Manju, 1997).

2.3 Yield of roots and chemical composition

According to Meera (1994), harvesting the plants at 18 months after planting resulted in maximum fresh weight of 80.80 g of roots. Manju (1997) reported the maximum yield in purple cordate type (65.60 g) of adapathiyan. When the root yield of adapathiyan as pure crop and inter crop was compared, the yield was maximum for pure crop though not significant (Kurian,1999).

CSIR (1959) published the economic importance of *Holostemma adakodien* with the uses and chemical composition. Analysis of the root powder revealed moisture (10.08%), protein (4.07%), sugar (24.0%), starch (32.54%), fibre (12.2%) and ash (3.07%). 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 ß-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 the lowest (56.60%) in 11 months old crop. The protein (10.00%) and alkaloid (1.40%) contents were the highest in 11 months old crop compared to 8 months (3.94 and 1.10%) and nine months old crop (8.95% and 1.25%). Meera (1994) studied the influence of planting materials and stage of harvest on quality components of adapathiyan. Both these factors significantly influenced the soluble carbohydrate content. Maximum content was recorded by seedling (4.92%) followed by root stump (4.86%), vine cuttings two nodes (4.81%), vine cuttings three 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 nine 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 free amino acids 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 nine months (0.02%).

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 (Manju, 1997).

Quality attributes of adapathiyan when compared as pure crop and inter crop, insoluble sugar content was slightly high for inter crop whereas soluble sugar content was maximum for pure crop. The mean protein and total free amino acid content showed not much difference between the two cropping situations (Kurian, 1999).

2.4 Compatibility studies

Incompatibility is the failure of plants with viable pollen and ovule to set seed due to some physiological hindrance which prevents fertilization (Crane and Lawrence, 1952). According to Brewbaker (1957) incompatibility mechanism is in operation in members of 66 plant families.

Cooper (1938) studied the various aspects of fruit set in apple and reported that all the varieties were self incompatible. It had also been reported by him that the system available in this crops was due to the presence of certain inhibitory substance of the pistil that considerably retarded the growth of pollen tube. Yaqub (1968) observed self incompatibility in *Capsicum pubescens.* Brewbaker and Gorrez (1967) reported self incompatibility in the monocot genera *Ananas* and *Gesteria.*

Kumar (1983) reported self incompatibility in sweet potato. Arab (1988) reported that in *Brassica oleraceae* incompatibility was manifested by the inability of the pollen tube to penetrate the stigmatic papillae due to callose deposits at the point of contact between the papillae and the pollen tubes. In *Dendrobium*, Johansen (1990) reported gametophytic self incompatibility and it is expressed by flower abscission and not by inhibition of pollen germination or pollen tube growth. This is because the auxin content in the pollinia triggers the incompatibility reaction. Moneur *et al.* (1991) reported that site of incompatibility is within the ovary in *Acacia mearnsii*. Lather and Dahiya (1992) reported pre-zygotic phenomenon of self incompatibility in chickpea. Kaur and Sareen (1992) reported self incompatibility in *Pterospermum aerifolium.* Studies of the floral biology, self and cross pollination indicated that incompatibility in *Raphanus sativus* operates at the sporophytic level of the pollen stigma interface with rejection of the pollen or pollen tube (Kaur *et al.*, 1992). Chichiricco (1993) reported that a gametophytic system of self incompatibility controls ovule fertilisation in crocus. Sage *et al.* (1994) reported ovarian self incompatibility in woody species. In an experiment conducted by Aneja *et al.* (1994) in cacao, it was found that in an incompatible genotype of cacao, self incompatibility expressed at two stages. The first is at the pollen germination stage and the second is at the gametic fusion stage. Brunn *et al.* (1995) observed incompatibility response at the border between the stigma and style in *Beta vulgaris*.

Tangmitcharoen and Owens (1997) studied the floral biology, pollination and pollen tube growth in relation to low fruit production of teak and reported the late acting gametophytic self incompatibility in teak. Li Xin Min *et al.* (1996) reported gametophytic self incompatibility in *Phalaris coerulescens.* Marcellan and Camadro (1996) reported self and cross incompatibility in *Asparagus officinalis*. Rugkhla *et al.* (1997) reported the pre and post-fertilization barrier in *Santalam spicatum* on selfing.

2.4.1 Incompatibility in *Asclepiads*

Moore (1946) ~~and Sparrow (1948)~~ reported a complete lack of self compatibility in *A. syriaca.* Sparrow (1948) confirmed the same result. 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.

2.4.2 Breaking of incompatibility barrier

A number of methods have been tried by various workers to induce fertility by breaking the incompatibility barrier in crop plants. They include surgical techniques, bud pollination, hormonal treatment, polyploidy, irradiation etc.

Mangelsdorf and Reeva (1931) obtained hybrid seed from a cross between two genera of *Zea* and *Tripsacum* by employing the technique of cutting off portion of the style before pollination. Davis (1957) used the technique of cutting off portions of the styles before pollination and crossed two incompatible species of *Lathyrus* and removal of upper third portion of the style caused greater increase in pod and seed formation. Goud *et al.* (1970) observed that incompatibility in *Capsicum* could be overcome by amputation of the style followed by pollination. Charles *et al.* (1974) investigated the nature of the incompatibility mechanism in two strains of the wild species of *Ipomoea trichocarpa* and eight cultivars of *I. batatas*. The incompatibility barrier was found to be located in the stigma and hence its removal resulted in successful seedset in *I. trichocarpa*. No such inhibition was found in *I. batatas* and hence, removal of the stigma failed to break the incompatibility barriers. Radhakrishnan (1976) pointed out that cross incompatibility in *Capsicum* was due to the inability of pollen tubes to grow beyond the upper region of the style. This was overcome by amputating the upper part of the style along with the stigma and pollinating the cut surface of the style after application of 5 per cent sucrose solution.

Togeri and Kawahara (1942) in their effort to induce pseudofertility in sweet potato by means of bud pollination, observed that since the stigma of the sweet potato flower would become receptive only a few hours before anthesis, bud pollination offered little prospect in overcoming self incompatibility. Haruta (1966) reported details regarding the use of bud pollination in *Brassica* and *Raphanus*.

According to Brewbaker (1957) stigma is the site of incompatibility for species with trinucleate pollen grains which remain viable only for a short period of time and do not germinate readily *in vitro*. The physiological explanation for this phenomenon was later given by Harrison *et al.* (1975). According to them the pollen is inhibited in the stigma for the simple reason that some kind of stimulus from the stigma which is essential for pollen germination is blocked by the incompatibility reaction.

Charles *et al.* (1974) reported the suppression of floral abscission by the application of 2,4-D (100 ppm) to the pedicels, which resulted in successful seed set. They reasoned that the application of 2,4-D gave adequate time for the pollen tube to penetrate the incompatible style and resulted in fertilization and seed set. So also, suppression of floral abscission by 2,4-D was thought to be another reason for the successful seed set.

Allard (1960) reported that end of season pollination was found to be effective in breaking the incompatibility barrier in crops like tobacco.

Gradziel and Robinson (1989) reported that stylar self incompatibility barriers in *L. peruvianum* could be avoided if pollen germination and growth through immature pistils was promoted under specific environmental condition approximately 2-3 days before the initiation of anthesis. In Brassica, self incompatibility can be overcome by Co_2 treatment (Nakanishi and Sawano, 1989).

Kashyap and Gupta (1989) reported that self incompatibility can be partially overcome *in vitro* by treating pollen and/or stigma with gibberellic acid (GA_3) in *Ipomoea cairica, Brassica campestris* and *Raphanus sativus* which was due to callose deposition in the stigmatic papilla and total inhibition of pollen germination.

Amaki and Higuchi (1992) found that when styles of some varieties of *Lilium longiflorum* treated with coenzymes and glutathione, promoted pollen tube growth of incompatible pollen. Treatment of pistils with okadaic acid (OA), an inhibitor of serine/threonine protein phosphatases causes breakdown of self incompatibility in *Brassica oleraceae.* Aneja *et al.* (1994) found out that Co_2 treatment partially overcomes self incompatibility in certain genotypes of cacao. Rai *et al.* (1995) studied the effect of spraying NaCl solution at different spraying times (8.30, 9.30, 10, 14 and 15 hours) during the flowering stage in Chinese cabbage combined with honeybee pollination and found that spraying of 3 per cent NaCl solution is effective to overcome self incompatibility. Ilieva and Alipieva (1996) reported that self incompatibility can be broken in certain lines of cabbage by means of pollen lazer treatment.

2.5 Pollen pistil interaction

Inhibition of pollen tube growth or its abnormally slow development inside the stylar column was reported to be one cause of incompatibility in angiosperm by Sears (1936).

Togari and Kawahara (1942) observed complete suppression of pollen germination after selfing in sweet potato.

The preliminary studies conducted by Miller (1938) in sweet potato revealed that lack of fertility might be due to the style being deficient in an unknown substance which might initiate pollen germination or it may contain some substances which act as inhibitor to pollen germination.

The cytoembryological studies of Banikova (1965) revealed that in an interspecific cross between *Nicotiana glutinosa* and *N. rustica*, not even a single pollen had reached the embryosac upto 12 days of pollination, and the embryosac finally degenerated.

Martin and Cabanillas (1966) in their observation on post pollen germination barriers to set seed, came to the conclusion that, in 3 cross combinations selected for good pollen germination, pollen tube failed to pass from the stigma to the style. Besides the incompatibility barrier inhibiting the pollen germination, a physiological barrier between stigma and style was also hypothesised. Martin and Ortiz (1966) made observations on the germination of pollen in relation to incompatibility and sterility in sweet potato. Using a fluorescent technique, it was found that the pollen in incompatible crosses failed to germinate entirely, in compatible crosses germinated well and in partially incompatible crosses germinated occasionally.

Wadderburn (1967) fairly succeeded in crossing two highly self incompatible varieties of *Ipomoea batatas* and *I. trichocarpa*. He observed good germination of pollen in all the pollinations, both compatible and incompatible. However, the pollen tube growth in incompatible matings was found to be abnormal.

Hogenboon (1972) reported abnormally slow growth of *Lycopersicon esculentum* pollen in styles of self compatible *L. peruvianum* and the hybrids of such crosses showed embryo abortion.

Nettancourt (1973) studied the ultrastructural aspects of self incompatibility mechanism in *Lycopersicon peruvianum* and reported that the incompatible pollen tube were not only slow in their growth through the style, but were destroyed of the innerwall and lysis of the tube occurred.

Harrison *et al.* (1975) concluded from their studies in interspecific incompatibility system of the sporophytic type that the inhibition of pollen or pollen tube was taking place on the stigmatic surface.

Radhakrishnan (1976) reported that the degree of germination of pollen grains was remarkably high when 5 per cent sucrose was applied on the cut end of the style before pollination in *Capsicum*. Venkateswarlu (1980) reported that self incompatibility in sweet potato is sporophytically controlled. The histological studies revealed that the inhibition of pollen grains was at the stigma level.

Chichirico (1990) reported that in *Crocus vernus* subsp. *vernus*, self-pollen tubes failed to react or to enter the ovule micropyle, while pollen tubes from cross pollinations grew normally to fertilise the ovules and ovary is the site of self incompatibility. Sareen and Kaur (1991) reported self incompatibility system in *Lagerstroemia parviflora* and studied pollen tube growth following self and cross pollinations. Tubes grew normally for the entire length of the style in cross pollinated flowers of both species, but in self flowers they reached only the middle part of the style before their tips swelled and they ceased to grow. Ram *et al.* (1992) reported that stigma is the site of action of the self incompatibility alleles in coffee. Lather and Duhiya (1992) reported pre-zygotic phenomenon of self incompatibility in chickpea where genes either restrict pollen germination or prevent the growth of the pollen tube.

Han (1994) reported that in *Hibiscus syriacus* pollen tube growth of the incompatible pollinations was inhibited in the style, two-third of the way from the stigma to the base at 9-12 h after pollination, pollen tube growth of compatible crosses reached to the base of the style. Bruun *et al.* (1995) studied the self incompatibility reaction in *Beta vulgaris* and reported that self incompatibility response was observed at the border between stigma and style. Tangmitcharoen and Owens (1997) studied the self incompatibility mechanism in teak and reported that several abnormalities occurred in pollen tube growth at various positions within the pistil. Abnormalities included reversing tubes, irregular or spiralling tubes and an increase of callose deposits resulting in swelling of the tube-tip. Roux *et al.* (1996) studied pollen pistil interaction in self incompatible *Gloriosa superba* and observed that pollen tubes grow in close association with the stylar secretion, reaching most ovules, even though pollen tube numbers decrease on their way down the stylar canal. Callose deposition in the nucellus of some self pollinated ovules could be indicative of a partly pre-zygotic gametophytic self incompatibility system.

Echarte *et al.* (1996) observed that in *Paspalum distichum* pollen grains germinated and developed pollen tubes after self pollination, but growth of the tube was arrested at different levels in the pistils. Egea and Burgos (1996) noted <2 per cent fruit set due to incompatible pollinations in apricot, and microscopic examination showed that pollen tube growth was arrested in the style, most frequently in its third quarter, and that the ovary was never reached. Rugkhla *et al.* (1997) observed that on selfing, growth of pollen tubes was arrested in the style, ovary and around the embryosac, a few penetrated the embryosac in *S. album.* Chichirico (1996) observed that the stigma-style tract of the crocus pistil is a mere promoter of pollen tube growth, discrimination of compatible and incompatible pollen occurs in the ovarian tract. According to Tangmitcharoen and Owens (1997), pollen tubes are arrested in the lower part of the ovary on selfing. Damri *et al.* (1998) observed swelling and bursting of pollen tubes into the stigma in selfed flowers of lemon. Gupta *et al.* (1998) observed that pollen grains germinated on the stigma, but pollen tubes failed to grow beyond the proximal one third of the style in *Commiphora wrightii.*

2.6 Heterosis

Adapathiyan being a cross pollinated crop, heterosis breeding is one of the important tools of crop improvement. The term heterosis refers to the phenomenon in which the F_1 obtained, by crossing two genetically dissimilar gametes or individual shows increased or decreased vigour over the better parent or over the midparental value.

Natarajan *et al.* (1984) reported that hybrids from crosses between the high yielding strains of snakegourd and the local variety had a significantly higher yield/plant and mean weight of fruit than the parents. In an experiment to study the variability in F_1 hybrids and open pollinated seed progenies of black pepper, significant heterosis for important yield related characters viz. number of spikes per vine, length of spike and number of developed berries per spike was shown by certain hybrids (Sujatha, 1991). Abusaleha and Dutta (1993) recorded heterosis over better parent in sponge gourd for all the characters except days to first female flower and fruit girth. Pitcharmuthu and Sirohi (1994) observed heterosis in bottlegourd for all the characters except days to first fruit harvest. According to Lal and Sharma (1995) in opium poppy, comparison of the latex and content of four alkaloid in the hybrids with that in the commercial variety revealed substantial economic heterosis for latex but negative heterosis for alkaloids in most of the hybrids.

In bittergourd, maximum and significant heterosis was observed for yield and fruit number/plant. Significant heterosis for seed yield was reported in opium poppy by Singh *et al.* (1999a). Singh *et al.* (1999b) noticed heterosis in opium poppy for major fatty acids palmitic acid, stearic acid, oleic and linoleic acid. Tewari and Ram (1999) reported heterosis for yield and other associated characters in bittergourd.

2.7 Selection criteria

The development of selection criteria helps to identify better seedlings based on early growth parameters which bears positive correlation with final yield.

In plantation crops like coconut (Liyanage, 1967; Sathyabalan and Mathew, 1983) and arecanut (Bavappa and Ramachander, 1967) selection criteria have been developed and used for the evaluation of seedlings in the nursery. In cocoa, yield was found to be significantly and positively correlated with number of pods per tree, height (2 year after planting) and girth (2 year after planting) of the tree (Sridevi, 1999). Correlation between biometric characters and dry root yield in adapathiyan by Meera (1994) indicated that selection for yield could be based on inter nodal length, diameter of the stem, number of branches and root volume.

Materials and Methods

MATERIALS AND METHODS

The present investigations on "Pre-zygotic incompatibility and evaluation of open pollinated and hybrid progenies of *Holostemma adakodien* Schult." was undertaken at the Department of Plantation Crops and Spices, College of Horticulture, Vellanikkara during 1997 to 2001. The studies were conducted under the following heads:

1. Study of reproductive mechanism, the incompatibility barrier and effect of different methods to overcome the incompatibility barriers
2. Study of pollen pistil interaction
3. Characterisation and evaluation of accessions and hybrid progenies
4. Formulation of selection parameters based on evaluation of open pollinated progenies

3.1 Study of reproductive mechanism, incompatibility barrier and effect of different methods to overcome the incompatibility barrier

Before studying the self incompatibility reaction, knowledge on floral biology and reproductive behaviour of the plant is essential. Hence, observations were noted in 15 accessions of adapathiyan about flowering pattern, floral biology and pollination mechanism as follows:

3.1.1 Flowering and floral characters

3.1.1.1 Pattern of flowering

Pattern of flowering was studied by observing the branches selected at random on each accession. Observations on season of flowering, position of inflorescence, number of inflorescence/plant and number of flowers/inflorescence were recorded in all the accessions.

3.1.1.2 Floral biology

Various aspects of floral biology viz. anthesis anther dehiscence, stigma receptivity and pollen characters of 15 accessions were studied.

3.1.1.3 Anthesis

Studies on anthesis by Manju (1997) showed that flower opens in the morning hours with the peak between 9.00 and 9.30 hours. In order to know whether the accessions differ in time of anthesis, 25 buds were tagged on each accession and observations were made at 30 min interval from 8.00 hours to 10.30 hours.

3.1.1.4 Anther dehiscence

The period of anther dehiscence was studied by tagging 25 buds of uniform size. 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 interval from 9.00 onwards to 13.00 hours upto fifth day after anthesis.

3.1.1.5 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 flowers (4^{th} day of anthesis) of male parent. Pollination was done at two hours before anthesis, at the time of anthesis, two hours after anthesis, six hours after anthesis and 24 hours after anthesis.

3.1.1.6 Pollen studies

The pollinia for the studies were collected from opened flowers on their first, second, third, fourth and fifth day of opening.

3.1.1.6.1 Pollen fertility

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 (Manju, 1997). 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.

3.1.1.6.2 *In vitro* pollen germination

Pollen germination in water, water + sucrose and Brewbaker and Kwack's medium was noted. The Brewbaker and 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 placed on a drop of medium kept on a clean glass slide. This was kept in a dessicator. Pollen germination was ascertained by examining pollen tube growth 24 hours after inoculation (Manju, 1997).

3.1.1.7 Pollination studies

3.1.1.7.1 Pollinating agents

To study the agents of pollination, the plants were closely observed during flowering season.

3.1.1.7.2 Mode of pollination

a) Self pollination

Individual flowers were covered with butter paper 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 pollinia from the same plant. The flowers were tagged after pollination. Pollinia were collected from flowers on fourth day of opening.

b) 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, kept tagged and fruit set was noted.

c) 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 flowers on fourth day of opening directly into the stigmatic cavities after lifting the membraneous covering on the stigma. Pollinated flowers were tagged and kept covered for four days and fruit set was observed.

3.1.2 Screening for incompatibility reaction

Twenty five flowers of each accession were covered with butter paper 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. Artificial selfing was also done using intact pollinia from flowers four days after opening from the same plant and also pollinia germinated in artificial medium.

3.1.3 Effect of different methods to overcome the incompatibility barrier

A number of methods have been tried by various workers to induce fertility by breaking the incompatibility barrier in crop plants. They include surgical techniques, delayed pollination, bud pollination, heat treatment etc.

In the methods listed below, flower buds which would open on the next day were selected and bagged. Pollinia was collected from flowers on fourth day of opening of the same plant. After pollination, flowers were bagged and labelled properly.

a) Bud pollination

Flower buds one day prior to opening were selected. Pollinia from the same plant was inserted into the stigmatic cavities after lifting memberaneous covering on the stigma.

b) Pollination after cutting the stigma

Stigmatic region of the flower was carefully cut and removed using a sharp blade and pollinia were transferred to the cut surface of the style.

c) Removal of the stigma and pollination with the help of sucrose solution

Sucrose solution was used on the cut stylar surface as a nutrient medium to facilitate the pollen germination. Stigmatic region was cut and removed and a drop of sucrose solution was placed on the cut surface of the style. Pollinia was placed on the cut end of the style.

d) Removal of stigma and pollination with the help of Brewbaker and Kwack's medium

After removing the stigma, a drop of Brewbaker and Kwack's medium was placed on the cut surface and transferred the pollinia.

e) Pollination after removal of the stigma and a part of style

In this method, stigma and top portion of the style were removed using a sharp blade without causing injury to the other floral parts and pollinia was placed on the cut surface.

f) Pollination with the help of sucrose solution after removal of the stigma and a part of style

After removing the stigma, along with the top portion of the style, a drop of sucrose solution was applied on the cut surface and pollinia was kept on the cut surface.

g) Pollination with the help of Brewbaker and Kwack's medium after removal of the stigma and a part of style

As in the above method, instead of sucrose, Brewbaker and Kwack's medium was applied on the cut surface and pollinia was transferred.

h) Pollination with *in vitro* germinated pollen grains

Pollen was germinated by taking the pollinia in Brewbaker and Kwack's medium on a clean glass slide and kept the slide inside the dessicator for 12 h. Pollination was done using the *in vitro* germinated pollinia.

i) Pollination at early, mid and late season

The month of June-July was fixed as the early season of flowering, August as the mid season and September-October as the late season. Selfing was done during the three seasons of flowering.

j) Application of 2,4-D

Immediately after pollination, a small bit of cotton dipped in 2,4-D solutions of 0.50 ppm and 1.00 ppm concentration was applied on the pedicel of the flower.

3.2 Pollen pistil interaction

Pollen germination and tube growth through stigmatic and stylar tissues after selfing, crossing and above 10 methods were studied using fluorescence technique proposed by Kho and Baer (1968) and Kho *et al.* (1980).

The pollinated flowers were fixed in FAA mixture (Formalin 10 ml, Acetic acid 10 ml and Ethyl alcohol 80 ml) at 24 and 48 h after pollination. After fixation, the materials were transferred into glass vials containing 1N NaOH for 8 h at room temperature in order to soften the tissue. The softened material was washed thoroughly with distilled water, then transferred into another glass vial containing 0.10% aniline blue in 0.1N K_2HPO_4 for 18 h. After staining, the gynoecium with style and stigma was mounted on a microscopic slide, gently pressed and viewed through fluorescence microscopy and photomicrographs were taken.

3.3 Characterisation and evaluation of accessions and hybrid progenies

3.3.1 Evaluation of accessions

Fifteen accessions of adapathiyan maintained at the Department of Plantation Crops and Spices were utilized for the study. Mounds were prepared at a spacing of 50 cm x 50 cm and rooted cuttings of adapathiyan were planted during October 1997 following Randomized Block Design with two replications and three plants per replication. Staking was given to the plants and the plants were maintained under uniform cultural and manurial practices.

3.3.1.1 Vegetative characters

Observations on various vegetative characters viz. pigmentation, leaf shape, length of vine, number of branches, collar girth of vine, inter nodal length and leaf number were taken three months after planting (early vegetative phase) and eight months after planting (active vegetative phase).

3.3.1.1.1 Leaf area

Length and breadth of five fully opened leaves from top of the vine were measured from each plant and average was taken. Leaf area was found out using the formula $Y = -0.16 + 0.995 LB$ (Meera, 1994) where Y is the leaf area, L and B are the length and breadth of the individual leaf.

3.3.1.2 Yield characters

Harvesting was carried out 24 months after planting. Plants were dug out separately taking care to collect the entire roots and cleaned with water to remove the adhering soil particles. Following observations were taken:

3.3.1.2.1 Length of root

The length of longest root was measured separately for each plant and expressed in centimetre.

3.3.1.2.2 Girth of main root

Girth of longest root was measured using a non elastic twine, measured in scale and recorded in centimetre.

3.3.1.2.3 Volume of roots

The entire roots from each plant was immersed in water taken in a measuring cylinder. The original level and the final level of water were noted and the rise in water level was expressed in millilitre.

3.3.1.2.4 Fresh weight of roots

After cleaning the roots, fresh weight was recorded separately for each plant and expressed in gram.

3.3.1.2.5 Dry weight of roots

After taking the fresh weight, the root samples were dried in an oven at 60°C to a constant weight and expressed in gram.

3.3.1.2.6 Driage

Based on the fresh weight and dry weight of roots the driage was worked out and expressed in percentage.

3.3.1.3 Chemical analyses

Root samples were analysed for total free amino acids, protein, soluble sugar and insoluble sugar. The methodology followed for each analysis is given below:

3.3.1.3.1 Protein

Protein estimation was done by Lowrey's method (Sadasivam and Manickam, 1992) and the content was expressed in percentage.

3.3.1.3.2 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 Manickam, 1992).

3.3.1.3.3 Soluble sugar

Phenol sulfuric acid method was followed to estimate the soluble sugars present in dry root powder (Sadasivam and Manickam, 1992).

3.3.1.3.4 Insoluble sugar

Content of insoluble sugar was determined by anthrone method using dried root powder (Malik and Singh, 1980).

3.3.2 Evaluation of hybrid progenies

The following parents and hybrids of adapathiyan maintained in the Department of Plantation Crops and Spices were used for the study.

A. Parents

Purple Cordate leaved	PC
Purple Elongate leaved	PE
Green Cordate leaved	GC
Green Elongate leaved	GE

B. Hybrids

H_1 - Purple elongate x Green elongate leaved (PE x GE)

H_2 - Purple elongate x Green cordate leaved (PE x GC)

H_3 - Purple cordate x Green elongate leaved (PC x GE)

H_4 - Purple cordate x Green cordate leaved (PC x GC)

H_5 - Green cordate x Purple elongate leaved (GC x PE)

H_6 - Green cordate x Purple cordate leaved (GC x PC)

H_7 - Green elongate x Purple elongate leaved (GE x PE)

H_8 - Green elongate x Purple cordate leaved (GE x PC)

The eight hybrids along with four parents were grown in a Randomized Block Design with three replication as inter crop and pure crops. The observations recorded were same as that described in Experiment 3.3.

3.3.2.1 Heterosis

Heterosis was worked out in terms of Relative heterosis and Heterobeltiosis.

Relative heterosis is the deviation of hybrid mean from the mid parent value

$$RH = \frac{\bar{F}_1 - MP}{MP} \times 100,$$

average value of the two parents in each cross was taken as the mid parental value (MP).

Heterobeltiosis is the deviation of hybrid mean from the better parent value.

$$HB = \frac{\bar{F}_1 - BP}{BP} \times 100$$

To test the significance of difference of F_1 mean over mid and better parent, critical difference (CD) was calculated from their standard error of difference as given below (Briggle, 1963).

To test the significance over mid parent

$$CD\ (0.05) = te'\ (0.05) \times \sqrt{\frac{3\ MSE}{2\ r}}$$

$$= te'\ (0.05) \times SE$$

To test the significance over better parent

$$CD\ (0.05) = te'\ (0.05) \times \sqrt{\frac{2\ MSE}{r}}$$

$$= te'\ (0.05) \times SE$$

where e = error degrees of freedom

MSE = error variance

r = number of replications

SE = standard error of difference between two means

3.4 Formulation of selection parameters based on evaluation of open pollinated progenies

The following four groups of open pollinated plants were used for the study and the characters studied were same as that described in Experiment 3.3.

P_1 - Female parent - Purple Elongate

P_2 - Female parent - Green Elongate

P_3 - Female parent - Purple Cordate

P_4 - Parent not known

Ten seedlings of each P_1, P_2 and P_3, and 50 seedlings of P_4 were grown in mounds at a spacing of 50 cm x 50 cm. The plants were maintained under uniform cultural and manurial practices. Seedling characters at one month after planting and mature plant characters at eight months after planting viz., number of branches, leaf area, collar girth and inter nodal length were correlated with yield to know whether selection parameters are useful in predicting productivity.

Results

RESULTS

4.1 Study of reproductive mechanism, incompatibility barrier and effect of different methods to overcome the incompatibility barrier

4.1.1 Flowering and floral characters

4.1.1.1 Pattern of flowering

Flowers appear in cymose inflorescence (Plate 1) in the leaf axils on current season shoots and hang downwards. Visual emergence of flower buds commenced in July in all the accessions. The accessions showed a range of 14-45 inflorescence per plant, 12-22 flowers per inflorescence and 198-855 flowers per plant. The peak flowering period was August-September. In HA. 1, HA. 2, HA. 7 and HA. 13, flowering continued up to October whereas in all other accessions it ceased by September (Table 1).

4.1.1.2 Floral biology

Adapathiyan flowers are bisexual and complete. Flowers are purplish crimson inside and pale pink outside (Plate 2). They are bracteate, actinomorphic, regular, hypogynous and pentamerous. Pedicels are 2.5 to 5 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. Pollen grains at maturity are seen as pendulous mass called pollinium. Each stamen bears two pollinia united by means of caudicles to form a translator (Plate 3). Pistil is bicarpellary, apocarpous and enclosed by the staminal tube. Two styles are free but united at the stigmatic head. Fruit is a follicle.

Table 1. Flowering pattern in different accessions of adapathiyan

Sl. No.	Accession No.	Season of flowering	No. of inflorescence per plant	No. of flowers per inflorescence	Total number of flowers per plant
1	HA. 1	July-October	27	12	324
2	HA. 2	July-October	16	21	336
3	HA. 3	July-September	35	22	770
4	HA. 4	July-September	26	18	468
5	HA. 5	July-September	19	16	304
6	HA. 6	July-September	27	15	405
7	HA. 7	July-October	45	19	855
8	HA. 8	July-September	38	21	798
9	HA. 9	July-September	24	18	432
10	HA.13	July-October	18	11	198
11	HA.16	July-September	42	19	798
12	HA.17	July-September	19	13	247
13	HA.19	July-September	40	20	800
14	HA.21	July-September	38	12	456
15	HA.23	July-September	14	18	252

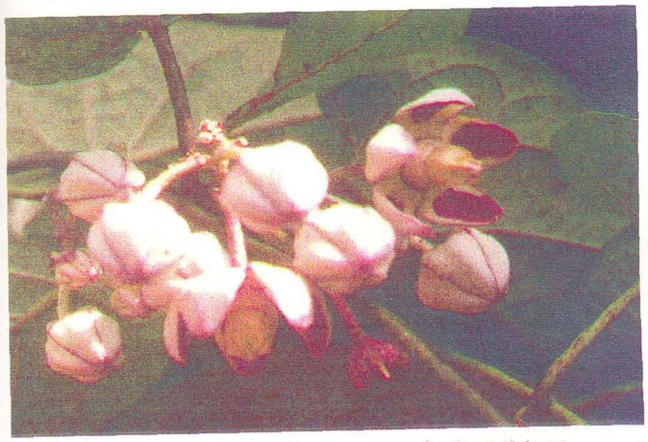

Plate 1. Cymose inflorescence of adapathiyan

Plate 2. Single flower of adapathiyan

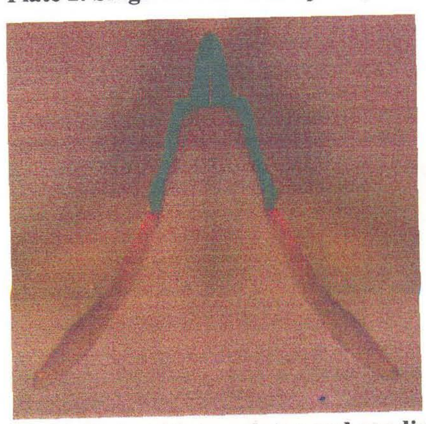

Plate 3. Pollinia with translator and caudicles

4.1.1.3 Anthesis

Anthesis of the accessions were recorded at half hourly interval from 8 a.m. to 10.30 a.m. Anthesis started from 8.30 a.m., maximum between 9.00 and 9.30 a.m. and completed by 10 a.m. in accessions HA. 4, HA. 5, HA. 7, HA. 9 and HA. 17 and by 10.30 a.m. in other accessions (Table 2).

4.1.1.4 Anther dehiscence

Anther dehiscence of the flowers occurred on the fourth day of opening (Table 3). At this time, flowers appeared wilted and the colour of stigma changed to deep yellow with dry appearance without nectar. But nectar was found at the bottom part of the gynostegium. Anther dehiscence started from 9.00 a.m. onwards on the fourth day and maximum dehiscence was noted from 11.00 to 13.00 a.m. irrespective of the accessions.

4.1.1.5 Stigma receptivity

Lateral sides of the stigma were found to be receptive (Plate 4) which is covered by tight membrane (Plate 5). Shiny surface and light cream colour indicate receptive stigma. Maximum fruit set was obtained when the flowers were pollinated between 9.00 and 11.00 a.m. indicating maximum receptivity. The trend was the same in all the accessions. Thus the study indicated that the stigma receptivity was very short and the receptivity lasted for only two hours after anthesis in all the accessions (Table 4). After the first day of flower opening, stigma gradually lost the fresh colour, appeared dried and turned light brown.

Table 2. Time of anthesis in different accessions of adapathiyan

Sl. No.	Accession No.	No. of flowers observed	Number of flowers opened				
			Time (a.m)				
			8.00-8.30	8.30-9.00	9.00-9.30	9.30-10.00	10.00-10.30
1	HA. 1	20	1	8	10	4	2
2	HA. 2	20	2	7	11	1	4
3	HA. 3	20	1	4	15	4	1
4	HA. 4	20	0	3	20	2	0
5	HA. 5	20	0	3	19	3	0
6	HA. 6	20	0	4	18	2	1
7	HA. 7	20	3	6	15	1	0
8	HA. 8	20	2	5	13	4	1
9	HA. 9	20	3	2	20	0	0
10	HA.13	20	0	5	16	3	1
11	HA.16	20	1	6	11	5	2
12	HA.17	20	1	6	15	3	0
13	HA.19	20	2	4	16	2	1
14	HA.21	20	1	2	18	3	1
15	HA.23	20	0	5	16	3	1

Table 3. Period of anther dehiscence

Time	No. of flowers observed/accession	No. dehisced	Dehiscence (%)
On the day of anthesis	20	0	0
II day of anthesis	20	0	0
III day of anthesis	20	0	0
IV day of anthesis	20	20	100

Table 4. Period of stigma receptivity

Time	No. of flowers pollinated	No. of fruit set	Fruit set (%)
2 hour before anthesis	50	0	0
At the time of anthesis	50	2	4
2 hour after anthesis	50	1	2
6 hour after anthesis	50	0	0
24 hour after anthesis	50	0	0

Table 5. Pollen fertility as influenced by days after anthesis

Days of flower opening	No. of pollen grain observed	No. of fertile pollen grains	Pollen fertility (%)
I day of anthesis	15	14	93.33
II day of anthesis	14	13	92.86
III day of anthesis	12	10	83.33
IV day of anthesis	5	3	60.00
V day of anthesis	5	2	40.00

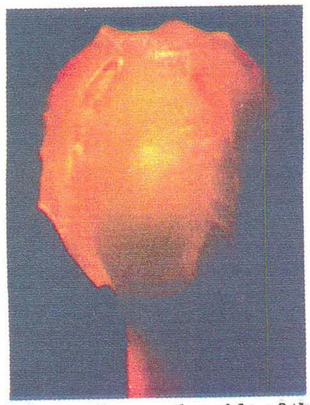

Plate 4. Lateral receptive side of the stigma

Plate 5. Stigma with membrane and translators inside the gynostegium

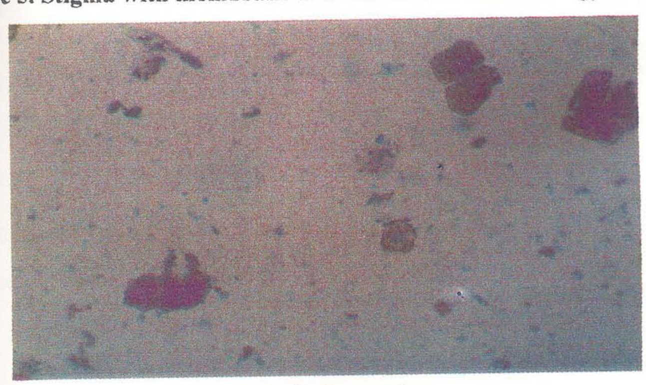

Plate 6. Pollen grains

4.1.1.6 Pollen studies

The two pollinia in one pair are attached by caudicles to the dark coloured pollen carriers or translators. Pollen grains were more or less circular or oval in shape without exine in all the accessions.

4.1.1.6.1 Pollen fertility

Pollen fertility was assessed using stain test (Alexander stain) from pollinia collected on first, second, third, fourth and fifth day after opening of flowers. In first and second day of opening of flowers, higher pollen fertility was observed (90.00-95.00%) (Table 5). Fertility decreased gradually thereafter in all the accessions. Proper staining was obtained only after dehiscing the pollinia by keeping in Brewbaker and Kwack's medium for two hours (Plate 6). There was not much difference in pollen fertility among the accessions. It varied from 94.44 to 100.00 per cent (Table 6).

4.1.1.6.2 *In vitro* pollen germination

In vitro germination of pollinia of all the accessions were attempted in Brewbaker and Kwack's medium. Very good pollen germination and pollen tube growth was recorded in all the accessions (Plate 7 and 8), but a slight decrease in pollen germination and pollen tube growth was noted in flowers of fourth and fifth day of anthesis. Among the different accessions, there was no difference in pollen germination and tube growth.

Table 6. Pollen fertility in different adapathiyan accessions

Sl. No.	Accession No.	No. of pollen grains observed	No. of fertile pollen grains	Pollen fertility (%)
1	HA. 1	18	18	100.00
2	HA. 2	19	19	100.00
3	HA. 3	18	18	100.00
4	HA. 4	19	19	100.00
5	HA. 5	21	20	95.24
6	HA. 6	19	18	94.74
7	HA. 7	18	17	94.44
8	HA. 8	19	19	100.00
9	HA. 9	20	19	95.00
10	HA.13	20	19	95.00
11	HA.16	20	19	95.00
12	HA.17	18	17	94.44
13	HA.19	20	19	95.00
14	HA.21	19	18	94.74
15	HA.23	21	20	95.24
Mean		19.27	18.6	96.58

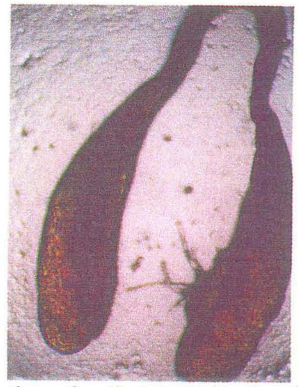

Plate 7. Initiation of pollen germination from pollinia

Plate 8. Profuse pollen tube growth

Plate 9. Pollinator of adapathiyan (carpenter bee) with pollinia attached to the body parts

4.1.1.7 Pollination studies

4.1.1.7.1 Pollinating agents

Carpenter bees were found to visit the flowers (Plate 9) and 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.

4.1.2 Screening for self incompatibility reaction

Natural and artificial selfing with *in vitro* germinated and intact pollinia resulted in no fruit set in any of the accessions showing that all the accessions are self incompatible.

Artificial crossing gave lower set compared to natural crossing (Table 7). Natural crossing gave a fruit set in the range of 6.00 to 10.00 per cent whereas in artificial crossing it ranged from 0.00 to 4.00 per cent. Accessions HA. 7, HA. 8 and HA. 17 recorded higher set of 10.00 per cent under natural crossing and HA. 1, HA. 7, HA. 8, HA. 13, HA. 19 and HA. 23 recorded higher set of 4.00 per cent under artificial crossing.

Fruit is a follicle (Plate 13). After anthesis flowers take 4 to 5 days to set fruits. Seeds are flat with a tuft of hairs (Plate 14). Number of seeds per fruit ranged from 200 to 450 in adapathiyan accessions. Seed germination ranged from 85 to 95 per cent.

Table 7. Fruit set under natural and artificial cross pollination in adapathiyan accessions

Sl. No.	Accession No.	No. of flowers observed	Fruit set (No.)		Fruit set (%)	
			Natural crossing	Artificial crossing	Natural crossing	Artificial crossing
1	HA. 1	50	4	2	8	4
2	HA. 2	50	4	1	8	2
3	HA. 3	50	4	1	8	2
4	HA. 4	50	4	0	8	0
5	HA. 5	50	3	0	6	0
6	HA. 6	50	4	1	8	2
7	HA. 7	50	5	2	10	4
8	HA. 8	50	5	2	10	4
9	HA. 9	50	4	1	8	2
10	HA.13	50	3	2	6	4
11	HA.16	50	3	1	6	2
12	HA.17	50	5	1	10	2
13	HA.19	50	4	2	8	4
14	HA.21	50	3	1	6	2
15	HA.23	50	3	1	6	4
Mean		50	3.87	1.2	7.73	2.53


Fig. 13. Fruits of adapathiyan

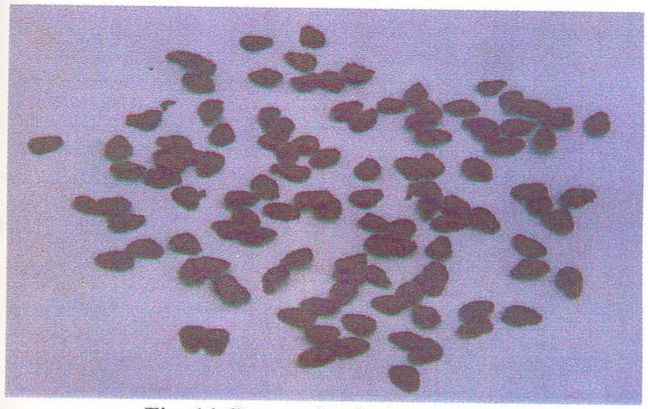
Fig. 14. Dry seeds of adapathiyan


Fig. 15. Fresh roots of adapathiyan

4.1.3 Different techniques for breaking incompatibility barrier

Ten different techniques were tried in all the accessions for overcoming the self incompatibility barrier. Thirty flowers of each accession were subjected to each of the technique and percentage of fruit set was assessed.

a) Bud pollination

Flower buds that would open on the next day morning were selected and bud pollinated. But no fruit set was observed in any of the accessions.

b) Pollination after cutting the stigma

The stigma was removed and pollination was done on the cut surface. Latex coagulation was observed at the cut surface and flowers failed to set fruits in all the accessions.

c) Removal of the stigma and pollination with the help of sucrose solution

The cut end of the style is not as suitable for pollen germination as the stigma. So, sucrose solution (1.00%) was used on the cut stylar surface as a nutrient medium to facilitate the pollen germination. Latex coagulated on the cut surface and no fruit set was observed.

d) Removal of the stigma and pollination with the help of Brewbaker and Kwack's medium

Brewbaker and Kwack's medium was found to be the best medium for *in vitro* pollen germination in adapathiyan (Manju, 1997). Pollination using *in vitro* germinated pollinia in Brewbaker and Kwack's medium also did not set fruits and latex coagulation was observed on the cut surface.

e) Pollination after removal of the stigma and a part of style

Removal of the stigma and a part of style also exuded latex at the cut surface and pollination failed to set fruits.

f) Pollination with the help of sucrose solution after removal of the stigma and a part of style

The upper part of the style along with the stigma was amputated followed by pollination at cut surface of the style along with 5 per cent sucrose solution.

As in other methods, all the accessions failed to set any fruits and latex coagulation was seen at cut surface.

g) Pollination with the help of Brewbaker and Kwack's medium after removal of the stigma and a part of style

No fruit set was observed in any of the accessions and latex coagulation was seen at cut surface.

h) Pollination with *in vitro* germinated pollen grains

The pollinia from the flowers were taken out and kept in a drop of Brewbaker and Kwack's medium on a clean glass slide and kept in a desiccator.

Pollen germination was ascertained by examining pollen tube growth 24 h after inoculation. Thus *in vitro* germinated pollinia was used for pollination. Here also, none of the accessions produced any fruit.

i) Pollination at early, mid and late season of the crop

Flowering season was found to be July to October in accessions HA.1, HA. 2, HA. 7 and HA. 13 and it was July-September in other accessions. The month of July was found to be the early flowering season, August-September the mid season and October the end of flowering season in accessions HA. 1, HA, 2, HA. 7 and HA. 13. In other accessions, July was the early season, August the mid and September the late season.

Pollination during early and mid season resulted in no fruit set. Among 15 accessions only two set fruits by end of season pollination. Fruit set was observed only in those accessions where flowering extended to October. Accession HA. 7 produced 6.66 per cent fruit set and accession HA. 13 produced 3.33 per cent fruit set (Table 8). All the other accessions failed to produce any fruits. But the size of selfed fruit was smaller.

j) Application of 2,4-D

None of the accessions showed any response to 2,4-D treatment, but the treated flowers were retained for one week more compared to untreated ones.

4.2 Pollen pistil interaction

Pollen pistil interaction under selfing, crossing and other methods to break incompatibility were studied by fluorescence technique 24 h and 48 h after pollination. The study revealed that under selfing, pollen grains were germinated on the stigma, pollen tube growth was normal through the style and they reached

Table 8. Fruit set under end of season selfing in adapathiyan accessions

Sl. No.	Accession No.	No. of flowers pollinated	Number of fruit set	Percentage of fruit set
1	HA. 1	30	0	0.00
2	HA. 2	30	0	0.00
3	HA. 3	30	0	0.00
4	HA. 4	30	0	0.00
5	HA. 5	30	0	0.00
6	HA. 6	30	0	0.00
7	HA. 7	30	2	6.66
8	HA. 8	30	0	0.00
9	HA. 9	30	0	0.00
10	HA.13	30	1	3.33
11	HA.16	30	0	0.00
12	HA.17	30	0	0.00
13	HA.19	30	0	0.00
14	HA.21	30	0	0.00
15	HA.23	30	0	0.00

the ovary but fertilisation was not taking place (Plate 10). In crossing, pollen tube growth was similar as in the case of selfing but pollen tube could enter inside the egg and effect fertilisation (Plate 11). But in some cases, pollen tube got inhibited in the ovary in crossing also (Plate 12). This indicates the chance of some extent of cross incompatibility prevailing in adapathiyan which is supported by the very low fruit set of 6.00-10.00 per cent and 0.00-4.00 per cent under natural and artificial crossing respectively.

In amputation methods, no pollen tube growth was noticed after pollination. Latex was found to be coagulated on cut surface which might have prevented the pollinial germination and subsequent growth. Moreover stigmatic exudation is necessary for pollen germination. In adapathiyan, it was found that stigma or style is not the site of incompatibility and hence there wont be any positive effect by cutting the stigma and style followed by pollination.

In bud pollination, pollen tube was not observed due to lack of stigma receptivity during bud stage.

Pollination with *in vitro* germinated pollen grain and pollination during early and mid flowering season resulted in no fruit set but pollen tube growth was normal.

Application of 2,4-D on the pedicel followed by pollination did not show any positive response to fruit set but the flowers treated were retained on the plant for further 7 days. Pollen tube growth was observed in this method also.

Plate 10. Pollen pistil interaction of selfed flower in adapathiyan

Plate 11 & 12. Pollen pistil interaction of crossed flowers in adapathiyan

4.3 Characterisation and evaluation of accessions and hybrid progenies

4.3.1 Evaluation of accessions

Fifteen accessions of adapathiyan collected from different parts of Kerala state and maintained at the Department of Plantation Crops and Spices were used for the study. The vegetative characters viz., length of vine, number of branches, collar girth, inter nodal length, leaf number and leaf area at 3 MAP and 8 MAP and yield characters viz., fresh weight of roots, dry weight of roots, volume of roots, root length, root girth and driage at harvest and biochemical characters were compared and presented.

4.3.1.1 Vegetative characters

Based on leaf shape, cordate and elongate and based on pigmentation, purple and green types were identified in the accessions. Among the 15 accessions evaluated, HA.3 and HA.5 were purple elongate types, HA.1 and HA.9 were green elongate, HA.6 green cordate and all other accessions were purple cordate type.

The analysis of variance showed significant difference among accessions for all morphological characters studied except number of branches and leaf area at 3 MAP and number of branches, inter nodal length and leaf area at 8 MAP (Table 9).

At 3 MAP, the length of vine ranged from 8.00 cm (HA. 4) to 30.00 cm (HA. 7). The accessions HA. 7, HA. 21, HA. 19 and HA. 17 showed higher vine length which were on par. Accessions HA. 5 and HA. 21 showed maximum number of branches (1.50) though not significant. Number of leaves ranged from

Table 9. Vegetative characters of adapathiyan accessions at 3 MAP

Sl. No.	Accession No.	Place of collection	Pigmentation	Leaf shape	Length of vine (cm)	No. of branches	No. of leaves	Collar girth (cm)	Internodal length (cm)	Leaf area (cm²)
1	HA. 1	Poojappura	Green	Elongate	11.00	1.00	11.00	0.50	1.50	16.54
2	HA. 2	Madakkathara	Purple	Cordate	15.00	1.00	7.00	0.80	1.00	24.80
3	HA. 3	Pazhayangadi	Purple	Elongate	20.10	1.00	24.00	0.65	1.50	26.87
4	HA. 4	Alwaye	Purple	Cordate	8.00	1.00	6.00	0.50	1.00	10.42
5	HA. 5	Odakkali	Purple	Elongate	12.00	1.50	15.00	0.55	2.00	10.70
6	HA. 6	Methala-1	Green	Cordate	12.00	1.00	19.00	0.50	1.00	12.95
7	HA. 7	Mundoor	Purple	Cordate	30.00	1.00	13.00	1.00	2.50	23.88
8	HA. 8	Ernakulam	Purple	Cordate	10.00	1.00	5.00	1.00	1.00	17.52
9	HA. 9	Methala-2	Green	Elongate	10.00	1.00	10.00	0.75	2.50	12.00
10	HA.13	Pampadi	Purple	Cordate	20.00	1.00	16.00	1.00	2.00	23.88
11	HA.16	Anakkayam	Purple	Cordate	12.00	1.00	10.00	0.80	2.00	9.68
12	HA.17	Mannarkad	Purple	Cordate	22.00	1.00	16.00	0.50	1.50	13.44
13	HA.19	Vyalathur	Purple	Cordate	24.00	1.00	23.00	0.80	2.50	15.92
14	HA.21	Palai	Purple	Cordate	25.00	1.50	13.00	1.00	1.00	17.50
15	HA.23	Thamaravellachal	Purple	Cordate	18.00	1.00	18.00	0.85	1.00	19.16
Mean					16.61	1.07	13.73	0.75	1.60	17.02
CD(0.05)					8.66	NS	9.49	0.37	1.13	NS

MAP - Months After Planting

5.00 (HA. 8) to 24.00 (HA. 3) and the mean value was 13.73. Collar girth recorded a maximum value of 1.00 cm in HA. 7, HA. 8, HA. 13 and HA. 21, significantly differed from HA. 1, HA. 4, HA. 6, HA. 17 and HA. 5. With respect to inter nodal length, all accessions were on par and the maximum value recorded was 2.50 cm (HA. 7, HA. 9 and HA. 19). Leaf area, though not significant, maximum value was shown by HA. 3 (26.87 cm^2) and minimum by HA. 16 (9.68 cm^2).

At 8 MAP, vine length showed the same trend as in 3 MAP and maximum was recorded by HA. 7 (340.00 cm) which was on par with HA. 17, HA. 21 and HA. 19. Minimum length recorded by HA. 4 (100.00 cm) (Table 10). Number of branches ranged from 1.00 to 2.00 with a mean value of 1.43. Accession HA. 4 recorded maximum number of leaves (80.00) which was on par with HA. 23, HA. 19 and HA. 21. Maximum collar girth was recorded by HA. 21 (3.10 cm) which was on par with HA. 17, HA. 7, HA. 13 and HA. 8. Minimum collar girth recorded was 1.00 cm by HA. 5, HA. 6, HA. 23 and HA. 9. Inter nodal length ranged from 2.25 cm (HA. 23) to 4.60 cm (HA. 8) and mean value was 3.49 cm. With respect to leaf area, maximum value was shown by HA. 13 (83.21 cm^2) and minimum was by HA. 5 (23.88 cm^2).

4.3.1.2 Yield characters

The accessions differed significantly for all yield characters namely fresh weight of roots, dry weight of roots, volume of roots, root length and root girth except driage (%) (Table 11).

Table 10. Vegetative characters of adapathiyan accessions at 8 MAP

Sl. No.	Accession No.	Place of collection	Length of vine (cm)	No. of branches	No. of leaves	Collar girth (cm)	Inter nodal length (cm)	Leaf area (cm²)
1	HA. 1	Poojappura	190.00	1.50	52.00	1.25	3.35	53.73
2	HA. 2	Madakkathara	200.00	2.00	38.00	1.50	2.50	34.56
3	HA. 3	Pazhayangadi	170.00	1.50	47.00	1.80	3.30	30.85
4	HA. 4	Alwaye	100.00	1.50	80.00	2.20	3.65	32.96
5	HA. 5	Odakkali	130.00	1.00	29.50	1.00	2.80	23.88
6	HA. 6	Methala-1	160.00	1.00	45.50	1.00	2.50	30.85
7	HA. 7	Mundoor	340.00	2.00	51.50	2.75	4.50	43.17
8	HA. 8	Ernakulam	200.00	1.50	49.00	3.00	4.60	62.19
9	HA. 9	Methala-2	250.00	1.50	46.50	1.50	3.75	30.35
10	HA.13	Pampadi	235.00	1.50	51.50	2.75	4.50	83.21
11	HA.16	Anakkayam	210.00	1.00	35.00	1.50	3.75	29.11
12	HA.17	Mannarkad	280.00	1.50	26.00	2.60	3.70	35.82
13	HA.19	Vyalathur	310.00	1.00	63.00	2.00	3.50	82.47
14	HA.21	Palai	300.00	2.00	72.00	3.10	3.75	53.55
15	HA.23	Thamaravellachal	230.00	1.00	62.50	1.00	2.25	24.88
Mean			220.33	1.43	50.47	1.93	3.49	43.44
CD(0.05)			63.70	NS	26.71	0.82	NS	NS

MAP - Months After Planting

Table 11. Yield characters of adapathiyan accessions

Sl. No.	Acce-ssion No.	Place of collection	Fresh weight of roots (g plant^{-1})	Dry weight of roots (g plant^{-1})	Vol. of roots (ml)	Root length (cm)	Root girth (cm)	Driage (%)
1	HA. 1	Poojappura	59.00	18.70	42.00	74.00	3.20	31.72
2	HA. 2	Madakkathara	38.00	12.00	30.00	70.00	4.35	31.84
3	HA. 3	Pazhayangadi	56.50	16.95	43.00	78.00	2.00	29.97
4	HA. 4	Alwaye	64.80	20.15	52.00	133.00	3.60	31.20
5	HA. 5	Odakkali	63.40	29.95	54.00	121.00	3.00	31.58
6	HA. 6	Methala-1	52.00	15.60	37.00	64.00	2.50	30.10
7	HA. 7	Mundoor	70.50	22.70	60.50	180.00	4.50	32.35
8	HA. 8	Ernakulam	71.10	22.70	59.00	154.00	4.00	31.84
9	HA. 9	Methala-2	44.00	13.85	35.00	71.00	2.00	31.38
10	HA.13	Pampadi	65.30	20.20	58.00	105.00	4.00	30.95
11	HA.16	Anakkayam	50.10	16.35	42.00	86.00	2.50	32.77
12	HA.17	Mannarkad	62.30	19.20	55.00	116.00	3.50	30.75
13	HA.19	Vyalathur	65.30	20.55	53.00	98.00	3.70	31.42
14	HA.21	Palai	70.00	21.90	61.00	164.00	4.20	31.11
15	HA.23	Thamaravellachal	58.20	18.20	45.00	81.00	3.00	31.24
Mean			59.37	18.60	48.43	106.33	3.34	31.36
CD(0.05)			9.82	2.73	4.62	22.24	1.07	NS

Maximum fresh weight of roots was recorded by HA. 8 (71.10 g) which was on par with HA. 7, HA. 13, HA. 5, HA. 17, HA. 4, HA. 21 and HA. 19. Accession HA. 2 recorded the minimum value (38.00).

Dry weight of roots was found to be maximum for HA. 5 (29.95 g). Accession HA. 2 recorded the minimum value (12.00 g).

Accession HA. 21 showed highest volume of roots (61.00 ml) which was on par with HA. 7, HA. 13 and HA. 8. Minimum value was shown by HA. 2 (30.00 ml).

The root length was maximum for HA. 7 (180.00 cm) which was on par with HA. 21 (164.00 cm). Minimum length was shown by HA. 6 (64.00 cm).

Root girth ranged from 2.00 cm (HA. 3 and HA. 9) to 4.50 cm (HA. 7). The accessions HA. 7, HA. 13, HA. 2, HA. 17, HA. 8, HA. 4, HA. 21 and HA. 19 were on par.

Driage showed a mean value of 31.36 per cent and it ranged from 29.97 per cent (HA. 3) to 32.77 per cent (HA. 16) though the difference was not significant.

4.3.1.3 Biochemical characters

Accessions showed significant differences for protein and total free amino acids but the differences for soluble sugar and insoluble sugars were not significant (Table 12). Accession HA. 8 showed maximum protein content (0.86%)

Table 12. Biochemical constituents of adapathiyan accessions

Sl. No.	Accession No.	Place of collection	Total free amino acids (%)	Protein (%)	Soluble sugars (%)	Insoluble sugars (%)
1	HA. 1	Poojappura	0.60	0.60	5.79	37.01
2	HA. 2	Madakkathara	0.61	0.70	6.39	37.00
3	HA. 3	Pazhayangadi	0.63	0.65	6.21	37.77
4	HA. 4	Alwaye	0.62	0.70	5.39	37.93
5	HA. 5	Odakkali	0.55	0.68	6.56	33.91
6	HA. 6	Methala-1	0.61	0.76	5.88	37.40
7	HA. 7	Mundoor	0.60	0.79	6.09	34.89
8	HA. 8	Ernakulam	0.62	0.86	6.33	35.26
9	HA. 9	Methala-2	0.61	0.68	6.31	33.72
10	HA.13	Pampadi	0.64	0.84	6.28	38.19
11	HA.16	Anakkayam	0.60	0.74	5.88	37.67
12	HA.17	Mannarkad	0.55	0.69	5.88	37.25
13	HA.19	Vyalathur	0.60	0.71	5.69	37.15
14	HA.21	Palai	0.58	0.77	6.05	37.17
15	HA.23	Thamaravellachal	0.60	0.65	5.43	35.80
Mean			0.60	0.72	6.01	36.58
CD(0.05)			0.05	0.13	NS	NS

which was on par with HA. 6, HA. 7, HA. 8, HA. 13, HA. 16 and HA. 21. Accession HA. 1 recorded minimum protein percentage (0.60%).

All the accessions were on par in total free amino acids content except HA. 5, HA. 17 and HA. 21. Mean value of amino acids content was 0.60 per cent. .

Though not significant, HA. 5 recorded maximum soluble sugar content (6.56%), whereas HA. 4 recorded the minimum (5.39%). Accession HA. 13 showed the highest insoluble sugar content (38.19%), while HA. 5 showed the lowest (33.91%).

4.3.2 Evaluation of parents and hybrids as inter crop and pure crop

Eight hybrids and four parents were grown as inter crop in coconut garden and as pure crop and the comparative performance in terms of vegetative, yield and biochemical characters were recorded.

4.3.2.1 Vegetative characters

The observations on vegetative characters recorded at 3 MAP and 8 MAP were length of vine, number of branches, collar girth, inter nodal length, leaf number and leaf area.

Within the pure crop and inter crop conditions, parents and hybrids differed significantly only for length of vine among the vegetative characters recorded at 3 MAP. Mean value of the parents and hybrids as pure crop was found to be high when compared with inter crop, for all the characters. The mean value of each hybrid and parent as pure crop and inter crop when compared, length of vine and leaf area alone showed significant difference (Table 13).

As pure crop, H_7 recorded maximum vine length (79.16 cm) closely followed by H_8 (66.83 cm) whereas length of vine was significantly low for H_7 and H_8 as inter crop. Between pure crop and inter crop, there was no significant difference in length of vine for all other hybrids and parents. When the mean performance as inter crop and pure crop was compared, H_7 showed maximum plant height of 51.08 cm which was on par with H_6 and H_8.

Maximum number of branches as pure crop was observed in H_7 and P_1 (1.66) whereas H_3 recorded the highest number (1.66) under inter cropped condition, though the variation was not significant. Mean value (1.58) was highest for H_3 followed by H_7 (1.41) while lowest value (1.00) was recorded for H_1 and H_5.

Maximum collar girth of vine (1.73 cm) was shown by H_7 and H_8 and minimum by P_2 as pure crop. As inter crop, highest value (1.41 cm) was observed in H_3 and H_7 and lowest (0.88 cm) in H_8. Mean value of collar girth was maximum in H_7 (1.57 cm) followed by H_2 (1.45 cm).

Inter nodal length was found maximum (4.06 cm) in H_8 followed by H_7 (3.35 cm) and minimum (1.65 cm) in H_3 as pure crop. As inter crop also, maximum inter nodal length was noticed in H_8 (3.03 cm) followed by H_3 (2.75 cm). Mean value was maximum in H_8 (3.55 cm) and minimum in P_4 (1.73 cm).

Table 13. Vegetative characters of hybrids and parents as inter crop and pure crop at 3 MAP

Treatments	Length of vine (cm)			Number of branches			Collar girth (cm)			Inter nodal length (cm)			Leaf number			Leaf area (cm^2)		
	Pure crop	Inter crop	Mean	Pure crop	Inter crop	Mean	Pure crop	Inter crop	Mean	Pure crop	Inter crop	Mean	Pure crop	Inter crop	Mean	Pure crop	Inter crop	Mean
P_1 (Purple elongate)	27.78	19.00	23.39	1.66	1.00	1.33	1.27	1.25	1.26	2.44	2.00	2.22	15.00	10.10	12.55	15.77	10.98	13.38
P_2 (Purple cordate)	28.78	21.00	24.89	1.39	1.20	1.29	1.11	1.00	1.05	2.02	2.16	2.09	12.66	13.86	13.26	12.42	13.66	13.04
P_3 (Green elongate)	17.00	24.33	20.66	1.00	1.16	1.08	1.22	1.16	1.19	1.77	2.26	2.02	14.66	19.33	17.00	12.86	13.47	13.16
P_4 (Green cordate)	28.00	24.33	26.16	1.33	1.33	1.33	1.33	1.06	1.20	1.75	1.72	1.73	13.83	19.20	16.51	12.23	11.33	11.78
H_1 (PE x GE)	36.33	28.43	32.38	1.00	1.00	1.00	1.30	1.26	1.28	2.16	2.38	2.27	9.16	4.86	7.01	19.32	20.19	19.76
H_2 (PE x GC)	36.50	26.83	31.66	1.50	1.00	1.25	1.58	1.33	1.45	2.08	2.25	2.16	20.83	6.00	13.41	33.25	28.75	31.00
H_3 (PC x GE)	30.00	28.50	29.25	1.50	1.66	1.58	1.41	1.41	1.41	1.65	2.75	2.20	11.66	5.50	8.58	12.02	10.19	11.11
H_4 (PC x GC)	37.16	23.66	30.41	1.22	1.33	1.27	1.64	1.08	1.36	2.35	2.66	2.50	9.43	6.67	8.05	19.47	10.29	14.88
H_5 (GC x PE)	18.33	17.00	17.66	1.00	1.00	1.00	1.50	1.08	1.29	1.83	2.41	2.12	7.33	6.33	6.83	34.06	15.86	24.96
H_6 (GC x PC)	31.16	44.83	38.00	1.00	1.16	1.08	1.33	1.13	1.23	2.50	2.66	2.58	12.33	5.66	9.00	12.11	10.07	11.09
H_7 (GE x PE)	79.16	23.00	51.08	1.66	1.16	1.41	1.73	1.41	1.57	3.35	1.81	2.58	36.63	7.66	22.15	17.33	14.32	15.83
H_8 (GE x PC)	66.83	10.10	38.46	1.22	1.00	1.11	1.73	0.88	1.31	4.06	3.03	3.55	15.10	5.53	10.31	20.03	6.76	13.40
Mean	36.42	24.25	30.33	1.29	1.16	1.23	1.43	1.17	1.30	2.33	2.34	2.33	14.88	9.22	12.05	18.406	13.82	16.11
CD (0.05) for comparison of hybrids/ parents within pure crop/inter crop																		
	23.72			NS			NS			NS			NS			NS		
CD (0.05) for comparison of hybrids/ parents																		
			16.33			NS			NS			NS			NS			10.58

MAP - Months After Planting

Highest number of leaves (36.63) was recorded for H_7 as pure crop whereas P_3 showed the highest number (19.33) as inter crop. Mean value was maximum for H_7 (22.15) and minimum for H_5 (6.83).

Leaf area was found to be maximum (34.06 cm^2) in H_5 closely followed by H_2 (33.25 cm^2) as pure crop. H_2 showed maximum leaf area (28.75 cm^2) as inter crop and minimum by H_8 (6.76 cm^2). Mean value was highest for H_2 (31.00 cm^2) which was on par with H_5.

At 8 MAP, the parents and hybrids differed significantly for length of vine and leaf area within pure crop and inter crop condition. Pure crop showed significantly higher value compared to inter crop for length of vine, leaf area and leaf number. Mean value of each hybrid and parent under pure and inter crop differed significantly for length of vine and leaf area (Table 14).

Length of vine was maximum (363.66 cm) in H_1 which was on par with all other parents and hybrids except P_1 and H_4 as pure crop. As inter crop maximum length of vine was noted in H_2 (299.33 cm). This was on par with P_1, H_1 and H_3. Minimum vine length was observed in P_1 (191.66 cm) as pure crop and in H_7 (83.66 cm) as inter crop. The mean value of length of vine was found to be maximum (308.16 cm) for H_1 which was on par with H_2 and H_3.

Though not significant, number of branches was found to be maximum (3.66) for H_1 as pure crop and H_4 recorded maximum number (2.66) as inter crop. The mean value was maximum (2.50) for H_1.

Table 14. Vegetative characters of hybrids and parents as inter crop and pure crop at 8 MAP

Treatments	Length of vine (cm)			Number of branches			Collar girth (cm)			Inter nodal length (cm)			Leaf number			Leaf area (cm^2)		
	Pure crop	Inter crop	Mean	Pure crop	Inter crop	Mean	Pure crop	Inter crop	Mean	Pure crop	Inter crop	Mean	Pure crop	Inter crop	Mean	Pure crop	Inter crop	Mean
P_1 (Purple elongate)	191.66	224.00	207.83	2.00	1.00	1.50	2.33	1.66	2.00	11.00	4.33	7.66	63.66	31.66	47.66	55.22	20.56	37.89
P_2 (Purple cordate)	243.66	159.33	201.50	2.33	1.66	2.00	2.00	1.50	1.75	7.66	3.00	5.33	71.00	18.66	44.83	44.61	10.44	27.53
P_3 (Green elongate)	243.33	107.33	175.33	2.00	1.66	1.83	1.66	1.33	1.50	8.33	3.00	5.66	78.33	24.33	51.33	29.68	11.61	20.65
P_4 (Green cordate)	280.66	89.33	185.00	2.33	1.33	1.83	2.16	1.33	1.75	7.00	4.00	5.50	87.33	16.66	52.00	58.87	8.46	33.66
H_1 (PE x GE)	363.66	252.66	308.16	3.66	1.33	2.50	2.16	2.00	2.08	5.16	4.33	6.41	100.33	29.00	64.66	67.49	77.94	72.72
H_2 (PE x GC)	258.33	299.33	278.83	2.33	1.00	1.66	2.33	1.66	2.00	7.33	2.50	6.08	58.00	45.33	51.66	43.78	53.56	48.67
H_3 (PC x GE)	322.66	262.66	292.66	1.66	1.66	1.66	2.66	1.50	2.08	9.66	4.50	5.91	73.33	29.00	51.16	92.20	50.08	71.14
H_4 (PC x GC)	230.00	130.33	180.16	1.33	2.66	2.00	2.00	2.00	2.00	8.50	2.83	4.00	67.66	37.33	52.50	62.19	26.04	44.11
H_5 (GC x PE)	263.33	114.66	189.00	2.00	1.33	2.00	1.66	1.50	1.58	2.83	3.60	3.25	59.66	22.66	41.16	34.25	21.23	27.74
H_6 (GC x PC)	246.66	100.00	173.33	1.33	1.60	1.50	2.00	1.00	1.50	4.66	3.50	4.08	46.33	13.00	29.66	79.43	11.44	45.44
H_7 (GE x PE)	334.33	83.66	209.00	2.00	1.33	1.66	2.00	1.33	1.66	4.33	2.66	3.50	52.66	21.66	37.16	73.79	10.61	42.21
H_8 (GE x PC)	294.66	130.66	212.66	1.33	1.33	1.33	2.00	1.33	1.66	4.66	3.33	4.00	54.33	16.66	35.50	41.80	18.24	30.02
Mean	272.75	162.83	217.79	2.08	1.50	1.79	2.08	1.51	1.79	6.76	3.47	5.11	67.72	25.50	46.61	56.94	26.69	41.81
CD (0.05) for comparison of hybrids/ parents within pure crop/inter crop																		
	122.04			NS			NS			NS			NS			29.45		
CD (0.05) for comparison of hybrids/ parents																		
			85.86			NS			NS			NS			NS			20.83

MAP - Months After Planting

Collar girth of vine was found to be highest (2.66 cm) for H_3 as pure crop. H_1 and H_4 recorded maximum value of 2.00 cm as inter crop. The mean value was maximum for H_1 and H_3 (2.08 cm).

Maximum inter nodal length (11.00 cm) was noted in P_1 followed by H_3 (9.66 cm) as pure crop. H_3 recorded maximum value of 4.50 cm as inter crop. The mean value was found to be highest (7.66 cm) for P_1 and lowest (3.25 cm) for H_5.

Leaf number was maximum (100.33) in H_1 and minimum (46.33) in H_6 as pure crop. H_2 recorded maximum (45.33) leaf number and H_6 recorded the minimum (13.00) as inter crop. H_1 recorded highest mean value of 64.66 and H_6 recorded the lowest number (29.66).

As pure crop, H_3 recorded maximum leaf area (92.20 cm^2) and it was on par with H_1, H_6 and H_7. Minimum leaf area (29.68 cm^2) was noticed in P_3 which was on par with P_1, P_2, P_4, H_2, H_5 and H_8. As inter crop, H_1 showed maximum leaf area of 77.94 cm^2 which was on par with H_2 and H_3. Mean value was maximum (72.72 cm^2) in H_1 and was on par with H_3. Minimum value (20.65 cm^2) was recorded by P_3 and was on par with P_1, P_2, P_4 and H_8.

4.3.2.2 Yield characters

Eight hybrids and four parents were compared for yield characters namely fresh weight of roots, dry weight of roots, volume of roots, length of root, girth of root and driage (Table 15).

Table 15. Yield characters of hybrids and parents as inter crop and pure crop in adapathiyan

Treatments	Fresh weight of roots (g plant^{-1})			Dry weight of roots (g plant^{-1})			Volume of roots (ml)			Length of roots (cm)			Girth of roots (cm)			Driage (%)		
	Pure crop	Inter crop	Mean	Pure crop	Inter crop	Mean	Pure crop	Inter crop	Mean	Pure crop	Inter crop	Mean	Pure crop	Inter crop	Mean	Pure crop	Inter crop	Mean
P_1 (Purple elongate)	62.80	34.73	48.77	20.50	11.07	15.78	49.17	23.00	36.05	96.67	61.50	79.08	3.73	2.57	3.15	32.60	31.77	32.19
P_2 (Purple cordate)	64.33	30.53	47.43	20.63	9.70	15.17	51.00	21.33	36.17	104.00	50.07	77.03	3.97	2.03	3.00	32.01	31.69	31.85
P_3 (Green elongate)	55.00	31.03	43.02	17.57	10.13	13.85	42.33	21.33	31.83	79.33	50.63	64.98	3.07	2.40	2.73	31.98	32.43	32.21
P_4 (Green cordate)	50.90	33.10	42.00	16.00	10.60	13.45	38.83	21.33	30.08	82.33	59.63	70.98	2.87	2.60	2.73	31.96	32.08	32.02
H_1 (PE x GE)	58.43	33.20	45.82	18.30	10.47	14.38	46.00	21.00	33.50	97.33	64.67	81.00	2.90	2.43	2.67	31.14	31.52	31.33
H_2 (PE x GC)	55.40	30.77	43.08	18.03	9.20	13.62	41.10	18.67	29.88	92.53	54.67	73.50	3.13	2.30	2.72	32.53	29.85	31.19
H_3 (PC x GE)	65.77	37.50	51.63	20.87	12.13	16.50	53.33	23.67	38.50	111.00	77.80	94.40	3.67	3.00	3.33	31.64	32.28	31.96
H_4 (PC x GC)	64.87	33.33	49.10	21.57	10.40	15.98	52.33	22.00	37.17	104.33	77.40	90.87	3.27	3.10	3.18	33.26	31.22	32.24
H_5 (GC x PE)	57.30	26.17	41.73	18.33	8.03	13.18	44.67	16.00	30.33	82.00	39.93	60.97	2.70	2.23	2.47	32.03	30.61	31.32
H_6 (GC x PC)	52.67	27.07	39.87	16.90	8.53	12.72	38.53	15.67	27.10	79.00	45.67	62.33	2.85	1.92	2.38	32.09	31.57	32.21
H_7 (GE x PE)	54.33	26.60	40.47	17.70	8.47	13.08	41.67	16.33	29.00	78.67	45.33	62.00	2.97	1.85	2.41	32.60	31.82	32.21
H_8 (GE x PC)	45.23	28.77	37.00	16.33	8.93	12.63	39.67	17.67	28.67	84.00	51.07	67.53	3.00	2.13	2.57	31.29	31.14	31.22
Mean	57.25	31.07	44.16	18.59	9.81	14.20	44.89	19.83	32.36	90.92	56.53	73.72	3.18	2.38	2.78	32.10	31.50	31.80
CD (0.05) for comparison of parents /hybrids within pure crop /inter crop																		
	5.78			NS			5.43			NS			0.49			NS		
CD (0.05) for comparison of parents/ hybrids																		
			4.08			1.76			3.84			12.14			0.35			NS

Within the pure crop and inter crop conditions, parents and hybrids differed significantly for the characters fresh weight of roots, volume of roots and girth of roots.

When mean performance was compared between pure and inter crop,. pure crop was significantly superior to inter crop for all the characters studied except driage.

With respect to the mean performance of each parent and hybrid as pure and inter crop, significant difference was noticed for all the characters except driage.

H_3 recorded maximum fresh root yield of 65.77 g which was on par with H_4, P_1 and P_2 as pure crop. As inter crop also H_3 recorded highest yield of 37.50 g which was on par with H_4, H_1, P_1 and P_4. H_3 recorded highest mean value for yield (51.63 g) which was on par with H_4, P_1 and P_2.

With regard to dry weight of roots, though not significant, H_4 recorded maximum value (21.57 g) and P_4 recorded minimum (16.00 g) as pure crop. As inter crop, H_3 recorded maximum of 12.13 g and minimum (8.03 g) for H_5. Mean value of dry weight of root was maximum (16.50 g) for H_3 which was on par with H_4, P_1 and P_2.

As pure crop, volume of root was maximum (53.33 ml) for H_3 which was on par with H_4, P_1 and P_2. H_6 recorded the minimum value (38.53 ml) which was on par with H_7, H_8, H_5, H_2 and P_3. As inter crop, H_3 showed the highest value (23.67 ml) which was on par with all the parents and H_1, H_2 and H_4. The maximum mean value of volume of roots (38.50 ml) was also shown by H_3 which was on par with H_4, P_1 and P_2.

With respect to root length, H_3 showed maximum length (111.00 cm) as pure crop and H_7 showed the minimum (78.67 cm). As inter crop, H_3 recorded the maximum value of 77.80 cm closely followed by H_4 (77.40 cm) and minimum by H_5 (39.93 cm). Mean performance was also maximum (94.40 cm) in H_3 followed by H_4 (90.87 cm).

Maximum root girth (3.97 cm) was observed in P_2 which was on par with P_1 and H_3 as pure crop. As inter crop H_4 showed maximum root girth (3.10 cm) on par with H_3. The mean root girth was maximum (3.33 cm) for H_3 and was on par with H_4, P_1 and P_2.

Though not significant, maximum driage was recorded by H_4 (33.26%) and minimum by H_1 (31.14%) as pure crop. As inter crop, maximum driage percentage was observed in P_3 (32.43%) and minimum in H_2 (29.85%). Mean performance was maximum by H_4 (32.24%).

4.3.2.3 Biochemical constituents

Biochemical constituents like total free amino acids, protein, soluble sugar and insoluble sugar of hybrids and parents were compared as pure and inter crop and the study revealed that they differed significantly except for insoluble sugar (Table 16).

The hybrids and parents raised as pure crop when compared with inter crop differed significantly in all biochemical constituents studied and pure crop registered higher values for all constituents except insoluble sugar. The mean value of hybrids and parents differed significantly for all constituents studied.

As pure crop, maximum amino acid content (1.03%) was recorded by H_8 which was on par with H_7. Maximum amino acid content (0.60%) was shown by H_7 as inter crop and was on par with H_8, P_1, P_4, H_1 and H_2. Mean content of amino acid was found to be 0.60 per cent. Mean value was maximum (0.81%) for H_8 which was on par with H_7.

As pure crop, H_1 was found to be significantly superior (0.79%) to all other hybrids and parents in protein content. As inter crop, H_3 was significantly superior (0.58%) which was on par with H_4. Mean value of H_1 (0.65%) was significantly superior to others and was on par with P_3 and H_2 (0.62%).

With respect to soluble sugar content, significantly superior performance was exhibited by H_2 (7.57%) and P_3 recorded the minimum value (4.52%) as inter crop. As pure crop, H_1 recorded maximum value (8.30%) and was on par with H_2 and P_1. On comparing the mean value, H_1 and H_2 were on par and showed higher values. H_8 recorded the minimum mean value (4.96%).

Table 16. Biochemical constituents of hybrids and parents as inter crop and pure crop in adapathiyan

Treatments	Total free amino acids (%)			Protein (%)			Soluble sugar (%)			Insoluble sugar (%)		
	Pure crop	Inter crop	Mean	Pure crop	Inter crop	Mean	Pure crop	Inter crop	Mean	Pure crop	Inter crop	Mean
P_1	0.31	0.53	0.42	0.60	0.50	0.55	7.92	6.43	7.17	38.84	49.83	44.34
P_2	0.82	0.39	0.60	0.56	0.37	0.46	6.52	5.74	6.13	41.50	50.86	46.18
P_3	0.79	0.42	0.60	0.73	0.51	0.62	5.80	4.52	5.16	31.26	43.62	37.44
P_4	0.87	0.57	0.72	0.57	0.45	0.51	5.70	4.92	5.31	31.90	46.19	30.05
H_1	0.35	0.52	0.44	0.79	0.52	0.65	8.30	6.91	7.60	31.14	46.62	38.88
H_2	0.32	0.57	0.44	0.73	0.52	0.62	8.13	7.57	7.85	30.28	43.81	37.04
H_3	0.78	0.37	0.57	0.62	0.58	0.60	6.02	5.99	6.00	39.22	51.62	45.42
H_4	0.85	0.40	0.62	0.60	0.54	0.57	5.93	5.53	5.73	38.36	50.75	44.56
H_5	0.79	0.41	0.60	0.54	0.45	0.49	5.50	4.59	5.04	30.34	45.42	37.88
H_6	0.79	0.39	0.59	0.55	0.38	0.46	5.45	4.62	5.03	31.05	41.48	37.26
H_7	0.96	0.60	0.78	0.52	0.41	0.46	5.54	5.37	5.45	28.04	42.93	35.48
H_8	1.03	0.58	0.81	0.53	0.45	0.49	5.40	4.53	4.96	27.48	41.90	34.69
Mean	0.72	0.48	0.60	0.61	0.47	0.54	6.35	5.56	5.95	33.28	46.25	39.77
CD (0.05) for comparison of parents /hybrids within pure crop /inter crop												
	0.09			0.04			0.57			NS		
CD (0.05) for comparison of parents/ hybrids												
			0.06			0.03			0.41			3.14

Hybrids and parents did not show significant difference in insoluble sugar content as inter crop and pure crop. As pure crop, P_2 showed maximum values (41.50%) and as inter crop H_3 showed maximum value (51.62%). Mean value was found to be 39.77 per cent.

4.3.3 Heterosis

Relative heterosis (RH) and heterobeltiosis (HB) were computed for the eight hybrids with respect to vegetative and yield characters (Tables 17 and 18).

a) Length of vine

Magnitude of relative heterosis varied between -0.12 (H_4) to 46.22 (H_8). Though positive heterosis was observed for all the hybrids except H_4, it was not significant.

Heterobeltiosis estimates indicated a variation from -20.42 (H_4) to 25.02 (H_8), but the variation was not significant. All the hybrids except H_4 and H_6 recorded positive values.

b) Number of branches

The range of relative heterosis was from -27.52 (H_7) to 15.46. Heterosis was not significant. Positive values were obtained for H_3, H_4 and H_8. Heterosis was nil for H_5.

Magnitude of heterobeltiosis ranged from -33.50 (H_7) to 33.00 (H_4). Heterosis was nil for H_5. Positive values were observed for H_3, H_4 and H_8.

Table 17. Mean, relative heterosis (RH) and heterobeltiosis (HB) for vegetative characters in hybrids of adapathiyan

Treat-ments	Length of vine (cm)			No. of branches			Collar girth (cm)			Inter nodal length (cm)			Leaf No.			Leaf area (cm^2)		
	Mean	Relative heterosis	Heter-obelti-osis	Mean	Relative heterosis	Heter-obelti-osis	Mean	Relative heterosis	Heter-obelti-osis	Mean	Relative heterosis	Heter-obelti-osis	Mean	Relative heterosis	Heter-obelti-osis	Mean	Relative heterosis	Heter-obelti-osis
P_1 (PE)	207.83			1.33			2.00			2.22			12.55			37.89		
P_2 (PC)	201.50			1.29			1.75			2.09			13.26			27.53		
P_3 (GE)	175.33			1.08			1.50			2.02			17.00			20.65		
P_4 (GC)	185.00			1.33			1.75			1.73			16.51			33.66		
H_1 (PE x GE)	308.16	19.42	19.27	1.00	-18.26	-25.00	2.08	12.61*	9.58*	2.27	-46.00	-95.53	7.01	-39.62	-39.85	72.72	21.51	-3.65
H_2 (PE x GC)	278.83	14.37	4.88	1.25	-17.00	-17.00	2.00	-0.20*	-8.50	2.16	9.90*	-4.71*	13.41	31.61*	16.24	48.67	104.61	83.92
H_3 (PC x GE)	292.66	35.34	15.72	1.58	15.46	9.58	2.08	15.31*	9.71*	2.20	-24.65	-35.55	8.58	12.43	9.87	71.14	11.47	84.46
H_4 (PC x GC)	180.16	-0.12	-20.42	1.27	52.00	33.00	2.00	6.66*	0.00	2.50	9.30*	-6.48	8.05	-17.79	-25.64	44.11	-1.33	-19.70
H_5 (GC x PE)	189.00	28.00	17.39	1.00	0.00	0.00	1.58	8.99*	0.00	2.12	-42.50*	-45.28	6.83	-7.42	-18.24	27.74	-19.68	-27.80
H_6 (GC x PC)	173.33	17.96	-6.00	1.08	-14.28	-25.00	1.50	-20.00	-25.00	2.58	-33.88	-43.44	9.00	-5.50	-14.53	45.44	-7.49	-24.71
H_7 (GE x PE)	209.00	1.80	1.69	1.41	-27.52	-33.50	1.66	12.61*	9.58*	2.58	-17.47	-21.43	22.15	1.48	1.11	42.21	71.65	-36.10
H_8 (GE x PC)	212.66	46.22	25.02	1.11	15.46	9.58	1.66	0.30	-4.57	3.55	-32.22	-42.02	10.31	-36.58	-38.03	30.02	12.83	-1.58

* - Significant at 5% level

Table 18. Mean, relative heterosis (RH) and heterobeltiosis (HB) for yield characters in hybrids of adapathiyan

Treatments	Fresh weight of roots (g $plant^{-1}$)			Dry weight of roots (g $plant^{-1}$)			Volume of roots (ml)			Length of roots (cm)			Girth of roots (cm)			Driage (%)		
	Mean	Relative heterosis	Heterobeltiosis	Mean	Relative heterosis	Heterobeltiosis	Mean	Relative heterosis	Heterobeltiosis	Mean	Relative heterosis	Heterobeltiosis	Mean	Relative heterosis	Heterobeltiosis	Mean	Relative heterosis	Heterobeltiosis
P_1 (PE)	48.77			15.78			36.05			79.08			3.15			32.19		
P_2 (PC)	47.43			15.17			36.17			77.03			3.00			31.85		
P_3 (GE)	43.02			13.85			31.83			64.98			2.73			32.21		
P_4 (GC)	42.00			13.45			30.08			70.98			2.73			32.02		
H_1 (PE x GE)	45.82	0.99	-6.05	14.38	-1.61	-8.87	33.50	1.27	-7.15	81.00	7.96	66.09*	2.67	-9.18	-15.24	31.33	-2.41	-2.67
H_2 (PE x GC)	43.08	-6.10	-11.67	13.62	-8.07	-13.69	29.88	-12.00	-17.18	73.50	2.04	-7.06	2.72	-7.48	-13.65	31.19	-3.13	-3.17
H_3 (PC x GE)	51.63	15.46*	8.86*	16.50	15.30*	18.77*	38.50	16.23*	6.44*	94.40	27.56*	22.55*	3.33	16.23*	11.00*	31.96	0.07	-0.18
H_4 (PC x GC)	49.10	8.50*	3.52	15.98	10.13*	5.34*	37.17	9.32*	2.76	90.87	27.98*	17.97*	3.18	10.99*	6.00*	32.24	0.65	0.09
H_5 (GC x PE)	41.73	-9.07	-14.44	13.18	-11.04	-16.48	30.33	-10.68	-15.94	60.97	-15.35	-22.90	2.47	-15.99	-21.58	31.32	-2.73	-2.76
H_6 (GC x PC)	39.87	-11.89	-15.94	12.72	-12.34	-16.15	27.10	-20.29	-25.07	62.33	-12.22	-19.08	2.38	-16.93	-20.67	32.21	-0.62	-1.18
H_7 (GE x PE)	40.47	-10.80	-17.02	13.08	-10.50	-17.11	29.00	-12.33	-19.62	62.00	-17.37	-21.59	2.41	-18.03	-23.49	32.21	0.32	0.06
H_8 (GE x PC)	37.00	-17.25	-21.99	12.63	-11.70	-16.74	28.67	-13.45	-20.74	67.53	-8.75	-12.33	2.57	-10.29	-14.33	31.22	-2.27	-2.53

* - Significant at 5% level

c) Collar girth

The range observed for relative heterosis was from -20.00 (H_6) to 15.31 (H_3). H_1, H_3, H_4, H_5 and H_7 recorded significant estimates. All hybrids except H_2 and H_6 recorded positive values.

The spectrum of heterobeltiosis varied between -25.00 (H_6) to 9.71 (H_3). H_1, H_3 and H_7 exhibited significantly high positive heterobeltiosis. Heterosis was nil for H_4 and H_5.

d) Inter nodal length

Magnitude of relative heterosis varied from -46.00 (H_1) to 9.90 (H_2). Significant positive values were obtained for H_2 and H_4.

Heterobeltiosis ranged between -95.53 (H_1) to -4.71 (H_2). All the hybrids showed negative heterobeltiosis.

e) Leaf number

The variation in relative heterosis was from -39.62 (H_1) to 31.61 (H_2). Positive relative heterosis was observed for H_2, H_3 and H_4 of which H_2 alone showed significant heterosis.

The magnitude of variation in heterobeltiosis ranged from -39.85 (H_1) to 16.24 (H_2). None of the hybrids showed significant heterobeltiosis, but H_2, H_3 and H_7 recorded positive values.

f) Leaf area

Relative heterosis values ranged from -19.68 (H_5) to 111.47 (H_3). Though H_1, H_2, H_3, H_7 and H_8 recorded positive values, heterosis was not significant.

Magnitude of heterobeltiosis varied between -27.80 (H_5) to 84.46 (H_3). Though not significant, positive values were shown by H_2, H_3 and H_7.

g) Fresh weight of roots

The spectrum of RH varied between -17.25 (H_8) to 15.46 (H_3). Significant positive heterosis was recorded by H_3 and H_4.

The variation in heterobeltiosis was from -21.99 (H_8) to 8.86 (H_3). Significant positive heterobeltiosis was exhibited by H_3 alone.

h) Dry weight of roots

Relative heterosis was observed to range from -12.34 (H_6) to 15.30 (H_3). Significantly positive relative heterosis was recorded by H_3 and H_4.

The values for heterobeltiosis ranged from -16.74 (H_8) to 8.77 (H_3). Significantly positive heterobeltiosis were exhibited by H_3 and H_4.

i) Length of root

Relative heterosis ranged from -17.37 (H_7) to 27.98 (H_4). Significant positive values were obtained for H_3 and H_4.

Heterobeltiosis was observed to range from -22.90 (H_5) to 66.09 (H_1). Significant positive values were recorded by H_1, H_3 and H_4.

j) Girth of root

The relative heterosis for this trait varied from -18.03 (H_7) to 16.23 (H_3).. Significant positive relative heterosis was obtained only for H_3 and H_4.

The limits for heterobeltiosis were -23.49 (H_7) to 11.00 (H_3). H_3 and H_4 alone showed significant positive heterobeltiosis.

k) Volume of roots

The relative heterosis for this trait varied from -20.29 (H_6) to 16.23 (H_3). Significant positive values were recorded by H_3 and H_4.

Heterobeltiosis was observed to range from -25.07 (H_6) to 6.44 (H_3). Only H_3 recorded significant positive heterobeltiosis.

l) Driage

Relative heterosis ranged from -3.13 (H_2) to 0.65 (H_4). H_3, H_4 and H_7 recorded positive values but not significant.

Heterobeltiosis ranged from -3.17 (H_2) to 0.09 (H_4). Only H_4 and H_7 recorded positive heterobeltiosis but it was not significant.

4.4 Formulation of selection parameters based on evaluation of open pollinated progenies

Correlation between seedling characters (1 month old) and root yield was worked out (Table 19). Correlation was also worked out between yield and mature plant characters (8 MAP) to assess the nature and degree of association of morphological characters with yield (Table 20).

Correlation coefficients indicated that significantly high positive correlation exists between leaf area and inter nodal length of seedling with dry root yield.

Correlation studies between mature plant characters and yield revealed that all the four morphological characters tested viz., number of branches, collar girth, inter nodal length and leaf area showed significant positive correlation with dry root yield, maximum among vegetative characters being for inter nodal length.

Table 19. Correlation between seedling characters and dry root yield

Characters	Correlation coefficient
1. Leaf area (cm^2)	0.401**
2. Collar girth of vine (cm)	0.069
3. Number of branches	-0.062
4. Inter nodal length (cm)	0.368*

* Significant at 5% level
** Significant at 1% level

Table 20. Correlation between mature plant characters and dry root yield

Characters	Correlation coefficient
1. Leaf area (cm^2)	0.608**
2. Collar girth of vine (cm)	0.703**
3. No. of branches	0.523**
4. Inter nodal length (cm)	0.824**
5. Fresh weight of root (g)	0.953**
6. Length of root (cm)	0.660**
7. Girth of root (cm)	0.321*
8. Volume of root (ml)	0.906**
9. Driage (%)	0.531**

* Significant at 5% level
** Significant at 1% level

Discussion

DISCUSSION

5.1 Study of reproductive mechanism, incompatibility barrier and effect of different methods to overcome the incompatibility barrier

5.1.1 Flowering and floral characters

Holostemma adakodien is a profuse flowering plant. Flowering occurs on current season shoots. Flowering started in July and peak flowering period was August-September. The flowering season ended in October in 4 accessions (HA. 1, HA. 2, HA. 7, HA. 13) whereas in other accessions flowering ceased by September. These observations are in concurrence with the report of Manju (1997) in adapathiyan.

Flowers appear in axillary cymes and hang down wards. The number of inflorescence per plant varied from 14-45 and number of flowers per inflorescence varied from 8-22. The number of flowers/plant ranged from 208 to 855 in different accessions.

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) also reported these characters in *Holostemma* flower. *Holostemma* flower represents a typical asclepiadaceous flower due to the presence of corona, pollinia and gynostegium (Vasishta, 1974).

Anthesis occurs between 8.30 and 10.30 am with the maximum between 9.00 and 9.30 am (Fig. 1) and completed by 10.00 am in accessions HA. 4, HA. 5, HA.7, HA. 8, HA. 9 and HA. 21 and by 10.30 am in other accessions. 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 am irrespective of the accessions. On fourth day, flowers were in the wilting stage and stigma appeared dry and deep yellow. Similar observations were reported by Manjü (1997) in *Holostemma*.

Stigma receptivity is indicated by shiny appearance, light cream colour and presence of nectar. Peak period of stigma receptivity coincided with maximum flower opening time from 9.00 to 11.00 am as proved by controlled pollination and the trend was same in all the accessions. The receptive side of the stigma was found to be the five lateral sides as reported by Sreedevi (1989) in other Asclepiads. It was tightly covered with a transparent membraneous covering. Position of the pollinia enclosed within the gynostegium, downward position of the flower and tight membrane covering the lateral receptive side of the stigma favour insect pollination and block other modes of pollination.

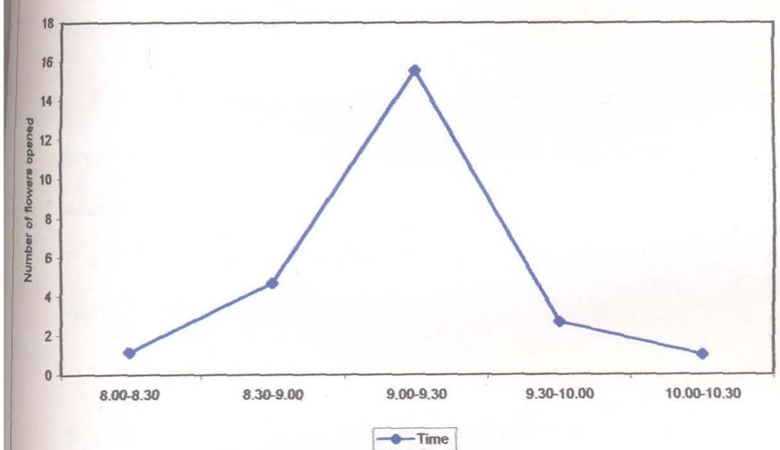

Fig. 1. Influence of time on anthesis in adapathiyan

5.1.1.1 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 in all the accessions. Similar observations were reported by Sreedevi and Namboodiri (1979) in *Asclepias* and *Dregea* and by Manju (1997) in *Holostemma.*

Pollen fertility was assessed by Alexander's stain test in each day of flower opening. In first and second day of flower opening, higher pollen fertility values were obtained (> 92.00%). Fertility decreased gradually thereafter and on 5th day only 40.00 per cent fertility was recorded (Fig. 2). Manju (1997) also made similar observation in Holostemma.

Pollinia were placed for *in vitro* germination in distilled water alone, distilled water + sucrose and Brewbaker and Kwack's medium and observed for germination. Pollen germinated when placed in Brewbaker and Kwack's medium but failed to germinate in other media. Pollinia collected from first, second, third, fourth and fifth day after opening of flowers were kept for *in vitro* germination and sparse germination was noted in flowers collected after four or five days of opening. It was observed that as the days after anthesis increased both fertility of pollen and germination of pollen were decreased.

Brewbaker and Kwack's medium contains high proportion of calcium, boron, magnesium and potassium. Calcium is very essential for pollen germination

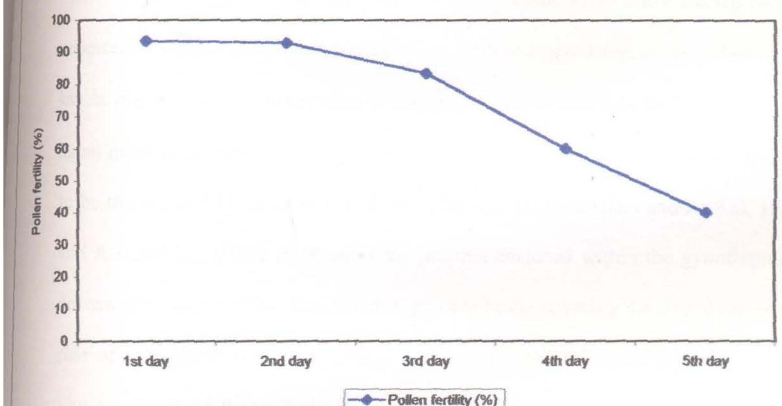

Fig. 2. Pollen fertility as influenced by days of anthesis in adapathiyan

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). The high proportion of these nutrient elements in Brewbaker and Kwack's medium might have promoted pollen germination in adapathiyan too.

5.1.2 Pollination studies

Pollination in adapathiyan is entomophilous as in other Asclepiads as reported by Rendle (1971). Presence of nectariferous gynostegium is in favour of insect pollination. Carpenter bees (*Xylocopa sp.*) were found to be the pollinating agent in adapathiyan as reported by Manju (1997). *Xylocopa sp.* were also reported to be the pollinating agent in *Calotropis gigantea* (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 in *Holostemma*. 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.

Pollination studies revealed that adapathiyan flower is solely cross pollinated and it is entomophilous. But the fruit set observed was very low (6.00-10.00%) even though they produced sufficient flowers (208-855 flowers/plant) and pollen production and germination are not limiting. These results are in concurrence with the finding of Manju (1997) that in adapathiyan fruit set is only

5.00-17.00 per cent. 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.

Various scientists attributed so many reasons for low fruit set in Asclepiads. Cabin *et al.* (1991) suggested that pollen limitation during pollination led to decreased fruit set. According to Manju (1997), pollen removal from the flower is not low, but its insertion into receptive stigmatic surface is very low because of tight membraneous covering on the stigma and pollinia were found misplaced on other parts of the flower. Sreedevi (1989) listed various reasons for low fruit set in Asclepiads. The most important reason is the low degree of removal and insertion of pollinia into the stigmatic chamber.

The present investigation reveals that the low fruit set in adapathiyan is mainly due to incompatibility barrier existing in adapathiyan. This is in conformity with the report of Sparrow (1948) that lack of pollination is not responsible for the poor setting of fruit in *Asclepias syriaca* but a large number of flowers are pollinated with incompatible pollen. Wyatt (1976) reported that the failure of pollen tube to penetrate the ovary and effect fertilisation and similar failures at various stages of developments are responsible for the physiological contribution to low fruit set.

5.1.3 Screening for incompatibility reaction

The data obtained during the course of the present investigation revealed failure of seed set under selfing. Chance for natural selfing is prevented by the innate protogynous nature of the flower, the restricted span of stigma receptivity and wide time lapse between stigma receptivity and anther dehiscence. Artificial selfing tried in 15 accessions also failed to set seed indicating the involvement of self incompatibility in adapathiyan.

Moore (1946) reported a complete lack of self compatibility in *A. syriaca*. Woodson (1954) maintained the opinion that all species of *Asclepias* to be self incompatible which was supported by Wyatt (1976) in *A. syriaca* and Wilson *et al.* (1979) 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*.

Natural cross pollination gave a fruit set of 6.00 to 10.00 per cent and artificial crossing also could not improve the fruit set (0.00-4.00%) in this study indicating the chance of cross incompatibility also in adapathiyan (Fig. 3). Sparrow (1948) reported a similar case where artificial crossing could not improve seed set (10.35%) over natural set (10.00%) in *Asclepias syriaca*.

5.1.4 Methods for breaking the self-incompatibility barrier

The effect of various methods tried for breaking the self incompatibility barrier are discussed as follows:

In the present study, bud pollination was found to be quite ineffective in breaking the self incompatibility barrier. Lack of stigma receptivity during the bud stage can be considered as the cause for the failure of this method. This is in line

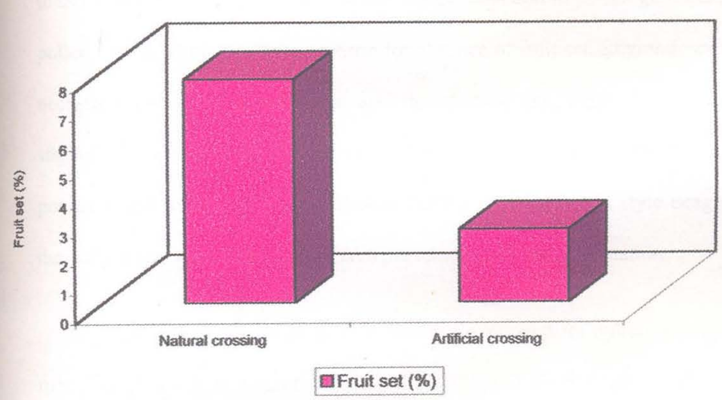

Fig. 3. Fruit set (%) under natural and artificial crossing in adapathiyan

with the reports made by Togeri and Kawahara (1942) and Kumar (1983). Gradziel and Robinson (1989) reported that self incompatibility can be broken down in *Lycopersicon peruvianum* by supplying pollen germinating medium followed by bud pollination.

The method of cutting the stigma and part of style were quite ineffective to overcome the incompatibility barrier. Large reduction in pollen germination and pollen tube growth may be the reason for absence of fruit set. Stigmatic exudate is necessary for pollen germination and the exudate get removed by cutting the stigma. These results are supported by Davis (1957) and Kumar (1983) in sweet potato. Latex exudation observed while cutting the stigma and style coagulate on the cut portion which also might have prevented pollinial germination.

Sucrose was found to be a favourable medium for pollen germination in many crop plants as reported by Kiss (1970) and Radhakrishnan (1976). But contradictory results were obtained in adapathiyan. In the present study, it was found that application of sucrose solution on the cut surface of the style was not at all favourable for pollen germination and seed set. Generally, stigmatic secretion is essential for pollen germination and it could be assumed that in addition to sugar, some other growth promoting substances may be present in stigmatic secretion, which are essential for pollen germination. Brewbaker and Kwack (1963) reported that Ca ion is very essential for pollen germination and tube growth. According to Addicott (1943), pollen tube requires inorganic salts apart from sugar for its growth.

In vitro pollen germination was observed in Brewbaker and Kwack's medium. This medium contains considerable amount of calcium, boron, magnesium and potassium. Brewbaker and Kwack's medium which contains the different elements in comparatively higher proportions, was found most suitable for pollen germination in adapathiyan as observed by Manju (1997). Suitability of this medium for pollen germination in another Asclepiad, *Calotropis gigantea* was reported (Viswanathan and Lakhsmanan, 1984).

Application of Brewbaker and Kwack's medium at cut surface followed by pollination also failed to set fruit. On cutting the stigma, latex exuding was found to coagulate and this might have hindered the pollen tube growth.

Pollination using *in vitro* germinated pollinia in Brewbaker and Kwack's medium also did not set fruits. The result indicates that lack of pollen germination is not the barrier in fruit setting. But there might be some inhibitory substances which controls the self incompatibility reaction. In sweet potato, Kumar (1983) reported that the inhibitory substances are situated on the stigma. According to Moncur *et al.* (1991) inhibitory substances are located within the ovary in *Acacia mearnsii.*

Artificial self pollination during early and mid season resulted in no fruit set in any of the accessions. The fruit set observed for the end of season pollination in HA. 4 (6.67%) and in HA. 13 (3.33%) indicated that the self incompatibility reaction was reduced at later season of flowering in these two accessions but not in other accessions. This may be due to the inadequate quantity of inhibiting substance during end of season (October) confirming the earlier report made by Allard (1960) in tobacco. Kumar (1983) also reported fruit and seed set in incompatible sweet potato variety by end of season pollination.

Application of 2,4-D on the pedicel followed by pollination did not show any positive response to fruit set. But the treated flowers were retained on the plant for further 7 days with no ovary enlargement followed abscission. Charles *et al.* (1974) reported the suppression of floral abscission by the application of 2,4-D to the pedicels, which resulted in successful seed set in *Ipomoea trichocarpa*. They reasoned that the application of 2,4-D gave adequate time for the pollen tube to penetrate the incompatible style and resulted in fertilisation and seed set. According to Kumar (1983), self incompatibility can be broken down by 2,4-D treatment in some varieties of sweet potato.

In adapathiyan, it was found that within 24 h of pollination, pollen tube reached the ovary and flowers will remain open for three more days after pollination and so there is no significance of extending the flower life by application of 2,4-D to the pedicel.

5.2 Pollen pistil interaction

The pollen pistil interaction was studied by the fluorescence technique 24 h and 48 h after pollination under selfing, crossing and ten methods to break incompatibility. In selfed flowers, it was observed that pollen germinate on the stigma, pollen tube grows through the style and reaches the ovary but fertilisation was not taking place. According to Sears (1937), there are 3 sites of incompatibility, stigma, style and ovary. Venkateswarlu (1980) reported that self incompatibility in sweet potato is sporophytically controlled and the histological studies revealed that the inhibition of pollen grains was at the stigma level. Ram *et al.* (1992) reported that stigma is the site of action of the self incompatibility alleles in coffee. Han (1994) reported that in *Hibiscus syriacus* L. pollen tube growth of the incompatible pollinations was inhibited in the style, two third of the way from the stigma to the base. Bruun *et al.* (1995) studied the self incompatibility reaction in *Beta vulgaris* and reported that self incompatibility response was observed at the border between stigma and style. Gupta *et al.* (1998) observed that pollen grains germinated on the stigma, but pollen tubes failed to grow beyond the proximal one third of the style in *Commiphora wrightii.* Unlike the above reports, pollen germination and tube growth were not inhibited by the stigma or style and since pollen tube growth was normal and reaching the ovary it is conclusively proved that late acting gametophytic self incompatibility is operating in adapathiyan and ovary is the site of incompatibility. This study reveals that stigma or style tract of adapathiyan pistil is a mere promoter of pollen tube growth and tube growth was profuse and discrimination of compatible and incompatible pollen occurs in the ovarian tract. The results are in confirmation with Chichirico (1990) who made similar observations in *Crocus vernus*.

Placental cells in the ovary produce the placental fluid. In the ovary, interactions exist between the pollen tubes and the placental fluid and between the pollen tube and the micropylar exudate (Willemse and Wittich, 1996). Some inhibitory substances may be present in placental fluid or micropylar exudate which might have prevented the pollen tube to enter the embryosac. Interaction between pollen tube and integuements was also reported to play a significant role in the stimulation of ovular development (Kostoff, 1930). The first report of incompatibility inhibition in the ovary was made by Stout and Chandler (1933) in *Hemerocallis* species. The ovarian site of incompatibility inhibition was later reported by Sears (1937) for *Gasteria verrucosa* and by Cope (1958) for *Theobroma cacao*. The ovarian site of incompatibility inhibition was reported by Sears (1937) for *Gasteria verrucosa*. Moncur *et al.* (1991) reported that site of incompatibility is within the ovary in *Acacia mearnsii*. Tangmitcharoen and Ownes (1997) found that selfing, pollen tubes are arrested in the lower part of the ovary in teak.

In crossed flowers also the growth of pollen tube was similar to that of selfed ones. This is in confirmity with the report of Sage and Williams (1991) in *Asclepias exaltata* that there was no temporal, structural or histochemical differences between self and cross pollen tube growth. In *Acacia retinodes*, self and cross pollen tubes appear similar through the stigma and style but self-pollen tubes were inhibited in the ovary (Tangmitcharoen and Owens 1997).

In this study, in crossed flowers, pollen tube entered the egg and effected fertilisation. But in some cases, in crossed flower also, pollen tube growth got inhibited in the ovary. This shows that some extent of cross incompatibility is also prevailing in adapathiyan. This might be the reason for the general low fruit set under natural crossing which is the principal mode of pollination reported earlier (Manju, 1997) and observed in this study. The low fruit set even under artificial crossing is in support of this finding. The result is in confirmity with the findings of Sparrow (1948) in *Asclepias syriaca* that lack of pollination is not responsible for the poor setting of fruit but a large number of flowers are pollinated with incompatible pollen. Wyatt (1976) reported that the failure of pollen tube to penetrate the ovary and effect fertilisation and similar failures at various stages of development are responsible for the physiological contribution to low fruit set.

In amputation methods, pollen tube growth was not observed after pollination. Latex coagulation found on cut surface might have prevented the pollinial germination and subsequent fruit set. Moreover, stigmatic exudate is necessary for pollen germination. In adapathiyan, it is observed that stigma or style is not the site of incompatibility but ovary is the site of incompatibility. Amputation methods are of significance in breaking self incompatibility of crops in which inhibition of pollen germination and pollen tube growth occurs on the stigma or in the style. In *Ipomoea trichocarpa* incompatibility barrier was found to be located in the stigma and hence its removal resulted in successful seed set (Charles *et al.,* 1974). Incompatibility in *capsicum* could be overcome by amputation of the style followed by pollination. In toria, removal of upper third portion of the style caused greater increase in pod and seed formation (Goud *et al.*, 1970).

In bud pollination no pollen tube growth was observed because of lack of stigma receptivity during bud stage. Togeri and Kawahara (1942) observed that since the stigma of the sweet potato flower would become receptive only a few hours before anthesis, bud pollination offered little prospect in overcoming self incompatibility. In adapathiyan, stigma becomes receptive only after anthesis and at the receptive stage gynostegium is filled with nectar.

Selfing with *in vitro* germinated pollinia, resulted in no fruit set because lack of pollen germination is not the cause for self incompatibility in adapathiyan.

Selfing during end of season resulted in 6.66 per cent fruit set in adapathiyan accession HA.7 and 3.33 per cent fruit set in HA 13. This indicates that inhibitory substance in the ovary which prevent fertilisation might get reduced in quantity during end of season leading to fruit set. Allard (1960) reported that end of season pollination was effective in breaking the incompatibility barrier in crops like tobacco. In some varieties of sweet potato, Kumar (1983) observed seed set for the end of season pollination and reported that incompatibility reaction was reduced at the end of growth season due to inadequate quantity of the inhibitory substance on the stigma.

Selfing during early and mid season of flowering failed to set fruit in adapathiyan. This might be due to adequate amount of inhibitory substance in the ovary which prevents fertilisation.

Application of 2,4-D on the pedicel followed by pollination did not show any positive response to fruit set. But the flowers treated were retained on the plant for further seven days with no ovary enlargement. Charles *et al.* (1974) reported the suppression of floral abscission by the application of 2,4-D to the pedicels, which resulted in successful seed set in *Ipomoea trichocarpa*. They reasoned that the application of 2,4-D gave adequate time for the pollen tube to penetrate the incompatible style and resulted in fertilisation and seed set. According to Kumar (1983), self incompatibility can be broken by 2,4-D treatment in some varieties of sweet potato.

In adapathiyan, it was found that within 24 h of pollination, pollen tube reached the ovary and flowers remained open for three more days after pollinaiton and so there is no significance of extending the flower life by application of 2,4-D to the pedicel.

5.3 Characterisation and evaluation of accessions and hybrid progenies

5.3.1 Evaluation of adapathiyan accessions

Success of any crop improvement programme primarily depends on the extent of genetic variation and diversity in a crop. To assess the extent of variation, 15 adapathiyan accessions were evaluated for their morphological, yield and biochemical characters.

Based on leaf shape, two types namely cordate and elongate and based on pigmentation, two types namely purple and green were observed. Purple cordate was found to be the predominant type among the four types, purple cordate, purple elongate, green cordate and green elongate.

Purple cordate was found to be the predominant type among the 15 accessions. Significant difference was observed among the accessions for morphological characters such as length of vine, number of leaves, collar girth and inter nodal length at 3 MAP and for characters length of vine, number of leaves and collar girth at 8 MAP.

The accessions showed significant variations for yield characters such as fresh weight of roots, dry weight of roots, volume of roots, root length and root girth which ranged from 38.00 to 71.10 g, 12.00 to 22.70 g, 30.00 to 61.00 ml, 64.00 to 180.00 cm and 2.00 to 4.50 cm respectively. Kurian (1999) studied twenty four *Holostemma* types and found that the types showed significant difference in all yield and yield related characters.

With respect to biochemical constituents, the accessions differed significantly for the contents of protein and total free amino acids but no significant differences were noticed in soluble sugar and insoluble sugar contents. Protein and total free amino acids ranged from 0.60% to 0.86% and 0.55% to 0.64% respectively. Soluble sugar content showed a variation of 5.39% to 6.56% and insoluble sugar varied from 33.72% to 38.19%.

The study revealed a wide range of variability for most of the characters in adapathiyan indicating great scope for selection. In terms of yield, HA. 8, HA. 7, HA. 13, HA. 21, HA. 4, and HA. 19 were found to be promising. The results are consistent with the earlier report that the accessions HA. 7, HA. 8, HA. 13, HA. 19 and HA. 21 were promising (Kurian, 1999).

The type HA. 8 recorded maximum protein, HA. 13 recorded maximum total free amino acid and insoluble sugar. HA. 2 showed maximum soluble sugar. According to Kurian (1999) the type HA. 13 recorded maximum total free amino acids and insoluble sugar and the type HA. 8 recorded maximum protein and soluble sugar.

5.3.2 Evaluation of hybrids and parents

Four parents and eight hybrids were grown as pure crop and as inter crop in coconut garden and they were evaluated for the vigour based on length of vine, number of branches, collar girth of vine, inter nodal length, leaf number and leaf area at 3 MAP (early growth phase) and 8 MAP (active vegetative phase). Yield characters compared were fresh weight of roots, dry weight of roots, volume of roots, length of root, girth of root and driage.

5.3.2.1 Vegetative characters

The study revealed that there was no significant difference between parents and hybrids in most of the vegetative characters studied. They differed significantly only for length of vine at 3 MAP and for length of vine and leaf area at 8 MAP. They showed slight difference for leaf number and leaf area also (Fig. 4).

The study also revealed that overall performance of adapathiyan as pure crop is superior to that as inter crop (Fig. 5). Pure crop showed significantly higher value for length of vine and number of branches at 3 MAP and for length of vine, leaf area and leaf number at 8 MAP.

Mean performance under pure and inter crop conditions showed significant difference only for characters such as length of vine and leaf area (Fig. 6).

At 3 MAP, H_7 and H_8 showed maximum value for most of the vegetative characters whereas at 8 MAP, H_1 and H_3 were found to be superior followed by H_4.

5.3.2.2 Yield characters

Among the six yield characters studied, parents and hybrids differed significantly for three characters namely fresh weight of roots, volume of roots and girth of roots.

Pure crop was significantly superior to inter crop for all characters studied, when overall mean performance was compared. The result is in confirmity with the report of Kurian (1999) that root yield of *Holostemma* was maximum for pure crop compared to inter crop.

With respect to mean performance of each parent and hybrid as pure and inter crop, significant difference was noticed for the characters, fresh weight of roots, dry weight of roots, length of roots and girth of roots.

H_3 recorded highest mean value for fresh weight of roots (51.63 g), dry weight of roots (16.50 g), volume of roots (38.50 ml), length of root (94.40 g) and girth of root (3.33) followed by H_4.

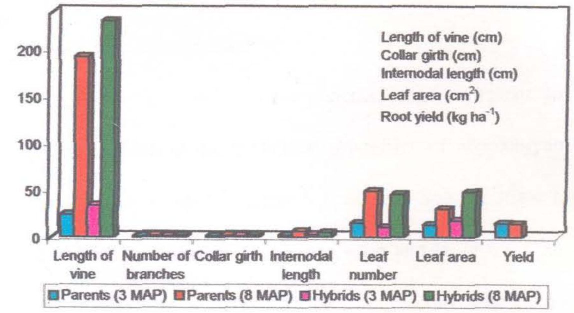

Fig. 4. Comparative performance of hybrids and parents of adapathiyan

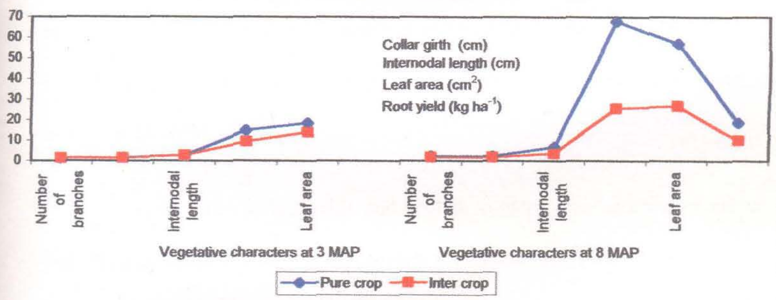

Fig. 5. Comparative performance of adapathiyan as pure crop and inter crop at 3 MAP and 8 MAP

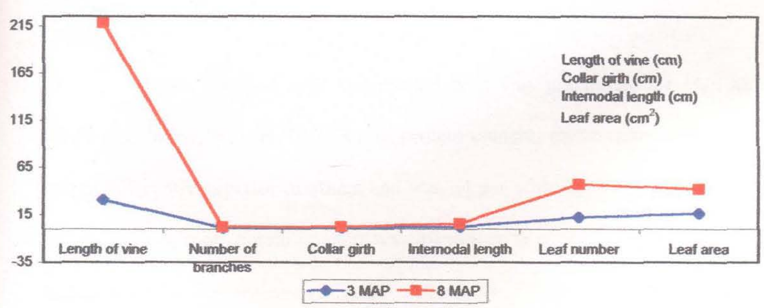

Fig. 6. Comparison of mean performance of hybrids and parents of adapathiyan at 3 MAP and 8 MAP

5.3.2.3 Biochemical constituents

Among the chemical components, sugars present in the roots are reported to contribute to the medicinal properties of adapathiyan (CSIR, 1959). Hence high content of soluble sugar is desirable. Soluble sugar recorded highest content in H_2 (PE x GC) (7.57%) as inter crop and H_1 (PE x GE), H_2 (PE x GC) and P_1 (PE) were on par in sugar content as pure crop. This is in confirmity with the observation of Manju (1997) that soluble sugar recorded highest percentage in purple elongate type (8.52%). Hence selecting the hybrids having PE as one of the parent is desirable due to high medicinal value of PE. Mean value of soluble sugar content was high under pure crop condition (6.35%) compared to inter crop condition (5.56%).

Total free amino acids and protein content recorded higher values as pure crop compared to inter crop. H_8 (GE x PC) and H_7 (GE x PE) were found to be superior in total free amino acid content. In protein content, H_1 (PE x GE) was found to be significantly superior (0.79%) as pure crop and H_3 and H_4 recorded higher values as inter crop.

Mean value of total free amino acid was maximum for H_8 (0.81%) which was on par with H_7 (0.78%). In protein content, mean value of H_1 (0.66%) was significantly superior to others and was on par with P_3 (0.63%). According to Manju (1997), root protein content was maximum in purple cordate (0.76%) and maximum total free amino acid content recorded in green elongate type (0.88%) and was on par with that in purple cordate type (0.83%).

Hybrids and parents did not differ significantly in insoluble sugar content both as pure crop and inter crop. The parent P_2 recorded maximum value of 41.51 per cent followed by H_3 (39.22%) as pure crop and as inter crop, H_3 recorded maximum value (51.62%).

The mean total free amino acids, protein and soluble sugar content of the hybrids and parents were significantly higher for pure crop compared to inter crop. Insoluble sugar content recorded higher value under inter cropped condition compared to pure crop. While comparing the performance of adapathiyan accessions as inter crop and pure crop, Kurian (1999) reported that insoluble sugar content was slightly high for inter crop whereas soluble sugar content was maximum for pure crop and the mean protein and total free amino acid contents of the types showed not much difference between two cropping situations.

Among the four parents, P_1 (purple elongate) and P_2 (purple cordate) and among the eight hybrids, H_3 (purple cordate x green elongate) and H_4 (purple cordate x green cordate) were found to be promising for yield characters. The superior performance of H_3 and H_4 may be due to the involvement of P_2 (purple cordate) as one of the parents. These observations suggest the utility of purple cordate type as seed parent in hybridisation programme of adapathiyan or collection of seeds from purple cordate types under open pollination.

Manju (1997) observed that hybrid progenies with purple cordate or purple elongate type as one of the parents showed better performance in adapathiyan.

5.3.3 Heterosis

Hybridisation is an important tool for crop improvement as it contributes to creation of genetic variability in crops. Heterotic expression of progenies is of concern in any hybridisation programme. No reports are available on heterosis in adapathiyan.

The data indicated that among the eight cross combinations, H_1, H_2, H_3, H_4, H_5 and H_7 expressed significant positive relative heterosis for one or more characters (Fig. 7). The hybrids H_3 and H_4 exhibited the highest relative heterosis for yield characters.

The hybrids which showed significantly positive values for heterobeltiosis were H_1, H_2, H_3, H_4 and H_7. H_3 exhibited high heterobeltiosis for yield characters.

The data indicated H_3 (PC x GE) as the best hybrid followed by H_4 (PC x GC) having high heterosis for yield. This may be due to the involvement of purple cordate as female parent in these crosses which was found to be the high yielding type in evaluation of accessions.

The performance of H_6 (GC x PC) was found very poor and it recorded a negative heterosis estimate for all the traits except length of vine. H_8 (GE x PC) also revealed a some what similar trend and not recorded significant positive estimate for any of the traits studied.

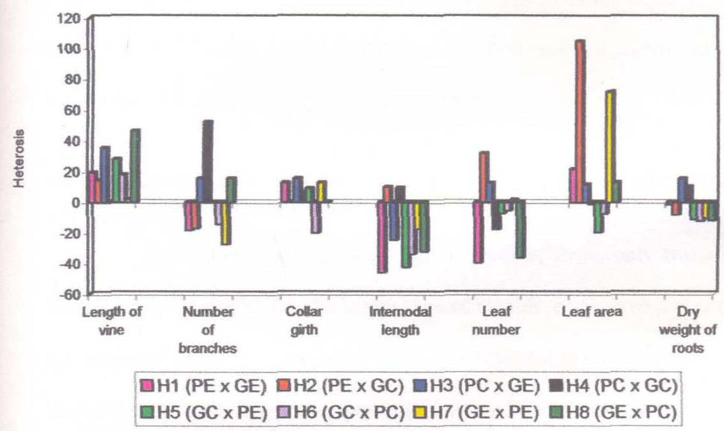

Fig. 7. Relative heterosis and heterobeltiosis in hybrids of adapathiyan

Adapathiyan, being a cross pollinated crop, heterosis breeding is one of the important tools of crop improvement. Janakiram and Sirohi (1992) reported heterotic performance for characters like vine length, days to first harvest, fruits per plant, fruit weight and yield per plant in bottlegourd. Tewari and Ram (1999) reported heterosis for yield and other associated characters in bittergourd. In cocoa, yield and number of pods per tree showed a very high heterotic expression in most of the hybrids (Sridevi, 1999). Singh *et al.* (1999b) reported significant heretosis for seed yield in opium poppy.

5.4 Formulation of selection parameters based on evaluation of open pollinated progenies

Correlation study revealed that in seedling stage, only two characters namely leaf area and inter nodal length showed significant positive correlation with dry weight of roots. Seedling which ultimately recorded the highest root yield also recorded significantly higher leaf area and inter nodal length.

In mature stage, the characters such as inter nodal length, collar girth of vine, leaf area and number of branches showed significant positive correlation with root yield. The studies indicated the scope for selecting plants based on these four characters for augmenting the root yield. This result is in confirmity with the results reported by Meera (1994) in adapathiyan that the characters such as number of branches, collar girth, inter nodal length and root volume showed significant positive correlation with root yield.

In perennial crops like coconut (Liyanage, 1967; Sathyabalan and Mathew, 1983) and arecanut (Bavappa and Ramachander, 1967) selection criteria have been developed and successfully being utilised for the evaluation of seedlings in the nursery. The vegetative vigour measured by the height and diameter of trunk in cocoa was found to be better correlated with precocity (Verghese, 1998).

Pollen pistil interaction studied by the fluorescence technique proved complete self incompatibility and gave evidence of cross incompatibility and ovary as the site of incompatibility in adapathiyan which is the primary reason for low fruit set leading to the restricted distribution or rare occurrence of the species. Indepth microscopic studies are essential to understand whether the incompatibility reaction within the ovary is taking place at the pre-fertilization or post-fertilization phase. The inhibitory substances which causes self incompatibility reaction need to be identified and its levels monitored at different stages of flower development to find out ways and means to manipulate it. Future studies are warranted to confirm the cross incompatibility reaction in different accessions. Heterotic vigour expressed by hybrids with purple cordate as female parent emphasize its utility for improvement of yield in adapathiyan. Identification of cross compatible types with purple cordate is a step further for efficient and economic production of hybrid seeds and exploitation of hybrid vigour.

Summary

SUMMARY

The present investigations on 'Pre-zygotic incompatibility and evaluation of open pollinated and hybrid progenies of *Holostemma adakodien* Schult.' was carried out at the Department of Plantation Crops and Spices, College of Horticulture, Vellanikkara during the period from 1997-2001. The study was aimed at understanding the incompatibility reaction in adapathiyan accessions, studying the pollen pistil interaction and to find out effect of different methods to break the incompatibility barrier. It was also intended to evaluate the germplasm accessions and to exploit the variability in hybrids to select ideal types, and to find out correlation between seedling characters and dry root yield for screening superior types in the seedling stage.

The results are summarised below:

Adapathiyan is a profuse flowering plant bearing cymose inflorescence in the leaf axils with 14-45 inflorescence per plant, 8-22 flowers per inflorescence and 208-855 flowers per plant. The usual flowering season was from July to September but it extended to October in a few accessions, HA.1, HA.2, HA.7 and HA.13.

Flowers are bisexual, regular, complete and actinomorphic, typical of Asclepiadaceae, the milkweed family. The peak anthesis time was between 9.00 and 10.00 am. Anther dehiscence was observed in flowers on fourth day of opening with maximum between 11.00 and 13.00 am. Stigma remained receptive

at the anthesis time on the first day of flower opening. The different accessions showed no variation in these basic reproductive biology. The receptive side of the stigma was found to be the five lateral sides. Position of pollinia enclosed within the gynostegium, downward position of the flower and tight membrane covering the lateral sides of the stigma are factors in favour of insect pollination in adapathiyan and totally block other modes of pollination.

Pollen fertility assessed by Alexander's stain test indicated that it is very high and ranged from 94.44 per cent to 100 per cent in different accessions. Brewbaker and Kwack's medium was found to be ideal one for *in vitro* pollen germination.

Adapathiyan plants are cross pollinated by insects. *Xylocopa* spp. (Carpenter bees) were identified to be the pollinating agents. The fruit set under natural condition was found to be very low (6.00-10.00%) and artificial crossing also failed to improve the fruit set (0.00-4.00%) in any of the accessions.

Among the ten different methods tried to break the incompatiblity reaction, end of season selfing alone was successful. Fruit set was obtained with end of season selfing in two accessions HA.7 and HA.13 with extended flowering and recorded 6.66 per cent and 3.33 per cent set respectively.

The study of pollen pistil interaction by fluorescence technique under selfing, crossing and ten methods to break incompatibility revealed that late acting gametophytic self incompatibility occurs in adapathiyan. In both selfing and crossing, pollen germinate on the stigma, pollen tube grows through the style and reaches the ovary. In selfed flowers, fertilisation is not taking place with egg in the ovary while in crossed flowers, fertilisation takes place leading to fruit set. But in some cases, even though pollen tube reaches the ovary, it fails to set fruit in crossed flowers also. The study has given evidence that ovary is the site of incompatibility. The study conducted in fifteen accessions has conclusively proved the involvement of complete self incompatibility and evidence for the occurrence of cross incompatibility in adapathiyan. This cross incompatibility may be the primary reason for low fruit set in adapathiyan which is naturally designed for cross pollination. In addition, the receptive lateral surface of the stigma covered by a tight membraneous covering is not within the easy reach of insects and in the act of foraging nector, the stigma and pollinia get injured and reduces the chance for successful pollination and fertilisation.

In amputation methods tried to break self incompatibility, pollen tube growth was not observed. Latex coagulation was observed at the cut surface which hindered the pollinial germination. Moreover, stigmatic exudate which is necessary for pollinial germination was removed. In bud pollination also, pollen germination was not observed. But pollen tube growth was observed in pollination with *in vitro* germinated pollinia, pollination during early and mid season and application of 2,4-D on the pedicel followed by pollination but the growth was inhibited in the ovary.

The incompatibility reaction may be due to some inhibitory substances in the ovary. The fruit set observed for the end of season self pollination in accession HA.7 (6.66%) and in accession HA.13 (3.33%) indicated that the incompatibility reaction might be reduced at the end of growth season due to inadequate quantity of inhibitory substance in the ovary at that time.

Characterisation of 15 accessions of adapathiyan revealed wide range of variability for most of the characters in adapathiyan, indicating great scope for selection. Purple cordate was the predominant type among the accessions. In terms of yield characters, the accessions HA.8, HA.7, HA.13, HA.21, HA.4 and HA.19 were consistently found to be promising. For quality components, superior accessions were HA.8 for protein, HA.13 for total free amino acids and insoluble sugar and HA.2 for soluble sugar.

Eight hybrid combinations along with four parents evaluated as pure crop and inter crop in coconut plantation revealed superior performance of adapathiyan as pure crop in terms of vegetative, yield and quality attributes except insoluble sugar.

The trait collar girth showed high relative heterosis and heterobeltiosis in most of the hybrids. Among the eight cross combinations, H_3 (purple cordate x green elongate) was found to be the superior one with high relative heterosis and heterobeltiosis for collar girth and for all the yield characters except driage percentage, followed by H_4 (purple cordate x green cordate).

Among the four parents, purple cordate (PC) and purple elongate (PE) and among the eight hybrids, H_3 (purple cordate x green elongate) and H_4 (purple cordate x green cordate) were found promising for yield characters. The superior performance of H_3 and H_4 may be due to the involvement of purple cordate (PC) as one of the parents.

Among the different biochemical constituents studied, soluble sugar recorded highest content of 8.13 per cent in H_2 (purple elongate x green cordate) as inter crop and H_1 (purple elongate x green elongate), H_2 (purple elongate x green cordate) and P_1 (purple elongate) were on par in soluble sugar content as pure crop. Since soluble sugar present in roots contribute to the medicinal properties of adapathiyan, selection of hybrids with purple elongate (PE) as one of the parents is desirable.

The above observations suggest the utility of purple cordate (PC) and purple elongate (PE) types as seed parent in hybridisation programme of adapathiyan or collection of seeds from purple cordate (PC) and purple elongate (PE) types under open pollination.

Correlation study between seedling characters and dry root yield revealed that ideal plants can be selected at seedling stage itself based on high inter nodal length and leaf area.

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PRE-ZYGOTIC INCOMPATIBILITY AND EVALUATION OF OPEN POLLINATED AND HYBRID PROGENIES OF *Holostemma adakodien* Schult.

By

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ABSTRACT OF THE THESIS

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ABSTRACT

The present investigations on "Pre-zygotic incompatibility and evaluation of open pollinated and hybrid progenies of *Holostemma adakodien* Schult." was carried out at Department of Plantation Crops and Spices, College of Horticulture, Vellanikkara during the period from 1997-2001. The study was aimed at understanding the incompatibility reaction in adapathiyan accessions, studying the pollen pistil interaction and to find out effect of different methods to break the incomaptibility barrier. It also envisages to evaluate the germplasm accessions and hybrid progenies to exploit the variability, to select ideal types, and to find out correlation between seedling characters and yield for screening superior types in the seedling stage.

Adapathiyan is a profuse flowering plant bearing cymose inflorescence in the leaf axils. Flowers are bisexual, regular, complete and actinomorphic, typical of asclepiadaceae, the milk weed family. The peak anthesis time was between 9.00 and 10.00 am. Anther dehiscence was observed in flowers on fourth day of opening with maximum between 11.00 and 13.00 am. Stigma remained receptive at the anthesis time on the first day of flower opening. The receptive surface of the stigma was found to be the five lateral sides. The different accessions showed no variation in these basic reproductive biology.

Naturally, adapathiyan is cross pollinated by insects but fruit set is very rare. Even with assisted pollination, the fruit set could not be increased.

Protogynous nature of the flower, position of pollinia enclosed within gynostegium, downward position of the flower and tight membrane covering the lateral receptive side of the stigma block other modes of pollination and favour insect pollination.

Study of incompatibility reaction in fifteen accessions showed that all the accessions are self incompatible. Ten different methods were tried to break the self incompatibility reaction and end of season (October) selfing alone was successful in two accessions out of fifteen evaluated.

The study of pollen pistil interaction by fluorescence technique revealed that pollen germinate profusely on the stigma, grows through the style and reaches the ovary in self as well as cross pollinations. This shows that late acting gametophytic self incompatibility occurs in adapathiyan and ovary is the site of incompatibility. The study conducted in fifteen accessions has proved the involvement of complete self incompatibility and evidence for the occurrence of cross incompatibility in adapathiyan. This cross incompatibility may be the primary reason for low fruit set in adapathiyan which is naturally designed for cross pollination.

The incompatibility reaction may be due to some inhibitory substances in the ovary. The fruit set observed for the end of season self pollination in accession HA. 7 (6.66%) and in accession HA. 13 (3.33%) indicated that the incompatibility reaction might be reduced at the end of growth season due to inadequate quantity of inhibitory substance in the ovary at that time.

Characterisation of fifteen accessions of adapathiyan revealed wide range of variability in vegetative and economic characters, indicating great scope for selection. Eight hybrid combinations along with four parents evaluated as pure crop and inter crop in coconut plantation revealed superior performance of adapathiyan as pure crop in terms of yield characters. Among the eight hybrid combinations, H_3 (Purple Cordate x Green Elongate) and H_4 (Purple Cordate x Green Cordate) were found to be more heterotic for yield characters. The superior performance of H_3 and H_4 may be due to the involvement of Purple Cordate (PC) as one of the parent. Among the different biochemical constituents studied, soluble sugar recorded highest content of 8.13 per cent in H_2 (Purple Elongate x Green Cordate) as inter crop and H_1 (Purple Elongate x Green Elongate), H_2 (Purple Elongate x Green Cordate) and P_1 (Purple Elongate) were on par in soluble sugar content as pure crop. Since soluble sugar present in roots contribute to the medicinal properties of adapathiyan, selection of hybrids with Purple Elongate (PE) as one of the parent is desirable.

The above observations suggest the utility of Purple Cordate (PC) and Purple Elongate (PE) types as seed parent in hybridisation programme of adapathiyan or collection of seeds from Purple Cordate (PC) and Purple Elongate (PE) types under open pollination.

Correlation of seedling characters with dry root yield revealed that leaf area and inter nodal length showed significant positive correlation with yield emphasising its use as selection indices in the seedling stage.