

MECHANISM OF SEED ABORTION IN
Syzygium cuminii (L.) Skeels. **AND THE ROLE OF**
SELF ORGANISED FLOW OF RESOURCES TO SINKS IN
DIFFERENTIAL DEVELOPMENT OF SEEDS IN PLANTS

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DEPARTMENT OF CROP PHYSIOLOGY
UNIVERSITY OF AGRICULTURAL SCIENCES
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DIFFERENTIAL DEVELOPMENT OF SEEDS IN PLANTS

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*Affectionately Dedicated to
My Beloved Parents*

DEPARTMENT OF CROP PHYSIOLOGY
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BANGALORE

CERTIFICATE

This is to certify that the thesis entitled "MECHANISM OF SEED ABORTION IN *Syzygium cumini* (L.) skeels. AND THE ROLE OF SELF ORGANISED FLOW OF RESOURCES TO SINKS IN DIFFERENTIAL DEVELOPMENT OF SEEDS IN PLANTS submitted by Mr. K.S. KRISHNAMURTHY, for the degree of DOCTOR OF PHILOSOPHY in CROP PHYSIOLOGY of the UNIVERSITY OF AGRICULTURAL SCIENCES, BANGALORE, is a record of research work done by him during the period of his study in this University under my guidance and supervision and the thesis has not previously formed the basis of award of any degree, diploma, associateship or other similar titles.

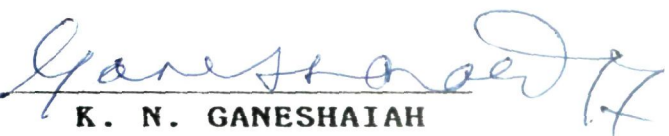
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INTRODUCTION

I. INTRODUCTION

Intra fruit seed abortion is a wide spread phenomenon in multiovulated species. The abortion may range from as high as 93 per cent (in *Kleinhovia hospita*; Uma Shaanker et. al., 1988) to none (in some members of Euphorbiaceae; Ganeshaiah and Uma Shaanker, 1992). Seed abortion in many species is consistent over seasons and locations (Arathi, 1990; Casper and Wiens, 1981 and Uma Shaanker et. al., 1988). In agriculturally important crop species, such as groundnut, chick pea, *Brassica*, pigeon pea etc., loss in yield due to seed abortion ranges from 5 to 55 per cent (Arathi, 1990). Even if 50 per cent of the existing extent of abortion is prevented, nearly 30 per cent increase in yield can be realised (Arathi, 1990). Several ecological correlates of seed abortion in plants have been identified. Seed abortion tend to increase with the habit from herbs to shrubs to trees (Wiens, 1984) and with breeding system from self to outbred (Bawa and Webb, 1984; Bawa and Buckley, 1989; Cumaraswamy and Bawa, 1989; Wiens, 1984; Wiens et. al., 1987). Species with fruit as the unit of dispersal show on an average high seed abortion than those with seed as the unit of dispersal (Ganeshaiah and Uma Shaanker, 1988; Uma Shaanker et. al., 1988).

Several hypotheses have been put forth to explain the low seed to ovule ratios observed in plants. These hypotheses attribute both proximate (pollen limitation,

resource limitation and genetic load hypotheses) and ultimate (bet hedging, risk aversion and sibling rivalry hypotheses) reasons for seed abortion.

Lack of sufficient pollen grains on the stigmatic surface to fertilize all the ovules in an ovary (Bierzzychudek, 1981, 1982a; Gross and Werner, 1983; Petersen et. al., 1982; Schemske, 1977, 1980a; Schemske et. al., 1978; Schemske and Pautler, 1984; Snow, 1982; Whelan and Goldingay, 1989; Wiens, 1984; Wilson and Schemske, 1980 and Zimmerman and Pyke, 1988) and lack of sufficient maternal resources (Lee and Bazzaz, 1982, 1986; Stephenson, 1981; Udovic and Aker, 1981; Wiens, 1984; Willson and Schemske, 1980; Willson et. al., 1987; Wyatt, 1981 and Zimmerman and Pyke, 1988) have been attributed to result in low seed set. Genetic load hypothesis assumes that recessive lethals in the embryos may result in their abortion (Wiens, 1984 and Wiens et. al., 1987). All these hypotheses have been criticised by several workers because they fail to explain the ecological correlates of seed abortion in plants (Uma Shaanker et. al., 1988; Ganeshaiah and Uma Shaanker, 1994) and have not stood up to test of critical experimentation.

Among the ultimate causes, the bet hedging hypothesis proposes that maternal parent bet her resources against environmental uncertainty, hedging this by over-producing ovules so that under favourable conditions all

ovules develop into seeds while under unfavourable environment, mother salvages the maximum possible ovules (Sutherland, 1986 and Kozolowski and Stearns, 1989). The risk aversion hypothesis assumes that maternal parent over produces as an insurance against the risk of losing some members due to predators, parasites or other causes (Casper, 1988; Ehrlén, 1991). These hypotheses however do not satisfactorily explain the ecological correlates of seed abortion mentioned above.

Ganeshaiah and Uma Shaanker (1988) and Uma Shaanker et. al. (1988) proposed that seed abortion in plants (especially in those species where entire fruit is dispersed as an unit through wind, water or animals) can be best viewed as a consequence of intra-fruit rivalry among developing sibs to be the lone survivors in the fruit to gain dispersal advantage. The sibling rivalry hypothesis satisfactorily explains most of the ecological correlates associated with seed abortion in plants.

While the above offer several proximate and ultimate causes of seed abortion, there have been very few concerted efforts in understanding the mechanistic basis of seed abortion in plants. Ganeshaiah and Uma Shaanker (1988) and Mohan Raju (1993) showed that in the wind dispersed species *Dalbergia sissoo* Roxb., where predominantly only one out of the four ovules mature to seed, the dominant among the embryos produces a chemical which kills the rest of the subordinate embryos. A

similar mechanism has been reported by Arathi (1990) in the animal dispersed species, *Syzygium cumini* (L.) Skeels., where invariably only one of the 30 ovules develops to maturity. She showed that abortion in *Syzygium* may be mediated by the production of a chemical diffusate by the dominant embryo which either directly or indirectly aborts the remaining embryos. None of these studies however sufficiently explain how the differential dominance among otherwise identical embryos arise.

Recently, Ganeshaiah and Uma Shaanker (1992, 1994) and Uma Shaanker et. al. (1995) proposed an alternate mechanism based on the process of self-organization to explain the emergence of dominance among developing embryos and thence to their differential development. Their model suggests a self-organised development of seeds within fruits involving an autocatalytic feedback process of resource flow into developing seeds. Starting from a set of identical sinks they showed that differential development (abortion) of sinks can arise purely by two simple process, a) random drift of resource resulting in initial asymmetry among the developing sinks and b) amplification of this initial asymmetry due to an autocatalytic feedback process. The rate at which the differential development (abortion) occurs is a function of the sink drawing ability (SDA) of the ovules. A major prediction of their model is that, everything else being constant, species or individuals with high SDA would have

a higher proportion of their ovules aborted. This model operates independent of the resource status and does not require any active interaction, chemical or otherwise, among the developing sibs.

The present study addresses two aspects of the mechanistic basis of seed abortion in plants. The first extends the studies of Arathi (1990) in understanding the mechanism of embryo abortion in the animal dispersed species, *Syzygium cumini*. Specifically, it intends to address the chemical interaction between the dominant and subordinate embryos leading to the abortion of the latter. Attempt shall be made to understand the nature of the chemical signal involved.

The second component of the study is aimed at testing the prediction of the theory of self organization, that seed abortion in plants would be directly influenced by the SDA of embryos (i.e. species with high SDA would be expected to exhibit a high degree of abortion and those with low SDA exhibit low seed abortion). This shall involve a) evaluating the relationship between the SDA and extent of embryo abortion within and across species b) manipulating the SDA of embryo in a few systems and examining its effect on embryo abortion and c) surveying the literature on the effect of exogenous application of growth regulators that alter the SDA on seed abortion.

REVIEW OF LITERATURE

II. REVIEW OF LITERATURE

Seed abortion wherein only a proportion of ovules in an ovary develop to maturity is a wide spread phenomena. The seed to ovule ratio ranges from as low as 7 per cent in *Kleinhovia hospita* to a high as 98 per cent in *Atylosia lineata*. In agriculturally important crops such as groundnut, chickpea etc., the loss in yield due to seed abortion ranges from 9 to 85 per cent. Understanding of the proximate and ultimate causes of seed abortion would contribute to the possibility of alleviating the abortion and thence to increase in the production. In the following section, the general pattern of seed abortion across taxa, the ecological correlates of seed abortion, the proximate and ultimate causes of seed abortion and finally the underlying mechanism leading to seed abortion are reviewed.

2.1 Patterns of seed abortion: Position and Stringency

Seed abortion within fruits could be either random or systematic with respect to the position of the aborted embryo. In species with linearly arranged ovules such as in the members of leguminosae, embryos at either the peduncular end (Bawa and Webb, 1984; Guth and Weller, 1986; Ganeshaiah and Uma Shaanker, 1988; Hossaert and Valero, 1988; Bawa and Buckley, 1989; Arathi, 1990 and Mohan Raju, 1993) or stigmatic end (Horovitz et.al., 1976; Meinke, 1982; Pechan and Morgan, 1985 and Sage and

Webster, 1990) or at both ends (Hedley and Ambrose, 1981) have been shown to systematically abort. Abortion of embryos randomly with respect to their position has rarely been reported (Casper and Wiens, 1981 and Arathi, 1990) this is partly because it is often difficult to characterise the pattern of spatial abortion in species where the ovules are arranged non-linearly in a sphere such as in solanaceous species. The positional bias in embryo abortion could be a consequence of the relative magnitudes of the resource and fertilization gradients across the position of ovule placement in an ovary (Radha et. al. 1993b). If the resource and fertilization gradients are equal in their magnitudes, then the probability of embryos being aborted at both the ends is high leaving behind embryos at the centre to develop to maturity. On the other hand, if the fertilization gradient is very steep for a given resource gradient, then the probability of the stigmatic embryos developing to maturity increases (Radha, 1990).

Based on the relative consistency in the extent of abortion, seed abortion could either be variable or stringent. For example, in species such as *Dalbergia sissoo* Roxb. which exhibit consistently a highly positively skewed distribution of seeds per pod are referred to exhibit variable extent of seed abortion (Ganeshaiah and Uma Shaanker, 1988 and Mohan Raju, 1993). In this species mean seed number per pod is low but not

invariant over trees and over seasons. On the other hand, species such as *Syzygium cumini* where invariably only one of 30 to 35 ovules develop to maturity are referred to exhibit stringent abortion (Arathi, 1990).

2.2 Ecological correlates of seed abortion:

Seed abortion in plants has been shown to be associated with several ecological features such as the unit and mode of dispersal, habit of the plant and breeding system. Uma Shaanker et. al. (1988) reported that the species in which the fruits per se are dispersed as an unit, either through wind, water or animal, exhibit a high degree of abortion compared to species in which the seed is the unit of dispersal. Wiens (1984) found that seed to ovule ratio decreased from herbs to shrubs to trees. It has been reported that species with a high degree of out crossing exhibit a higher rate of abortion than those which are inbred (Bawa and Webb, 1984; Bawa and Buckley, 1989; Cumaraswamy and Bawa, 1989; Schoen, 1982b; Wiens et. al., 1987). In a comparative study, Wiens et. al. (1987) found that species of *Epilobium* that were inbred showed a high seed to ovule ratio compared to their congeneric species that were out crossed.

2.3 Proximate and ultimate causes of seed abortion

2.3.1 Proximate causes

2.3.1.1 Pollen limitation hypothesis:

Lack of sufficient pollen grains on the stigma to fertilize all the ovules in an ovary has often been argued to limit seed production in many species (Bierzychudek, 1981, 1982a; Gross and Werner, 1983; Petersen et. al., 1982; Schemske, 1977, 1980a; Schemske et. al., 1978; Schemske and Panther, 1984; Snow, 1982; Whelan and Goldingay, 1989; Wiens, 1984; Wilson and Schemske, 1980 and Zimmerman and Pyke, 1988). In support of this view, these studies have reported an increase in seed set on supplementing pollen loads on stigma. A few workers have also attributed poor seed set due to lack of genetically good quality pollen grains (Nybom, 1987). However, a number of studies in several systems have shown that supplemental pollination does not always lead to increased seed set (Casper, 1983; Guth and Weller, 1986; Uma Shaanker and Ganeshaiah, 1984). These studies have questioned the validity of pollen limitation hypothesis in explaining seed abortion across taxa. The pollen limitation hypothesis further does not substantiate the association between the extent of seed abortion and several ecological correlates such as unit and mode of dispersal, habit and breeding system.

2.3.1.2 Resource limitation hypothesis:

This hypothesis seeks to explain seed abortion as a consequence of lack of sufficient resource in the plant to provide for seed development (Lee and Bazzaz, 1982, 1986; Stephenson, 1981; Udovic, 1981; Wiens, 1984; Willson and Schemske, 1980; Willson et. al., 1987; Wyatt, 1981 and Zimmerman and Pyke, 1988). In support of this hypothesis a few workers have demonstrated a reduction in seed to ovule ratio subsequent to reducing the resource status of the plant through either defoliation or imposition of stressful environments (Udovic, 1981). However, several studies that have attempted to enhance the resource status of the plant through fertilization have failed to increase the seed set (Bawa and Webb, 1984; Ho, 1980; Michael and Beringer, 1980 and Ojehomon, 1970).

Uma Shaanker et. al. (1988) argued that resource may not be limiting seed set in many species that exhibit consistently low seed to ovule ratios such as in wind, water or animal dispersed species. In most of these species, embryo abortion occurs immediately after fertilization of ovules at which stage limitation of resources is unlikely. Moreover, in many of these species, the extent of abortion remains consistent over environments and seasons. Further, exogenous supply of resources failed to improve seed set in most of these species.

Finally resource limitation hypothesis fails to explain several patterns associated with seed abortion such as stringency of abortion and non random abortion associated with position. This theory also does not explain several ecological correlates associated with seed abortion such as with habit, mode and unit of dispersal and breeding system.

2.3.1.3 Genetic load hypothesis:

Wiens (1984) and Wiens et. al. (1987) proposed that low seed set could be due the genetic load of the system. According to this hypothesis, developmentally recessive lethals in the embryos may result in their abortion. This hypothesis however, has been criticised by Uma Shaanker et. al. (1988) and Bawa et. al. (1989). Since embryos at any position in the fruit could receive the recessive lethal alleles according to the genetic load hypothesis, seed abortion should occur randomly across the position of the fruit. However, as already discussed, seed abortion is non-random with respect to position in many species. Further, contrary to that predicted by the genetic load hypothesis, embryos that are otherwise destined to be aborted have been demonstrated to develop till maturity in a few species (Weigel and Hughes, 1986; Ganeshaiah and Uma Shaanker, 1988; Mohan Raju, 1993).

The genetic load hypothesis also fails to explain the ecological correlates with seed abortion mentioned earlier.

2.4 Ultimate causes of seed abortion

2.4.1 Risk aversion hypothesis:

This model also referred as "egg insurance hypothesis" was first proposed for the masked booby (*Sula dactylactra*) where siblicide is an obligate feature. The model suggests that the birds produce additional eggs as an insurance against the risk of losing some of the members of the brood to predators, parasites and other causes. Production of additional offspring as backup under such situations (Casper, 1988) would be favored if the second offspring has a higher value as a replacement than the cost involved in producing it (Anderson, 1990) i.e. if the rewards from producing a second egg are greater than the cost of the first egg.

These arguments might be directly applicable to plants because mortality occurs at various stages from early embryogenesis to seedling establishment due to pathogens, parasites, predators and genetic load (Wiens, 1984) or unpredictable external mortality (Ehrlén, 1991). High mortality select for the production of backup ovules leading to the over production of ovules than the fruit might be capable of developing to maturity.

The risk aversion hypothesis does not seem to explain all the observed patterns of seed abortion in plants for several reasons. First, it explains the production of very few supernumerary ovules but several plants produce many more ovules than can mature into seeds. Second, the egg insurance hypothesis emphasises that abortion is generally due to fraticide (Mock, 1984) and that the maternal parent is passive or connives with elder sibs (Anderson, 1990). The consequences therefore cannot be differentiated from abortion induced by sibling rivalry. Third, the model does not address the association of brood reduction with breeding system and dispersal mode. Fourth, the model implies that abortion is independent of the genetic quality of the offspring which again is not upheld by the data in plants. And, finally, the risk aversion hypothesis does not explain why certain plants have stringent and others variable seed abortion.

2.4.2 Bet hedging hypothesis:

This model suggests that the maternal parent is an opportunist in unpredictable and varying environments. Though the argument was proposed to explain the over production of flowers (Sutherland, 1986), it has been extended to address the production of supernumerary ovules (Kozolowski and Stearns, 1989). Assuming that the productivity of breeding episodes is affected by environmental variations and that maternal parents would

not know in advance the number of offspring that could be reared to maturity in each season, maternal strategy would be to "bet" her resources against environmental uncertainty, "hedging" this by overproducing, so that under poor or unfavourable environments the mother salvages the maximum possible ovule production.

Both the risk aversion and bet hedging hypotheses predict that plants should respond to variations in resources by changing their seed to ovule ratios. Nevertheless, bet hedging hypothesis fails to explain several features of seed abortion (low seed to ovule ratios). As with the risk aversion hypothesis, it does not explain the association of seed abortion with breeding system and dispersal mode. Furthermore, the bet hedging hypothesis does not account for any bias in the position of seeds aborted which is a common feature in plants. It also does not explain the stringency in brood reduction insensitive to environmental variations. Finally, it does not explain why the abortion of seeds is associated with their genetic quality.

2.4.3 Sibling rivalry hypothesis:

Ganeshaiah and Uma Shaanker (1988) and Uma Shaanker et. al., (1988) proposed that seed abortion in plants can be profitably viewed as a consequence of sibling rivalry to gain dispersal advantage. According to this hypothesis, in multi-ovulated species where the entire

fruit is dispersed as an unit through wind, water or animals, the dispersal efficiency is inversely related to the wing loading (ratio of weight to surface area in wind and water dispersed species) or pulp to seed weight ratio (in animal dispersed species). The latter two parameters however are inversely affected by the seed number per fruit. They argued that under such a scenario, selection to enhance the dispersal efficiency of fruits will favour packing fewer seeds in a fruit. One of the means by which this could be achieved is through sibling driven competition among each other in order to be the lone survivor in the fruit and thus gain maximum dispersal efficiency. Their theory therefore predicts that species with fruit as an unit of dispersal should be expected to have a higher degree of seed abortion than those in which seed per se is the dispersal unit (such as in passively dispersed species). They provided evidence in this regard by surveying the extent of seed abortion in species with different unit and modes of dispersal.

The sibling rivalry hypothesis explains most of the ecological correlates of seed abortion mentioned earlier. It explains the association of seed abortion with unit and mode of dispersal and also the association with breeding system. For instance it is argued that when developing sibs within a fruit are genetically heterogenous (because of cross pollination), sibling rivalry would be more severe as under such circumstances, the inclusive fitness

of the lone survivor will be higher than when sibling rivalry occurs among related individuals (Charnov, 1979; Trivers, 1974).

In summary, among the various hypotheses described above, sibling rivalry hypothesis seems to explain satisfactorily the consistent patterns of seed abortion observed in plants; it also explains rest of ecological correlates of seed abortion.

2.5 Mechanisms underlying seed abortion in plants

Four major mechanisms leading to differential development of embryos and seed abortion in plants have been reported. Each of them is briefly discussed here.

2.5.1 Passive/Active prevention of fertilization of ovules leading to seed abortion

In several systems, degeneration of ovules immediately after megasporogenesis renders them incapable of being fertilized leading to their abortion. The degeneration of ovules can occur either passively or actively. Uma Shaanker and Ganeshaiah (1989) reported an unique mechanism of abortion in *Kleinhovia hospita*, a wind dispered tree. Of the 20 ovules, predominantly only one develops to maturity. They showed that the abortion is due to an active exclusion of fertilization of ovules by the early fertilized ovule through the production of indoleacetic acid. The early germinating pollen grain and the early fertilized ovule produces IAA which prevents the

later arriving pollen grains from germinating and growing down the style. In other words, the early fertilized ovule plugs the style for later arriving pollen grains and thus leads to abortion of other ovules. In *Quercus*, early formed embryo suppresses the growth and development of other ovules by preventing their fertilization by inhibiting the pollen tube entry into the style (Mogenson, 1975). In *Phytolacca americana*, the early fertilized ovules prevent the fertilization of the other ovules by inhibiting the pollen tube entry into the embryosac (Mikesell, 1988). In *Macadonia*, the early fertilized ovule prevents the fertilization of the remaining ovules by physically crushing the latter (Sedgley, 1981).

2.5.2 Starving the young

Abortion of embryos subsequent to their fertilization can occur through starvation. In *Phaseolus vulgaris* for example, Sage and Webster (1990) observed that during very early developmental stages, aborting seeds exhibited rapid starch depletion and vacuolation of integumentary and nucellar cells indicating the disruption of the nutritional balance.

In *Derris indica*, a wind/water dispersed tropical tree, of the two ovules, only one develops to maturity. Arathi (1990) showed that the abortion is due to the development of a dominance hierarchy among the developing embryos and subsequently due to the starvation of the

subordinate embryos of resources by the developing embryo. She proposed that the dominant of the two embryos compete for resources and starve the sub-ordinate. In a clear illustration to this effect she found that in presence of the dominant embryo, the ability of the sub-ordinate embryo to take up exogenously supplied assimilate was severely inhibited. However, in absence of the dominant embryo in the pod, the sub-ordinate embryos exhibited an uptake rate equal to that exhibited by the dominant embryos.

In another study Mohan Raju (1993) demonstrated a similar mechanism of abortion in a wind dispersed tree, *Dalbergia sissoo*.

2.5.3 Chemically killing the young

This mechanism was originally proposed to explain the low fruit to flower ratios in plants. It is argued that in an inflorescence, the dominant among the fruits produces a chemical that diffuses out and inhibits the development of younger sub-ordinate fruits (Cornforth et. al., 1966; Tamas et. al., 1979; Van Steveninck, 1959). The central argument of such a mechanism could also be extended to abortion of seeds within fruits. Recently a few workers have attempted to provide experimental verification of the possibility of chemically mediated abortion of developing seeds within fruits. Ganeshaiah and Uma Shaanker (1988) showed the possibility of

chemically mediated abortion in *Dalbergia sissoo*. In this species, of the 4 to 5 ovules per ovary, predominantly only one develops to maturity leading to highly positive skewed distribution of seed number per pod. Ovules at the stigmatic end that are presumably first to be fertilized, gain dominance and develop while those at the peduncular end abort. By surgically removing the stigmatic ovules, they showed that the peduncular ovules are also equally capable of development. Aqueous extracts of dominant embryos were found to cause the abortion of fertilized subject embryos significantly greater than that from maternal tissue. Ganeshaiah and Uma Shaanker (1988) suggested that, immediately after fertilization, the dominant stigmatic embryos produce a chemical that diffuses out and kills the later formed embryos. In a later work, Mohan Raju (1993) showed that the chemical responsible for the abortion could be a low molecular weight, heat stable, non-proteinaceous compound. He proposed that the compound could be an indole compound or its derivatives.

In *Syzygium cumini*, an animal dispersed species, flowers contain 25 to 30 ovules of which invariably only one develops to maturity. Investigating the proximate mechanisms of reduction in seed to ovule ratio in this species, Arathi (1990) showed that lack of adequate pollen grains (lack of fertilization) does not completely explain the single seededness of all the fruits. She hypothesised

that dominant embryos might produce an abortive chemical which diffuse into other embryos leading to the abortion of other embryos directly or indirectly.

2.5.4 Self organization hypothesis

The resource limitation hypothesis and chemically mediated embryo abortion require the development of an initial hierarchy among the developing embryos. But both the hypotheses do not explain the process of its emergence. In some species, such hierarchy might be built up due to intrinsic factors such as position of the ovules within the fruit (Harris, 1915; Rocha and Stephenson, 1990; Radha, 1990; Radha *et. al.* 1993a). For example in legumes where the ovules are arranged linearly along the ovary, those near the stigma are likely to gain a temporal head-start as they might be fertilized ahead of others while those at the base might derive an advantage due to the proximity to resource supply point (Horovitz *et. al.* 1976; Bawa and Webb, 1984; Hill and Lord, 1986; Bawa and Buckley, 1988; Radha 1990; Radha *et. al.* 1993a and 1993b). But these resource and fertilization gradients leading to dominance hierarchy are not common to all species; there are a number of species where ovules are placed radially around the stigma (style) and resource supply point. Further, these gradients cannot explain the consistent rates of seed abortion observed in some species (Radha, 1990, Radha *et. al.*, 1993 a and b).

Recently Ganeshaiah and Uma Shaanker (1992 and 1994) proposed a simple mechanism that can generate such an initial hierarchy among the developing embryos which can lead to seed abortion independent of any gradients or limitations of resource and pollen or chemical mediation of embryo abortion. Their model suggests a self organized development of fruits involving an autocatalytic feedback process of resource flow into the developing seeds. The model is based on the process of self organization, a concept originally developed in physics and chemistry and recently extended to explain several aspects in biology such as evolution of genetic code (Wills, 1993) and organized behaviour in insects (Goss and Deneubourg, 1988; Deneubourg et. al., 1989; Goss et. al., 1989; Camazine, 1990 and Ganeshaiah and Kumar, 1993).

The process of self organization can be best illustrated by the way in which members of a certain group of ants select one of the two identical paths offered to them to reach the food source from the nest. Deneubourg et. al. (1989) connected the nest of *Iridomyrmex* sp. to the food source with two identical paths and allowed the ants to forage through these paths (fig 1). Although initially the ants used both paths, after a certain period they invariably switched to only one. Such asymmetry in using the paths was explained by Deneubourg et. al. (1989) by using two basic rules specific to these ants; a) ants deposit pheromones on their path as they pass by and b)

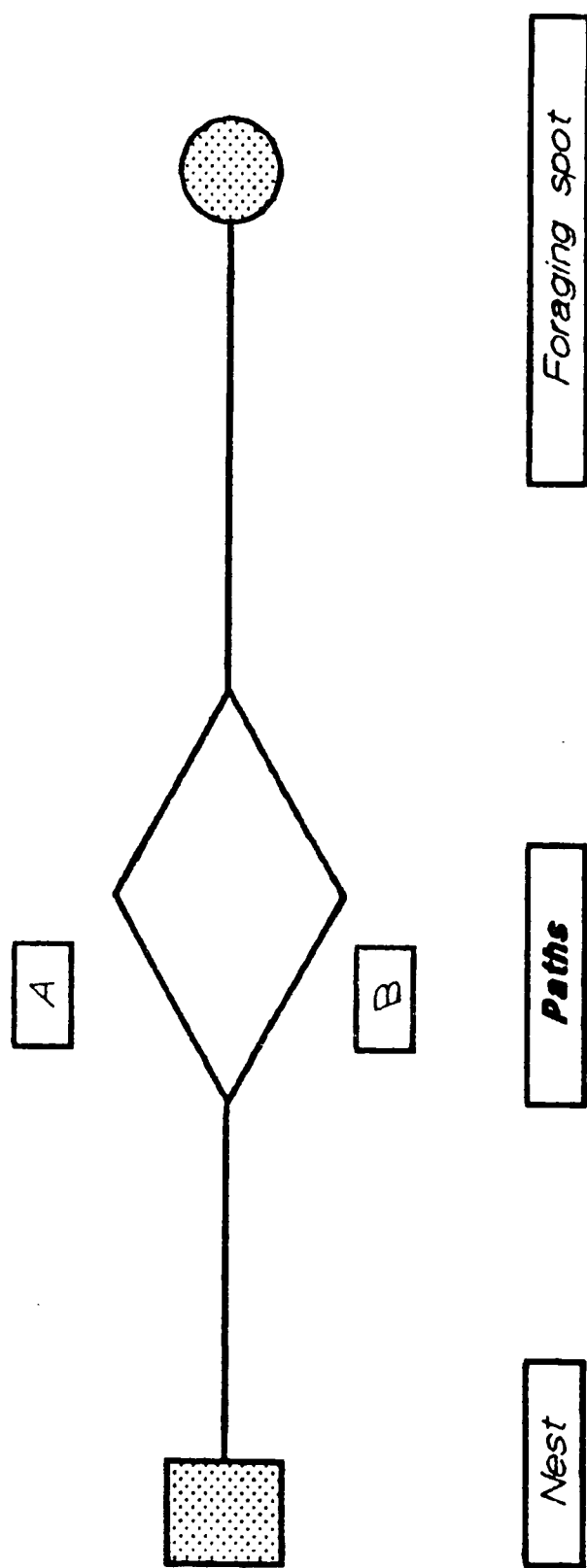


Fig. 1. Two identical paths A and B connecting the nest of the ants to the foraging spot

given a choice between two paths, ants prefer that with the relatively higher pheromone concentration. Given these rules, it is clear that a simple random drift in the movement of ants to one of the paths in the initial stages would lead to an increased concentration of pheromone on it and hence to the preference of that path by subsequent ants. This further increased the pheromone concentration in that path and such a process of positive autocatalytic feedback reinforces its use by the ants. Thus the emergence of order (asymmetry in using the paths) in the system is because of the differential probabilities with which ants prefer paths of varying pheromone concentration and is given by a non-linear relation familiarly known as the polya urn equation (Arthur, 1987).

Pods as self organizing pots

The differential development of seeds within fruits can also be viewed to be a consequence of the process of self organization by merely translating the forked paths offered to the ants by Deneubourg et. al. (1989) as sieve tubes supplying resources to the ovules and the ants moving on these paths as the resource molecules moving to the ovules (fig 2). The rule intrinsic to the system would be that every molecule that flows into an ovule increases the probability of subsequent molecules moving into it. This rule has its parallels in hormone physiology and can be viewed as the ability of the

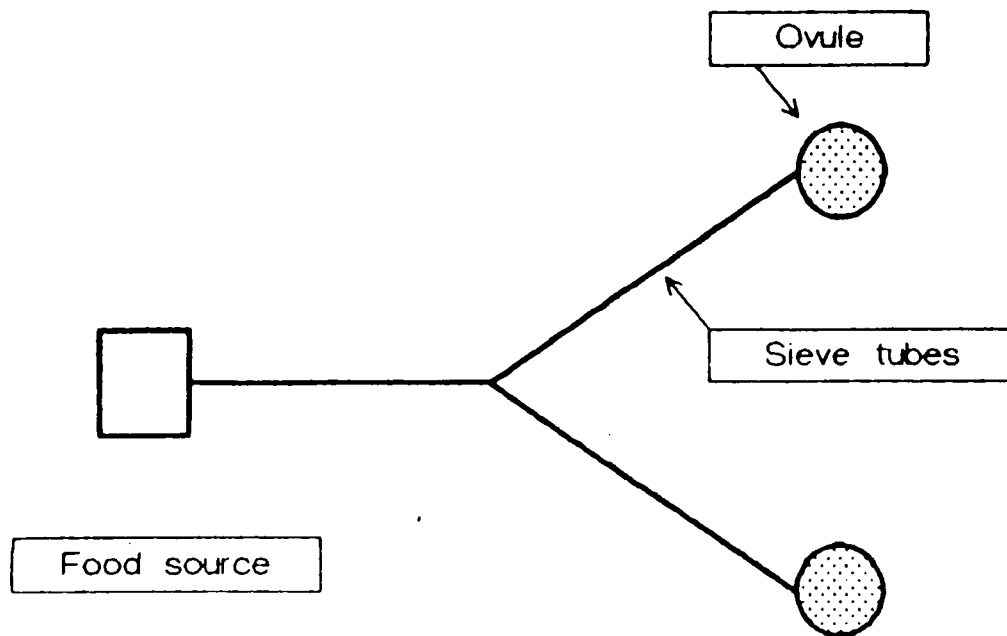


Fig. 2. Translation of the paths connecting the nest to the foraging spot to sieve tubes connecting ovules in plants

resource flow to an ovule to induce sink drawing ability in it. This might happen either by the differentiation of sieve tubes or by conversion of the resource molecules to hormones (fig. 2).

Under such conditions, an initial random drift in the movement of molecules into one of the two ovules increases the probability of further resources moving to it. Autocatalytically, this leads to the dominance of that ovule and the eventual abortion of the other. However, the extent to which this random drift leads to a dominance hierarchy (and hence to abortion) is a function of the sink strength induced by the drift. If sink strength induced is very high the dominance hierarchy built up is stronger and faster and if it is low, the hierarchy might never be established. This relation can be clearly expressed by the Polya urn equation that defines the probability $P(a)$ with which a given ovule acquires the new molecule of resource and is given by

$$P(a) = \frac{(R+Y_a)^x}{(R+Y_a)^x + (R+Y_b)^x}$$

where R = the initial resource status of the ovule before fertilization; Y_a and Y_b = the resource status of the ovule a and b respectively, and x = the sink drawing ability (SDA) of the ovules.

Clearly, $P(a)$ depends upon the current relative resource status of the ovule (i.e. $R+Y_a$ and $R+Y_b$) and x . x is the most important parameter that defines the

extent and the rate at which asymmetry is built. And in the context of the development of seeds per fruit, x can be interpreted to indicate the SDA of ovules. In the beginning, since the resource status of both ovules is similar, $Y_a = Y_b = \emptyset$ and hence $P(a) = P(b) = 0.5$, that is the two ovules are equally likely to obtain the resources. During this initial stage, if one of the ovules receives few additional molecules of resources purely as a consequence of random drift, the probability of that ovule obtaining further resources increases at a rate defined by x . Consequently, one of the two ovules eventually dominates and develops to maturity while the other starves; eventually the latter aborts because of lack of resources. It is important to realise that, this starvation is not akin to that due to resource limitation. The model does not assume any limitation of resource but only offers it in discrete bouts. The starvation (abortion) is purely due to the asymmetry generated among the ovules. However, whether or not such asymmetry builds up between the ovules is to a greater extent determined by the value of x , the SDA of the ovules; it is possible to see that for low values of x (close to $\emptyset$), despite the random drift in the resource mobility, both ovules are favoured throughout ($P(a) = P(b) = 0.5$) and develop to maturity. But for higher values of x , even the minor differences in resource movement leads to dominance hierarchy and hence to the underdevelopment (or abortion)

of one of the two ovules. In other words, it is possible to generate fruits with different mean seed numbers by merely varying x .

Simulating this process for varying ovule numbers per flower, Ganeshaiah and Uma Shaanker (1992) showed that irrespective of the initial ovule numbers, the per cent seeds set in a fruit decreased with increase in x . The simulation showed that given an ovule number and x value, the process of self organization resulted in a specific mean seed number per fruit and a specific pattern of frequency distribution of seeds per fruit. They compared the observed frequency distributions of 18 species having a constant ovule number but with varying mean seed number per fruit with those simulated. They found that the frequency distribution of seeds per fruit obtained through simulation corresponded almost exactly with those observed with respect to their coefficient of variation and skewness coefficient, suggesting that the process of self organization is probably the mechanism underlying seed abortion.

A major prediction that emerged from their simulation is that keeping other things constant, seed abortion increased with the increase in sink drawing ability of the embryos within a fruit. These abortion patterns arise even if the resource and pollen loads are abundant and when all embryos are genetically similar.

As seen from the above discussion, differential development of seeds leading to their abortion seems to be satisfactorily explained by the process of self organization involving the random drift of resources and its subsequent autocatalytic feedback. The mechanism does not require any extraneous regulation nor active interaction such as chemically killing/starving the developing seeds by the dominant embryos within the fruit. However, it is likely that other mechanisms (starving the young and chemically killing the young) if occurring would only further accentuate the differential development of seeds within fruits triggered by the process of self organization.

MATERIAL AND METHODS

III. MATERIAL AND METHODS

The study is aimed at a) understanding the mechanism of embryo abortion in an animal dispersed species *Syzygium cuminii* and b) providing test of predictions of the theory of self organized flow of resources to developing ovules. The materials used and the approaches taken in conducting various experiments to accomplish the objectives of this study are presented in this chapter.

3.1 Mechanism of embryo abortion in an animal dispersed species *Syzygium cuminii*

3.1.1 The System

Syzygium cuminii (L.) Skeels., Myrtaceae, an animal dispersed species, is widely distributed in India. The tree is grown for its timber and also for fuel wood purposes. The trees bear flowers which are borne on a cymose inflorescence (plate 1a). The ovary is inferior with numerous stamens. The trees flower and fruit during May and August. The fruit is a fleshy berry with a single seed. The flowers contain 25 to 30 ovules of which only one develops into maturity (Arathi, 1990). At fertilization, all embryos are similar in size. But a week after fertilization, one of the embryo attains dominance while the rest degenerate gradually. Investigating the proximate mechanisms of reduction in seed to ovule ratio in this species, Arathi (1990) showed that lack of adequate pollen grains (lack of

fertilization) does not completely explain the single seededness of all the fruits. She hypothesised that dominant embryos might produce a chemical which diffuses into other embryos and leads to their abortion either directly or indirectly. In the present study, the proximate mechanism of seed abortion in *Syzygium cumini* is investigated.

3.1.2 Study site:

The study was conducted on trees available in the Botanical Garden, University of Agricultural Sciences, GKVK, Bangalore during 1991-1993.

3.1.3 Terminologies

Several terminologies frequently used in the various experiments are given below.

Dominant embryo: The embryo which is distinctly larger in size than the rest within a fruit (plate 1b). Dominant embryos start appearing in the fruits 8 to 10 days after fertilization of ovules.

Aborted embryos: Those embryos which are very small compared to dominant embryos (plate 1c). At later stages (20 days after fertilization) these are completely sclerotised and turn brown. Sclerotised aborted embryos were used as aborted embryos for experiments whenever required unless otherwise mentioned.

Subject embryos: Embryos from fruits one or two days after fertilization (plate 1c). Subject embryos equal in size

and weight were used in conducting various assay experiments.

Experiment 3.1.4.1: Effect of extract of dominant embryos on the abortion of subject embryos (by visual scoring)

Developing fruits (10 and 20 day old) were randomly collected from trees. The dominant embryos from 10 day old fruits and aborted embryos from 20 day old fruits were dissected out into cavity slides and were used for preparation of extract or collection of diffusate unless otherwise mentioned. Twenty mg each of dominant embryos and aborted embryos were ground separately in double distilled water in cavity slides with the help of a glass rod. As a control, maternal tissue (fruit coat tissue of equal weight) was also taken. After centrifugation at 4000 rpm for 8 to 10 min. the supernatant was collected. The supernatant constituted the extract solution for various treatments. Ten subject embryos were incubated in plastic wells (in 4 replicates) of 0.5 cm dia. and 0.5 cm depth containing 0.6 % agar with 20 ul of extract from either dominant or aborted embryos, fruit coat tissue or distilled water. The subject embryos were incubated in humid chamber (75 % RH, 30°C) and number of subject embryos aborted were counted regularly at 10 h interval starting from 6 h to 46 h after incubation. The embryos that appeared dark brown in colour under stereo microscope were counted as aborted and those that appeared green as

healthy (plate 1d) and expressed as percent abortion of embryos.

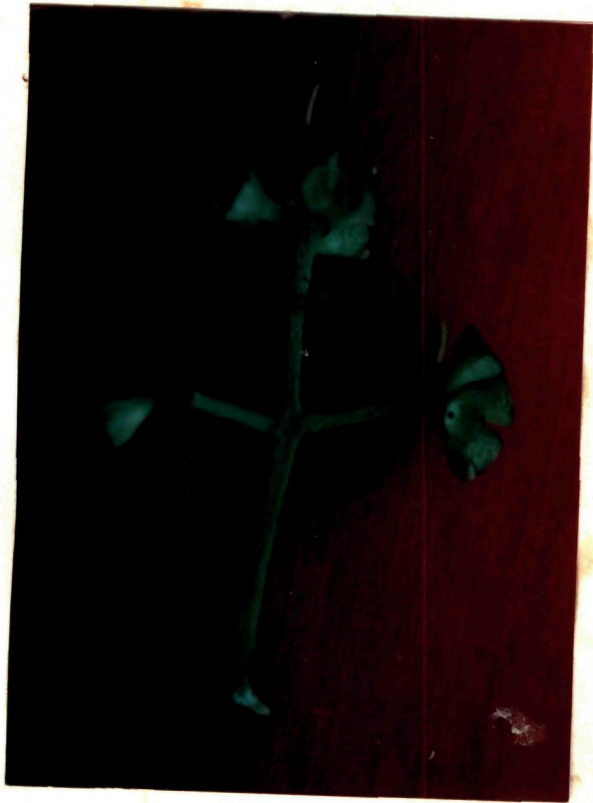
Experiment 3.1.4.2: Effect of extract of dominant embryos on the uptake of labeled sucrose by subject embryos

The above experiment is based on the visual scoring of abortion of the subject embryos. However, this technique may not be highly reliable. Here an effort was made to develop a rapid assay method for examining the effect of extract of dominant embryos on the uptake of labeled sucrose by subject embryos.

Equal quantity (by fresh weight) of dominant embryos, aborted embryos and fruit coat tissue were ground separately in cavity slides and extracts were prepared as described earlier in experiment 1. Ten μ l of extracts in triplicate were dispensed into cavity slides. About 20 subject embryos were put into each of the cavities containing the extract and incubated in humid chamber (75 % RH, 30°C) for 6 h as pretreatment. A control where subject embryos were incubated in buffer was also maintained in triplicate. The uptake assay was conducted as described below.

Ten μ l of 25 μ Ci sucrose solution (uniformly labeled, BARC, Bombay) was added to the cavity slides and incubated for 12 h. After this period, embryos were thoroughly washed with distilled water for 3 h to remove the surface activity (Mohan Raju, 1993) and ground in 2 ml of Bray's

- Plate 1:**
- a) Inflorescence of *Syzygium cumini*
 - b) 1 = dominant embryo
2 = aborted embryos
 - c) Subject embryos
 - d) Healthy and aborted (dark brown) embryos



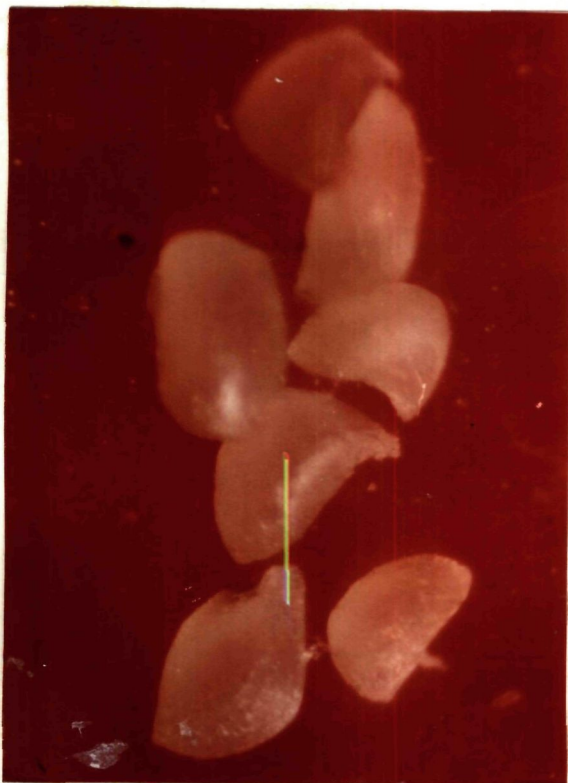
a



1

b

2



c



d

solution. Labeled sucrose taken up by embryos was measured using liquid scintillation system (Model LSS 20) at 1130 KV operating voltage. The uptake was expressed either as cpm/embryo or cpm/mg fresh weight. Two way ANOVA was conducted to compare among the treatments.

Preparation of labeled sucrose feeding solution:

Uniformly labeled ^{14}C sucrose solution of activity 0.1 mCi was obtained from Bhabha Atomic Research Centre (BARC), Bombay. For experimental purpose, 25 uCi activity was used which was obtained by diluting the 0.1 mCi solution four times with distilled water.

Preparation of Bray's Solution (Scintillation liquid) for estimating radioactivity:

Bray's solution was prepared by dissolving the following chemicals in 1,4 Dioxane

Naphthalene	60 g
PPO (2,5 diphenyl oxaazole)	4 g
Dimethyl POPOP (1,4 bis 2,4 methyl phenyl oxazole)	0.2 g
Methanol	100 ml
Ethylene glycol	20 ml

The final Volume was made upto one litre using 1,4 Dioxane

Experiment 3.1.4.3: Effect of diffusate of dominant embryos on uptake of labeled sucrose by subject embryos

The assumption that dominant embryos produce a chemical that kills the subordinate embryos (Arathi, 1990)

implies that the chemical produced by the dominant embryos is diffusible. In this experiment, the diffusate from dominant embryos were examined for their effect on the inhibition in the uptake of labeled sucrose by subject embryos.

Equal quantity (by fresh weight) of dominant and aborted embryos were incubated in double distilled water overnight. The diffusate was collected by centrifuging the contents in eppendorf tubes at 5000 rpm for 10 minutes and the volume was made upto 40 ul using 5mM MES buffer (pH 6.5). Ten ul of each of these diffusates or buffer in triplicates was dispensed into the cavity slides. About 20 subject embryos were put into each of the cavities containing diffusate or buffer and incubated for 6 h as pretreatment. The uptake assay was conducted as described in the earlier experiment. Student's t-test was conducted to compare the mean uptake rates between the treatments.

Experiment 3.1.4.4: Nature of the diffusate of dominant embryos causing inhibition in the uptake of labeled sucrose

Several experiments were conducted to investigate the nature of the chemical present in the diffusate of dominant embryos which apparently causes the inhibition in the uptake of labeled sucrose by subject embryos. The methodology followed for these experiments is given below.

Experiment 3.1.4.4.1: Effect of heat treatment of the diffusate of dominant embryos on the inhibition in the uptake of labeled sucrose by subject embryos

This experiment was conducted to examine if the chemicals present in the diffusate of dominant embryos causing inhibition in the uptake of labeled sucrose is heat labile.

Diffusates from equal weights of dominant embryos, aborted embryos and fruit coat tissue were collected as described in earlier experiment. 40 ul of the diffusates were dispensed into the cavity slides and heated repeatedly over an open flame. Care was taken to see that the contents of the cavity slides did not dry up during heat treatment. After heat treatment, the volume was made upto 40 ul using MES buffer in all cases. 10 ul of heat treated diffusates, untreated diffusates (no heat treatment), and control (buffer) was dispensed into the cavity slides in 3 replicates. At least 20 subject embryos were put into all the cavities and incubated for 6 h as pretreatment. Later 10 ul of labeled sucrose was added and uptake assay was conducted as explained earlier. Completely randomised design (CRD), Two factor factorial analysis was conducted to compare the mean uptake between the treatments.

Experiment 3.1.4.4.2: Effect of trypsin digestion of dominant embryos diffusate on the uptake of labeled sucrose by subject embryos

This experiment was conducted to examine if the chemical present in the diffusate of dominant embryos causing the inhibition in the uptake of labeled sucrose by subject embryos is proteinaceous.

Trypsin solution (1mg/ml) was prepared in 5 mM MES buffer. Equal quantity (by fresh weight) of dominant embryos, aborted embryos and fruit coat tissues were ground in trypsin solution and incubated for 30 minutes. Controls, one trypsin blank and the other where dominant embryos, aborted embryos and fruit coat tissues were ground in buffer were also maintained. After 30 minutes, treatments having trypsin were heated over a flame to denature the added trypsin and the volume was made upto 40 μ l using MES buffer in all the treatments. 10 μ l of treatment extracts in three replicates was dispensed into the cavity slides. Twenty subject embryos were put into each of these cavities and incubated for 6 h as pretreatment. At the end of 6 h, 10 μ l of 25 μ Ci sucrose was added into all the cavities and incubated for 12 h in humid chamber. Further, embryos were processed and the radio activity measured as described earlier. CRD, two factor factorial analysis was conducted to compare between the mean uptake rates among the treatments.

Experiment 3.1.4.4.3: Determination of the absorption maxima of the diffusate of dominant embryos

Bangerth (1989) in his hypothesis of primigenic dominance proposed that transport of diffusible auxin by the dominant fruit prevents the diffusion of auxin from the remaining later forming fruits. Such inhibition of the movement of diffusible auxin from later formed fruits may lead to their death directly (because of poor nourishment resulting from the poor differentiation of sieve elements connecting the fruit) or indirectly (build up of endogenous levels of auxin resulting in the production of ABA and ethylene). Engvild (1987; 1989) reported the presence of chloroindole-acetic acid in the developing pea seeds and suggested that this might act as death hormone causing the abortion of young developing pea pods. These indole compounds have their absorption maxima at 222 and 280 nm. Hence the diffusate from dominant and aborted embryos were tested for their absorption maxima at 222 and 280 nm.

Diffusate from dominant (n = 200) and aborted (n = 800) embryos were collected and the volume of the diffusates were made upto to 1 ml. The absorption spectrum of the diffusates were scanned between 200 and 600 nm wave length range using a double beam spectrophotometer (Model Shimadzu UV 200S). As a standard, 10^{-4} M indoleacetic acid (IAA) solution was also

scanned between the same wave length range. The experiment was repeated twice.

Experiment 3.1.4.5: Organic solvent partitioning of the diffusate of dominant embryos

To further examine if the diffusate did contain indole compounds an experiment was conducted wherein the diffusate was fractionated in organic solvent such as butanol which selectively separates indole compounds. The methodology followed was as follows. Diffusates were collected from equal weights of dominant embryos, aborted embryos and fruit coat tissue and the volume was made upto 200 ul using MES buffer (5 mM) of pH 6.5. 150 ul of butanol was added to all the diffusates, mixed well using a cyclomixer and centrifuged at 5000 rpm for 5 minutes. Later, butanol and buffer fractions were separated. Butanol was evaporated and the resultant residue was redissolved in 200 ul of buffer. 10 ul of each of the solutions were dispensed into cavity slides in 4 replicates. Twenty subject embryos were put into each cavity and incubated for 6 h. Ten ul of 25 uCi sucrose solution was added to all the cavities and uptake assay was conducted. As a control, the experiment was repeated with diffusates collected in buffer solution. Two factor factorial ANOVA was conducted to compare among the treatments.

Experiment 3.1.4.6: Thin layer chromatography (TLC) analysis of the diffusate of dominant embryos

An experiment was conducted to detect chromatographically the presence of indole compounds in the diffusate from dominant embryos using silica gel and cellulose TLC. Diffusate from 100 dominant embryos was collected and the volume was made upto 50 ul. 20 ul of the sample or standard was used for spotting. Standards used were tryptophan, phenylalanine, indole, indole acetic acid, indole acetonitrile and tyrosine. Solvent system used was isopropanol:ammonia:water in the ratio of 8:1:1. Solvent was allowed to run for about 4/5th of the plate. After that, the plates were taken out and allowed to dry in an oven at 60°C.

When dried, they were sprayed with Ehrlich reagent prepared by dissolving 1g of 4-diaminobenzaldehyde in a mixture of conc. HCl, n-butanol and water in the ratio 30:30:180 (for cellulose plate) or 0.05M FeCl₃:5 % perchloric acid mixture (for silica gel plate). The plates were kept for colour development and the colour developed in the sample as well as the relative flow (Rf) values were compared with that of standards.

Experiment 3.1.4.7.1: High performance liquid chromatography (HPLC) analysis of the diffusate of dominant embryos

To examine the specific chemical nature of the diffusate obtained from dominant embryos the diffusate was

subjected to HPLC analysis. The solvent system used was 30 % methanol with 1 % acetic acid. C-18 reverse phase bondapak column was used for IAA detection.

The diffusate was collected from 200 dominant embryos. 20 ul of the diffusate was loaded to HPLC column along with 30 % methanol and 1 % acetic acid. The peak retention time was detected at 280 nm wave length. Standard IAA was also run for comparison and the peak retention times were recorded.

Experiment 3.1.4.7.2: Effect of various HPLC fractions of the diffusate from dominant embryos on the uptake of labeled sucrose by subject embryos

100 ul of the diffusate from dominant embryos was loaded to HPLC with 30 % methanol set for detection at 280 nm wave length. One ml fractions of the diffusate coming out of the column after detection were collected. Using a fraction collector a total of 15 one ml fractions were collected corresponding to a total run time of 15 minutes. The fractions so collected were pooled as follows.

For experiment 1,

- a) fractions corresponding from 1 to 3 minutes and from 9 to 15 minutes which didnot show any absorbance and b) fractions corresponding from 4 to 8 minutes which exhibited a peak absorbance at 4 minutes

For experiment 2,

- a) fractions corresponding from 1 to 2 minutes which showed no absorbance

- b) fractions corresponding from 3 to 4 minutes which showed low absorbance
- c) fractions corresponding from 5 to 7 minutes which showed peak absorbance at 4.5 minutes
- d) fractions corresponding from 8 to 10 minutes which showed low absorbance and
- e) fractions corresponding from 11 to 15 minutes which showed no absorbance.

The pooled fractions were later subjected to lyophilization to evaporate methanol. On complete evaporation of methanol the residue in the flasks were redissolved in MES buffer of pH 6.5 and the volume was made up to 40 μ l in each case and was used in uptake assay. 10 μ l of the above fractions in three replicates were dispensed to cavity slides. At least twenty subject embryos were put into each cavity and incubated for 6 h. Later 10 μ l of 25 μ Ci sucrose was added to each cavity and the uptake assay was conducted. Two way ANOVA was conducted to compare between the treatments.

Experiment 3.1.4.8.1: Quantification of indole compounds in the diffusate collected from different tissues

The objective of this experiment was to determine the amount of indole compounds present in the different tissues such as dominant embryos, aborted embryos, unfertilized embryos and pod coat tissue. 8 mg (by fresh

weight) of each tissue was used for diffusate collection and the final volume was made upto 250 ul. Indirect ELISA for IAA/indole compounds (Anitha Naren, 1993) was conducted to quantify the amount of indole compounds present in the diffusate.

Indirect ELISA for the quantification of indole compounds in the diffusate

Principle: In this method, labeled anti-I (secondary antibody conjugated with alkaline phosphate) are allowed to bind with primary antibodies (raised against IAA) complexed to antigen (IAA-casein) attached to a solid phase. After the complex has been washed with buffer, the enzyme substrate was added and the enzyme activity measured by reading the colour developed using ELISA reader.

Reagents:

- 1) Hapten carrier protein conjugate (IAA-casein)
- 2) Primary antibodies (Antibody raised against IAA-BSA conjugate)
- 3) Secondary antibody: Goat anti-rabbit conjugated with alkaline phosphate
- 4) Coating buffer (0.05 M, pH 9.5): Coating buffer was prepared using the following chemicals.

Na_2CO_3 - 1.59 g

NaHCO_3 - 2.93 g

NaN_3 - 0.2 g

Final volume made upto one litre using distilled water.

- 5) Washing buffer: Washing buffer was prepared using the following chemicals

NaCl	- 8 g
KCl	- 0.2 g
KH_2PO_4	-0.2 g
Na_2HPO_4	-1.15 g
Tween 20	- 0.5 ml

and the volume made upto 1 litre using distilled water

- 6) Dilution buffer (blocking buffer, pH 7.4)

Casein	- 1 g
Washing buffer	- 100 ml

Procedure:

1. Coating the ELISA plate with antigen (IAA-casein):
Antigen was diluted using coating buffer (100 ng/200 ul) and 200 ul of it was added to each well. The plate was incubated at 4°C overnight. After the incubation, the contents were discarded and washed thrice with washing buffer at three minutes interval each time.
2. Blocking the non-specific sites in the wells: The wells were filled with blocking buffer and the plate was incubated for 90 minutes at 37°C. Then the wells were washed thrice with washing buffer as mentioned above.

3. Addition of primary antibody: Primary antibody of 200 ul diluted with dilution buffer was added to the wells and the plates were incubated at 37 ° C for three hours to allow the IAA antibodies to bind to the IAA molecules bound to each well. The wells were washed thrice with washing buffer as explained earlier.
4. Addition of secondary antibody: Secondary antibody (Goat anti-rabbit antibody) of 200 ul diluted 1:1500 times with dilution buffer, was added to the wells. The plate was incubated at 37 ° C for two hours and washed thrice with washing buffer.
5. Detection: The substrate for alkaline phosphatase, para nitrophenol phosphate (PNPP) was prepared in coating buffer without azide (1 mg/ml) and 200 ul was added to each well. The plate was incubated at 37 ° for colour development (15 minutes). Reaction was stopped by adding 25 ul of 5 N KOH. PNPP added to two empty wells was taken as control. The absorbance was read at 405 nm using ELISA reader.

Construction of standard curve:

Materials used:

IAA casein conjugate

Primary antibody

IAA standards (working range 0 to 1000 picomoles)

(0, 0.02, 2, 20, 50, 100, 200, 400, 600, 800, 1000 pmoles)

0 to 1000 pmoles IAA solutions were prepared from 0.1 m

stock solution by using Tris Buffer Saline (TBS), pH 7.5

Secondary antibody (Goat anti-rabbit antibodies)

substrate (PNPP)

Buffers:

a) Coating buffer

b) Dilution buffer

c) Washing buffer

d) Tris buffer saline (TBS) 7.5 pH: TBS was prepared using the following chemicals

Trizma base - 3.03 g

NaCl - 5.84 g

MgCl₂·6H₂O - 0.2 g

Volume was made upto 1 litre using distilled water

Procedure:

In step 3 of earlier procedure, instead of 200 ul of diluted serum, the IAA antibody was mixed with TBS buffer, before being added to the coated microtiter well. The free IAA will bind to the specific antibodies in solution and prevent their subsequent binding by competitive inhibition to the IAA/casein attached to the plate. To this, 200 ul of diluted secondary antibody to which the chromogenic enzyme is attached, was added to the wells which binds to the IAA antibody. Thus, the intensity of the final colour in each well is inversely proportional to the concentration of free IAA added to the well. That is, the more free IAA that is added, the less intense is the colour reaction.

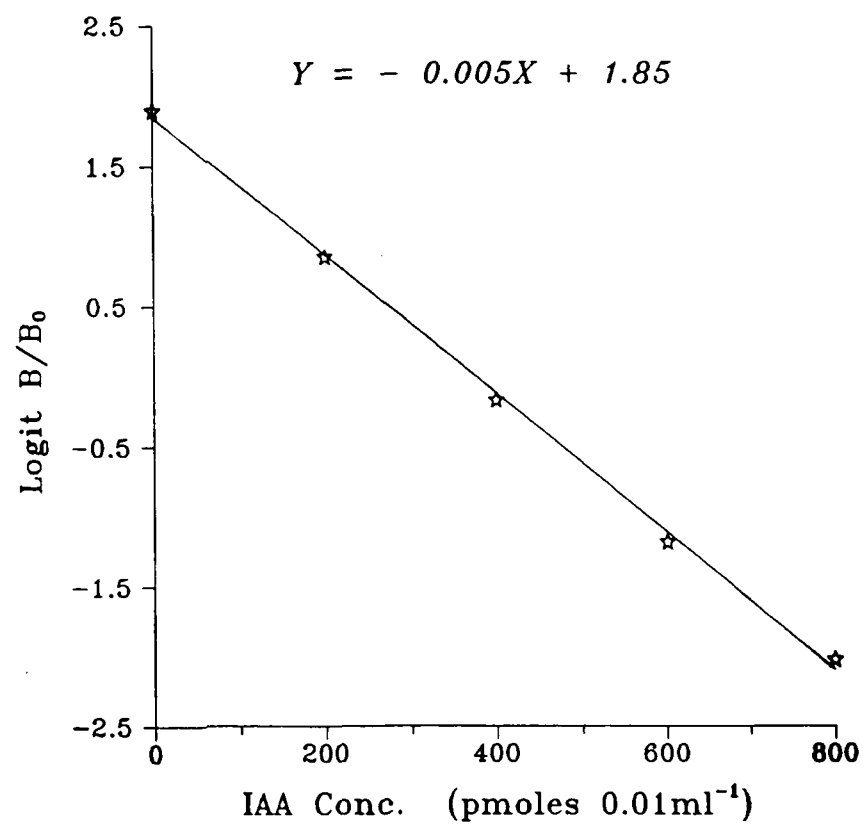


*Fig. 3: Standard curve for IAA
using indirect ELISA*

Calculations:

Optical densities of standards were recorded.

Average of optical densities of duplicate standards was taken.

Per cent binding of each standard was calculated using the formula

$$\text{Per cent binding} = \frac{\text{standard OD} - \text{ZB OD}}{B_0 \text{ OD} - \text{ZB OD}} \times 100$$

B_0 = 100 % binding

ZB = Zero % binding

Per cent binding was converted into logit B/B_0

$$\text{Logit } B/B_0 = \ln \{ (B/B_0 \%) / (100 - B/B_0) \}$$

Standard curve was drawn using IAA (standards) against logit B/B_0 values.

Experiment 3.1.4.8.2: Quantification of indole compounds in different developing stages of dominant and aborted embryos

This experiment was conducted to investigate how the quantity of indole compounds vary with different stages of development of dominant embryos as well as aborted embryos both on fresh and dry weight basis. The moisture percentage of embryos (both dominant and aborted) at each stage was also determined.

The dominant and aborted embryos were categorised into six stages starting from 2 days before to 20 days after anthesis.

Stages	Days before/after anthesis (DBA/DAA)	Stage description
1	2 DBA	Unopened flowers, whitish in colour, all ovules are similar in size and weight
2	2 DAA	Whitish flowers, anthers have fallen style retained, all embryos are similar in size and weight
3	10 DAA	Green fruit, style has dropped, no bulging of the ovary wall, one embryo attains dominance and the rest small in size
4	14 DAA	Green fruit, slight bulging of the ovary wall is seen, dominant embryo increases in size and the rest start turning brown
5	18 DAA	Green fruit, bulging of the ovary wall is apparent, dominant embryo grows bigger and the rest turn brown completely
6	20 DAA	Green fruit, bulging of the ovary wall is increased, dominant embryo still gains weight and the rest are completely aborted

Eight mg (by fresh weight) of embryos from each stage was used for diffusate collection. Indirect ELISA was conducted to quantify the amount of indole compounds present in the diffusates.

3.2: Test of predictions of the theory of self organization

Recently, Ganeshaiah and Uma Shaanker (1992, 1994) proposed an alternate mechanism based on the theory of self organisation to explain the differential development of seeds within fruits. According to this model, initial random drift in the mobilisation of resources to any one of the ovules and its subsequent autocatalytic feedback would result in the asymmetrical development of ovules, wherein one of the ovules become dominant and others are suppressed. This model operates independent of the resource status of the tree and does not assume any active interaction among the ovules (such as production and diffusion of an abortive chemical). The major prediction of their model is that keeping other things constant, seed abortion would increase with sink drawing ability (SDA) of the ovules in a fruit. In this section, three approaches were followed to provide test of this prediction namely

- a) manipulating the SDA of embryo in a few systems and examining its effect on embryo abortion
- b) evaluating the relationship between the SDA and per cent embryo abortion within and across species and
- c) surveying literature on the effect of exogenous application of growth regulators on seed abortion

Manipulating the SDA of embryo in a few systems and examining its effect on embryo abortion

Growth regulators are known to enhance the SDA (Lis and Antozewski, 1982; Goodwin et. al., 1978). In this experiment, SDA of embryos in a few species was manipulated using growth regulators and its effect on embryo abortion examined. The methodology followed is given below.

Experiment 3.2.1: Effect of growth regulators on SDA of embryos of *Phaseolus vulgaris* L. and *Syzygium cuminii*

This experiment was conducted to confirm the earlier finding that growth regulators enhance the mobilization of nutrients to sink. *Phaseolus vulgaris* plants were grown in pots and all recommended package of practices for the crop were followed. Two day old pods of *Phaseolus vulgaris* were treated by dipping their pedicels in different concentrations of growth regulators (100:100:10 ppm, 50:50:5 ppm, 25:25:0.5 ppm and 10:10:0.1 ppm of GA:NAA:BA) or distilled water for 6 h. The pods were then incubated in 20 ul of 25 uCi labeled sucrose at 30° C and 75 % RH for 16 h. After thoroughly washing the pods with water the embryos were excised, weighed and radio activity using liquid scintillation system. From this, specific activity of embryos was determined. Three replications of 15 to 20 embryos from at least 3 to 4 pods were included in each treatment.

A similar protocol was also used for *Syzygium cumini*. But here instead of pods, embryos excised from 2 day old fruits were directly pretreated with growth regulators solution after weighing and then transferred to labeled sucrose solution.

Experiment 3.2.2: Effect of exogenous application of growth regulators on embryo abortion in a few species

a) *Crotalaria medicaginea* Lam.

Crotalaria medicaginea plants were selected from the Botanical Garden of University of Agricultural Sciences, GKVK, Bangalore and 500 μ l of growth regulators solution (100:100:10 ppm of NAA:GA:BA) was injected into *Crotalaria medicaginea* pods ($n = 8$) 24 h after their fertilization to enrich the embryos directly. Control pods ($n = 5$) were injected with equal volume of double distilled water. The treatment was continued for four days. Three days later, embryos were observed for their development. Those that were very small in relation to the average size of the embryos were counted as aborted and expressed as per cent abortion.

b) *Solanum seaforthianum* Andr.

In *Solanum seaforthianum* young developing fruits immediately following their fertilization were dipped in growth regulator solution (100 : 100 : 10 ppm of NAA:GA:BA)

for 30 seconds a day for 10 days and observed for the development of embryos 15 days later.

c) *Vigna unguiculata* Walp. and *Phaseolus vulgaris* Linn.

Vigna unguiculata and *Phaseolus vulgaris* plants were grown in pots and all the recommended package of practices for their cultivation were followed. The SDA of embryos of *Vigna unguiculata* and *Phaseolus vulgaris* were modified using growth regulators. Following fertilization, young developing pods were dipped in solutions of varying concentrations of growth regulators (100:100:10, 50:50:5, 25:25:0.5 and 10:10:0.1 ppm of NAA:GA:BA) or distilled water for 20 seconds a day for eight (*Phaseolus*) and ten (*Vigna*) days. Ten days (for *Phaseolus*) and 15 days (for *Vigna*) after the last day of treatment, number of embryos aborted in each pod was recorded by visually scoring for small sclerotised embryos and expressed as per cent abortion. Three replications of six pods each for *Phaseolus* and 12 pods each for *Vigna* were used for each treatment.

Experiment 3.2.3: Effect of exogenous application of growth regulators on embryo abortion and final seed weight in *Phaseolus vulgaris*

This experiment was conducted to examine the seed weight variations within pods by growth regulator treatment. SDA of the embryos was altered by dipping the pods of *Phaseolus vulgaris* in different concentrations of growth regulators (same as earlier experiment) or

distilled water for 20 seconds a day for eight days. Treatment started following a day after fertilization. At least three replicates of six pods each were used for each treatment. At maturity, pods were harvested and individual seed weights were determined separately for each treatment. Mean seed weight, coefficient of variation for seed weight and per cent abortion of embryos were calculated. Embryos less than 20 % of mean seed weight were considered as aborted. The relation between the SDA and the various seed features were computed.

Effect of decreasing SDA on embryo abortion in *Syzygium cumini*

Triiodobenzoic acid (TIBA) is a commonly used auxin transport inhibitor. Application of TIBA to developing fruits inhibits the basipetal movement of auxins. Such fruits tend to exhibit a reduction in the SDA. In the following series of experiments, developing sinks in a few systems were pretreated with TIBA (to decrease SDA) and its effect on embryo abortion was studied.

Experiment 3.2.4: Effect of exogenous application of TIBA on the uptake of labeled sucrose by *Syzygium cumini* embryos

Embryos from two day old *Syzygium* fruits were excised and enriched with different concentrations of TIBA (10^{-6} , 10^{-5} and 10^{-4} M) or distilled water for 6 h, transferred to 25 uCi sucrose solution and were incubated for 12 h under humid conditions (75 % RH and 30°C) in

three replicates of at least 20 embryos each. Embryos were thoroughly washed with water for 3 h, ground in 2 ml of Bray's solution and the radio activity determined using liquid scintillation system and expressed as cpm/mg fresh weight. Two way ANOVA was conducted to compare among the treatments.

Experiment 3.2.5: Effect of exogenous application of TIBA on development of embryos in *Syzygium cuminii*

Following fertilization fruits of *Syzygium cuminii* were implanted in agar block. Ten μ l of TIBA (10^{-6} , 10^{-5} and 10^{-4} Molar) or distilled water was added into the flower cavity (fig. 4) and maintained in humid chamber for 6 days. Observations were recorded from at least 30 fruits in each treatment on the number of medium and small sized embryos developing within the fruit once in 3 days by opening the fruits and observing under stereo microscope. Care was taken to see that the solution (TIBA/distilled water) was always maintained in the cavity.

In another experiment, developing fruits were either sprayed with TIBA (0.1 mM) or distilled water for 9 days starting from a day after fertilization. After 13 days (4 days after the termination of the treatment) observations on the number of dominant (big), sub-dominant (medium) and small embryos developing within a fruit were recorded on at least 30 fruits in each treatment. Chi-square test was

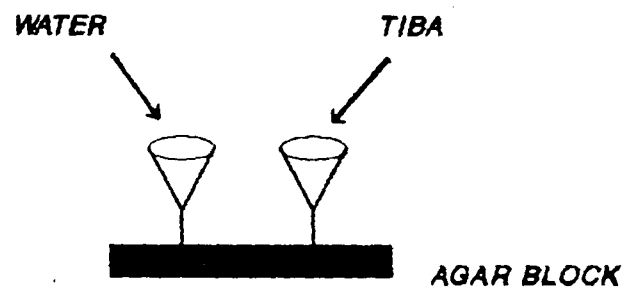


Fig. 4: Experimental set up for the application of Tri-iodo benzoic acid to Syzygium fruits

conducted to compare between the observed and expected frequencies in each treatment and to test the significance between the treatments.

b) Evaluating the relationship between the SDA and per cent embryo abortion within and across species

Earlier studies have shown wide variation in the extent of embryo abortion both within and across taxa. In this study, the relation between embryo abortion and SDA across and within taxa is addressed.

Experiment 3.2.6: Relationship between SDA and embryo abortion across 15 species

The relationship between SDA of embryos and rate of seed abortion was examined in 15 species. The methodology followed is given below.

For each of the 15 species (please see fig. 15 for a list of the species used) following fertilization, embryos were excised, weighed and incubated at 30° C and 75 % RH for 12 h in cavity slides containing 20 μ l of 25 μ Ci sucrose. After repeated washing of embryos in distilled water for 3 h to remove free space activity, they were ground in 2 ml of Bray's solution and the radio activity determined using liquid scintillation system and expressed as cpm/mg fresh weight. For each estimate, embryos from at least 3 to 6 pods replicated thrice were used. The ovule number per ovary and seed number per pod were counted for all 15 species in at least 20 pods/fruits each

and the seed abortion was estimated and regressed against the respective SDA.

Experiment 3.2.7: Relationship between sink drawing ability (SDA) and embryo abortion in *Derris indica*.

Derris indica (Fabaceae) is an oil yielding tree. Flowers generally contain two ovules. However, at maturity, pods contain predominantly only one seed. The trees differ markedly in the extent to which their pods exhibit such asymmetrical development of the two embryos within the pod. Such asymmetry within a given tree was estimated and referred to as asymmetry ratio.

From 12 randomly selected trees of *Derris indica* from the Botanical Garden, University of Agricultural Sciences, GKVK, Bangalore, about 30 pods from each tree were cut open eight to ten days after fertilization. The number of pods showing asymmetrical development of seeds (where the seeds were unequal in their sizes was calculated as follows:

$$\text{Asymmetry ratio} = \frac{\text{No. of pods showing asymmetrical development of embryos}}{\text{Total number of pods screened}} \times 100$$

From each of the 12 trees, one day after fertilization, pods were randomly selected, excised and embryos dissected, weighed and incubated in 10 ul of 25 uCi sucrose for 12 h in humid chamber (75 % RH and 30° C). Later, the embryos were transferred to distilled water,

washed for 3 h to remove surface activity, ground in 2 ml of Bray's solution and the sucrose uptake was measured using liquid scintillation system and expressed as cpm/mg fresh weight. Correlation analysis was conducted to examine the nature of relationship between SDA and asymmetry ratio.

Experiment 3.2.8: Relationship between SDA of the embryos and the extent embryo abortion in a few accessions of *Cajanus cajan* Linn.

Four germplasm accessions of *Cajanus cajan* (Acc. No 7896, 10903, 7332 and 11145) that were shown to exhibit certain consistent levels of seed abortion (Cumaraswamy and Bawa, 1989) were obtained from the International Crop Research Institute for Semi Arid Tropics (ICRISAT), Hyderabad, India and grown in pots. All recommended package of practices for its cultivation were followed. For each of the accessions, following 24 h of fertilization, embryos were excised, weighed and incubated in cavity slides containing 15 μ l of 25 μ Ci sucrose. After repeated washing of embryos in distilled water for 3 h to remove free space activity, they were ground in 2 ml of Bray's solution and the radio activity determined using liquid scintillation system and expressed as cpm/mg fresh weight. For each estimate, embryos from at least 3 to 6 pods replicated thrice were used. The ovule number per ovary and seed number per pod were counted for all the accessions in at least 20 pods and the seed abortion was

estimated. Two way ANOVA was conducted to compare the treatments.

Experiment 3.2.9: Surveying literature on the effect of exogenous application of growth regulators on seed abortion

Literature survey was conducted to examine if there exists any relation between the exogenous application of growth regulators and hence increased SDA of embryo and the extent of seed abortion. Literature pertaining to the effect of exogenous application of growth regulators and seed abortion was collected from CD ROM (which included Agris index and CAB index). The number of trials/experiments where in application of growth regulators resulted in either enhanced/reduced/no change in seed set was recorded and statistically analysed. It is important to note that only studies where application of growth regulators was done after fertilization were considered and not those where the growth regulator treatments were given before/during anthesis. Literature pertaining to the effect of Triiodobenzoic acid on seed set was also collected and analysed in a similar manner.

RESULTS

IV. RESULTS

The results of the experiments conducted are presented in two sections. The first section deals with the mechanism of embryo abortion in an animal dispersed species, *Syzygium cuminii* and the second deals with the test of predictions of the theory of self organization as applied to the differential development of seeds within fruits.

4.1: Mechanism of embryo abortion in an animal dispersed species, *Syzygium cuminii*

In *Syzygium cuminii*, of the 30 ovules in an ovary, only one develops to maturity. Arathi (1990) found that lack of pollination/fertilization does not completely explain the single seededness of fruits. She hypothesised that abortion of embryos could be due to the production and diffusion of a chemical killing agent by the dominant among the embryos. In this study, several experiments were conducted to test the above hypothesis and examine the chemical nature of the diffusate.

Experiment 4.1.1: Effect of extract of dominant embryos on the abortion of subject embryos (by visual scoring)

The objective of this experiment was to examine if the extract of dominant embryos cause the abortion of subject embryos. The abortion was assessed visually by the degree of browning of subject embryos on treatment with the extract of dominant embryos. Incubation in the

aqueous extract of dominant embryos caused a greater percentage abortion of subject embryos compared to that by either extract of aborted embryos, fruit coat tissue or water (Fig. 5). However, these differences were significant only at 46 h after incubation; the extract of dominant embryos caused 50 % abortion compared to 13.2 by that of fruit coat tissue, 11.8 by that of aborted embryos and 9.4 in control (table 1). These results indicate that in *Syzygium cumini* abortion of embryos could be due to the production of a chemical by the dominant embryo.

Experiment 4.1.2: Effect of extract of dominant embryos on the uptake of labeled sucrose by subject embryos

The aim of this experiment was to develop a relatively precise assay for studying the effect of extract of dominant embryos on the subject embryos (as a substitute for visual scoring). The assay involved estimating the effect of extract of dominant embryos on the uptake of labeled sucrose by subject embryos. As seen from table 2, the extract of dominant embryos caused significantly higher inhibition in the uptake of labeled sucrose by subject embryos (two way ANOVA, $P = 0.01$). Extract of dominant embryos caused 58.7 % inhibition while extract from aborted embryos caused 43.7 % inhibition in the uptake of labeled sucrose by subject embryos. Interestingly, extracts of unfertilized ovules and fruit

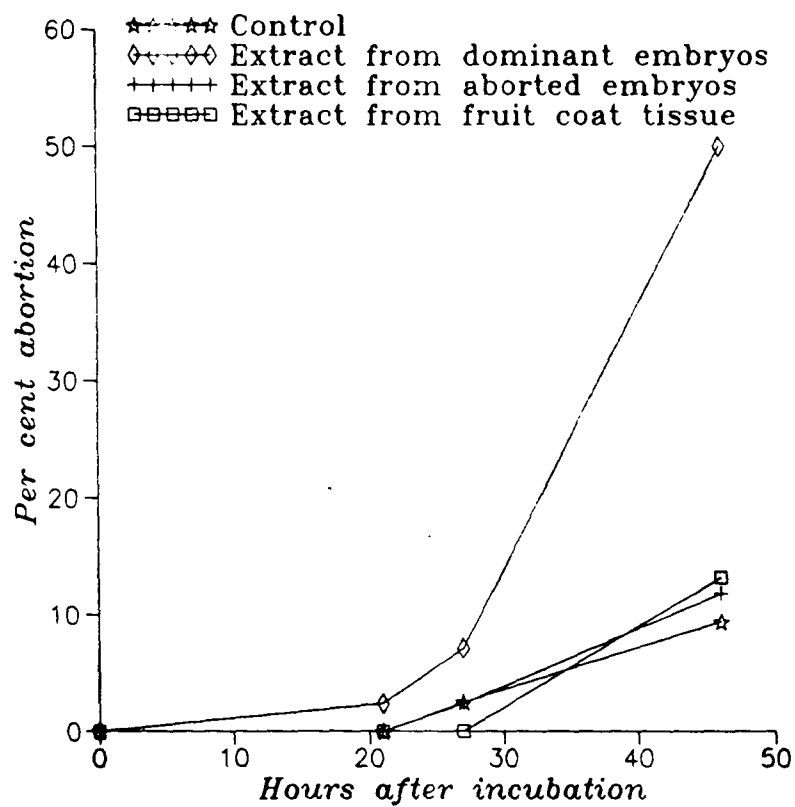


Fig. 5: Effect of extracts of dominant embryos, aborted embryos and fruit coat tissue on the abortion of subject embryos

Table 1: Effect of extract of dominant embryos, aborted embryos and fruit coat tissue on the abortion of subject embryos at different hours after incubation (HAI)

Treatment	Abortion percentage					
	21 HAI	SE	27HAI	SE	46 HAI	SE
Control	0	0	2.4	3.5	9.4	3.1
Extract from dominant embryos	2.4	3.5	7.1	3.3	50	6.5
Extract from aborted embryos	0	0	2.4	3.5	11.8	2.9
Extract from fruit coat tissue	0	0	0	0	13.2	3.1
test	two way anova		two way anova		two way anova	
CD (at 5%)	NS		NS		3.5	

Note : Each value is a mean of 3 replicates each containing at least 20 subject embryos

Table 2: Effect of extract of dominant embryos, aborted embryos, fruit coat tissue and unfertilised ovules on the uptake of labeled sucrose by subject embryos

Treatment	Absolute uptake (cpm/ovule)	% inhibition over control
Control	312.5 $\pm$ 34.5	0.0
Dominant embryos extract	124.3 $\pm$ 21.4	58.7
Aborted embryos extract	172.3 $\pm$ 32.5	43.7
Fruit coat tissue extract	204.3 $\pm$ 24.5	34.0
Unfertilised ovule extract	243.4 $\pm$ 14.6	18.0

Test Two way ANOVA
 Probability value $P < 0.01$
 CD Value 34

Note: Each value is a mean of 3 replicates and in each replicate at least 20 subject embryos were maintained

coat tissue did result in substantial inhibition in uptake of labeled sucrose by the subject embryos though the inhibition caused by them was significantly less compared to dominant embryos extract. Such reduction in the uptake by these tissues could be due to non-specific inhibition. However, these results suggest that for the same weight of tissues, extract from dominant embryos result in significantly greater inhibition in the uptake of labeled sucrose by subject embryos.

Experiment 4.1.3: Effect of diffusates from dominant embryos on the uptake of labeled sucrose by subject embryos

The hypothesis that dominant embryos produce a chemical responsible in aborting the subordinate embryos demands that such chemicals are easily diffusible. In this regard, an experiment was conducted to examine the effect of diffusate of dominant embryos on uptake of labeled sucrose by subject embryos.

Diffusate from dominant embryos caused significantly greater inhibition in the uptake of labeled sucrose compared to that from aborted embryos (table 3, Two way ANOVA, $p < 0.0001$). On an average, diffusates from dominant embryos caused 69.6 per cent inhibition while it was only 16.1 with the diffusate from aborted embryos. Diffusate from fruit coat tissue resulted in nearly 38 per cent inhibition in the uptake of labeled sucrose by the subject embryos, perhaps due to non-specific inhibition. The

Table 3: Effect of diffusate of dominant embryos, aborted embryos and fruit coat tissue on the uptake of labeled sucrose by subject embryos

Treatment	Absolute uptake (cpm/ovule)	% inhibition over control
Control	141.73 $\pm$ 4.86	—
Diffusates from dominant embryos	43.05 $\pm$ 2.41	69.6
Diffusates from aborted embryos	118.85 $\pm$ 6.33	16.1
Diffusates from fruit coat tissue	88.25 $\pm$ 4.4	37.7

Test: : Two way ANOVA
 Probablity value : P < 0.0001
 CD : 15.62

Note: Each value is a mean of 3 replicates each containing at least 20 subject embryos

results of this experiment indicates the possibility of the presence of some diffusible chemical in the dominant embryos that may be involved in the abortion of the subordinate embryos either directly or indirectly.

Experiment 4.1.4: Nature of the diffusate from dominant embryos causing inhibition in the uptake of labeled sucrose by subject embryos

Experiment 4.1.4.1: Effect of heat treatment of diffusate of dominant embryos on inhibition in the uptake of labeled sucrose by subject embryos

From the previous experiments it is clear that both the extract and diffusate from the dominant embryos cause a significantly greater inhibition in the uptake of labeled sucrose by subject embryos compared to the extracts and diffusates from other tissues. In this experiment, heat lability of the diffusate causing inhibition in the uptake of labeled sucrose was tested. The diffusates collected from the dominant and aborted embryos as well as from the fruit coat tissue were heated to 80° C and the ^{14}C uptake assay conducted. Inhibitory nature of the diffusate was retained despite the heat treatment. Heated diffusate caused inhibition in labeled sucrose uptake as effectively as non-heated control (table 4, F-test, non-significant). As in earlier experiments, diffusate from dominant embryos caused significantly higher inhibition in labeled sucrose uptake under both control and heat treatments over that caused by diffusates from other tissues. Significant interaction effect was

Table 4: Effect of heating the diffusate of dominant embryos on uptake of labeled sucrose by subject embryos

Factor 1	Factor 2	Absolute uptake (cpm/ovule)	% inhibition over control
Control	Control	163.2 $\pm$ 36.7	
	Diffusate from dominant embryo	81.7 $\pm$ 4.5	49.9
	Diffusate from aborted embryo	83.3 $\pm$ 12.7	49.0
	Diffusate from fruit coat tissue	95.6 $\pm$ 7.3	41.4
	Overall mean	105.96	46.9
Heat treatment	Control	134.1 $\pm$ 15.5	
	Diffusate from dominant embryo	67.5 $\pm$ 1.5	49.7
	Diffusate from aborted embryo	104.2 $\pm$ 16.5	22.3
	Diffusate from fruit coat tissue	77.5 $\pm$ 2.4	42.2
	Overall mean	95.79	38.2

Test : Two factor factorial
 CD Value : Factor 1 NS
 Factor 2 26.7
 Probability value P = 0.03

Note: Each value is a mean of three replicates. At least 20 subject embryos were used in each replicate for uptake assay.

observed between the heat treatment and different diffusate treatments. These results indicate that the chemicals contained in the diffusate responsible for the inhibition in uptake of labeled sucrose may be heat stable.

Experiment 4.1.4.2: Effect of trypsin digestion of the diffusate of dominant embryos on uptake of labeled sucrose by subject embryos

Trypsin digested diffusate did not alter the extent of inhibition in the uptake of labeled sucrose by subject embryos compared to that by untreated diffusates (table 5, F-test, NS). Diffusates from dominant embryos, both in trypsin digested and control, again caused significantly greater inhibition in the uptake of labeled sucrose by subject embryos compared to the diffusate from other tissues. The results of this experiment suggest that the chemical causing inhibition is probably non-proteinaceous.

Experiment 4.1.4.3: Determination of the absorption maxima of the diffusate of dominant embryos

Results of the earlier experiments indicate that the inhibitory chemical present in the diffusate of dominant embryos could be a small molecular weight, non-proteinaceous diffusible compound. Engvild (1987) demonstrated the presence of chloroindole-acetic acid in the developing pea seeds and suggested that this might act as death hormone causing the abortion of subordinate sinks. Bangerth (1989) hypothesised that abortion of

Table 5: Trypsin digestion of the diffusate of dominant embryos on the uptake of labeled sucrose by subject embryos

Factor 1	Factor2	Absolute uptake (cpm/ovule)	% inhibition over control
Control	Control	301.2 $\pm$ 11.3	--
	Diffusate from dominant embryo	129.0 $\pm$ 7.9	57.1
	Diffusate from aborted embryo	191.8 $\pm$ 6.4	36.3
	Diffusate from fruit coat tissue	216.83 $\pm$ 11.7	28.0
	Overall mean	190.3	40.5
Trypsin digested	Control	277.5 $\pm$ 16.2	--
	Diffusate from dominant embryo	120.7 $\pm$ 11.1	56.6
	Diffusate from aborted embryo	159.0 $\pm$ 10.22	42.7
	Diffusate from fruit coat tissue	203.9 $\pm$ 16.5	26.6
	Overall mean	209.7	42.0

Test : CRD, Two factor factorial
 CD : Factor 1 NS
 Factor 2 50.3
 Probability value [P =0.0047]

Note: Each value is a mean of three replicates. At least 20 subject embryos were used in each replicate for uptake assay.

subordinate fruits could be mediated by the process of primigenic dominance involving a competitive inhibition in the diffusion of auxin from the subordinate embryos by the dominant embryo. Both these imply a role for indole compounds in the abortion of subordinate fruits.

Indole compounds have absorption maxima at 222 and 280 nm. The absorption maxima of the diffusate collected from both the dominant and aborted embryos coincided with that of indole compounds. The diffusates had a peak absorption at 220 and 278 nm (table 6) suggesting the presence of indole compounds in the diffusate.

Experiment 4.1.5: Organic solvent partitioning of the diffusate from dominant embryos

To further examine if the diffusate did contain indole compounds an experiment was conducted wherein the diffusate was fractionated in organic solvent such as butanol which selectively separates indole compounds. The butanol and water fractions were separated and the uptake assay was conducted. Results of the experiment are presented in table 7. Butanol and water fractions did not differ in the extent of inhibition in the uptake of labeled sucrose by subject embryos (Table 7, F-test, NS). On an average the water fraction caused 18.5 % inhibition as against 9.1 by butanol fraction. But water fraction from diffusates of dominant embryos caused significantly higher inhibition (Two way ANOVA, $p < 0.01$) in the uptake

Table 6: Absorption maxima of the diffusate from dominant and aborted embryos

Treatment	Expt I		Expt II	
	λ (nm)	OD	λ (nm)	OD
IAA (10^{-4} M)	222	1.31	221	1.68
	278	0.58	278	0.50
Diffusate from dominant embryos	222	1.08	216	1.42
	278	0.15	276	0.26
Diffusate from aborted embryos	222	1.08	216	1.38
	278	0.14	276	0.24

Note: At least 100 dominant and 200 aborted embryos were used for diffusate collection

Table 7: Effect of organic solvent partitioning of the diffusate from dominant embryos on labeled sucrose uptake by subject embryos

Fraction	Diffusate	Absolute uptake (cpm/ovule)	% inhibition over control
Water	Control	887.6 ± 17.2	--
	Dominant	568.2 ± 29.5	36.0
	Aborted	842..0 ± 34.5	5.2
	Pod coat tissue	762..8 ± 85.1	14.1
	Mean	765.15	18.43
Butanol	Control	818.4 ± 49.2	--
	Dominant	673.8 ± 12.5	17.5
	Aborted	748.7 ± 10.64	8.5
	Pod coat tissue	807.2 ± 49.3	1.4
	Mean	762.02	9.1

TestTwo factor factorial

Probalility valueP < 0.02

CD ValueFractions NS

Diffusates 66.7

Note: Each value is a mean of three replicates. At least 20 subject embryos were included per replication

of labeled sucrose (36 %) than that from butanol fraction (17.5 %). The results suggest that the chemical present in the diffusate is equally soluble in both water and butanol which is in fact a property of most of indole compounds.

Experiment 4.1.6: Thin Layer Chromatography (TLC) analysis of the diffusate of dominant embryos

This experiment was conducted to test the presence of indole compounds in the diffusate of dominant embryos. Cellulose plate TLC with Ehrlich reagent spray did not indicate the presence of any indole compounds in the diffusates from dominant embryos. But cellulose plate TLC sprayed with ninhydrin indicated the presence of tryptophan which is the precursor for indole acetic acid (IAA) synthesis (Fig. 6a).

Silica gel TLC with FeCl_3 + Perchloric acid spray revealed the presence of IAA (R_f value of 0.42) along with a highly diffusible substance (R_f value of 0.51) belonging to the same group (Fig. 6b) in the diffusate from dominant embryos. Other standards used in the experiment such as phenylalanine and tryptophan were not noticed in the diffusate of dominant embryos.

Experiment 4.1.7: High performance liquid chromatography (HPLC) analysis of the diffusate of dominant embryos

To confirm the results of the previous experiment, HPLC analysis of the diffusate from dominant embryos was

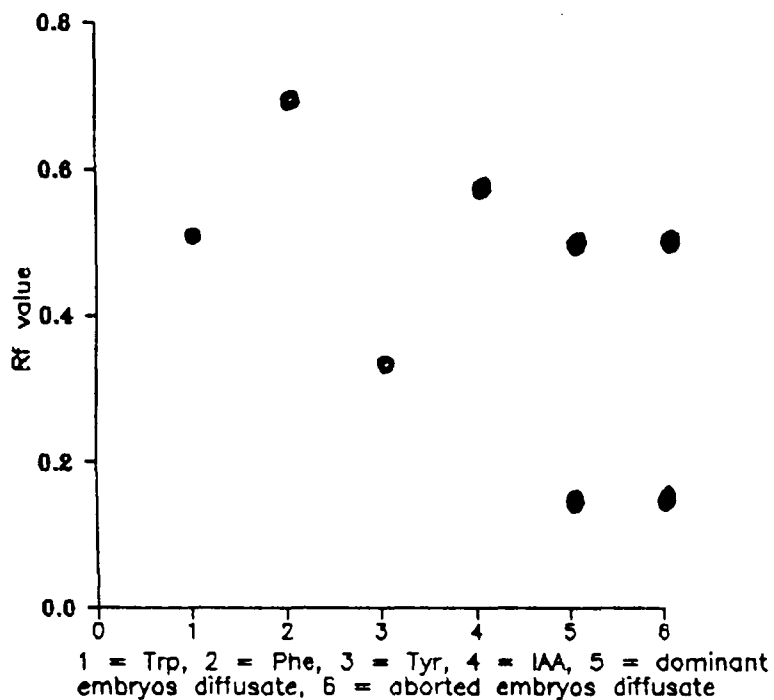


Fig. 6a: Cellulose TLC showing the presence of Tryptophan in the diffusates of dominant and aborted embryos

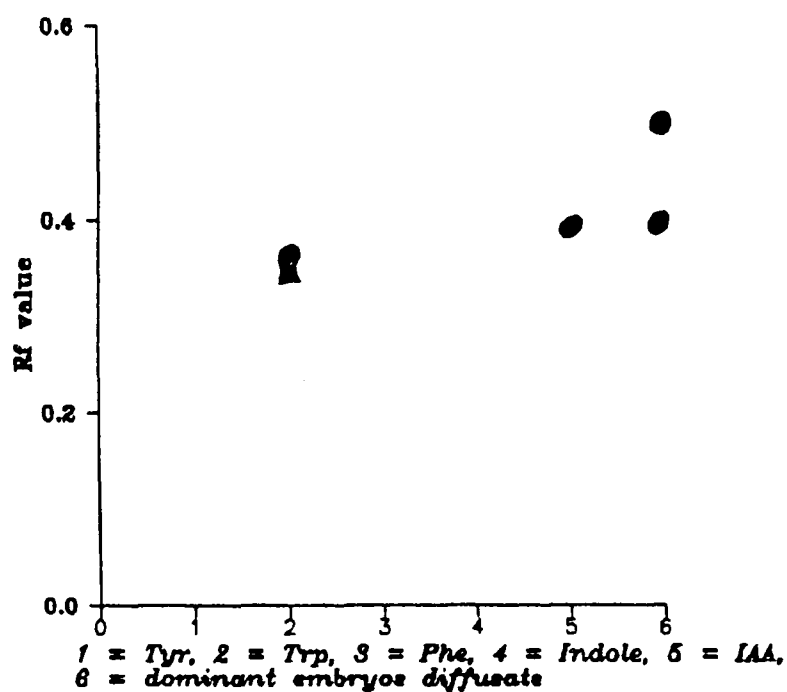


Fig. 6b: Silica gel TLC showing the presence of IAA in the diffusate of dominant embryos

conducted at 280 nm which detects IAA. The retention time of standard IAA was 4.7 min. The diffusate exhibited a single peak with a retention time of 3.55 min ; this is different from the retention time exhibited by IAA (Fig. 7 a and b). However there is a substantial overlap in the retention time of the diffusate and that of standard IAA indicating the possibility of the presence of indole compounds in the diffusate as well.

An uptake assay was carried out from the various fractions collected during HPLC analysis. Fractions comprising those exhibiting the peak retention time caused significantly higher inhibition in the uptake of labeled sucrose than the rest of the fractions. On an average, fractions pooled over the peak retention time caused 94 % inhibition as against 63 % from a pooled sample from rest of the fractions (Table 8a; two way ANOVA, $p < 0.05$).

Similar results were obtained in another experiment. Pooled fractions corresponding to the peak retention time (fractions 5 to 7) and fractions 8 to 10 resulted in higher inhibition (table 8b, two way ANOVA, $P < 0.001$) in labeled sucrose uptake by subject embryos than the rest of fractions. Fractions 1 and 2 resulted in least inhibition (Fig. 8).

These results indicate that the chemical present in the diffusate having a peak retention time of 3.55 min.

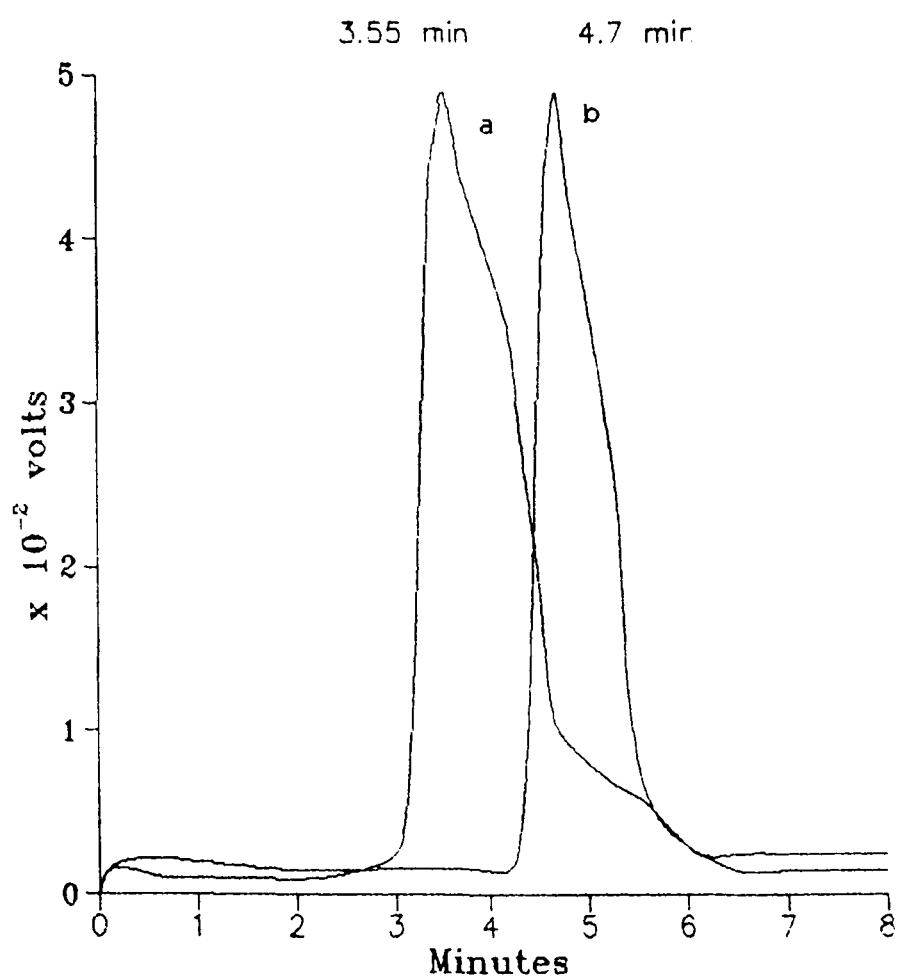


Fig. 7: HPLC analysis of the diffusate from
a) dominant embryos
b) standard IAA

Table 8a: Effect of different HPLC fractions of diffusate from dominant embryos on the inhibition in the uptake of labeled sucrose by subject embryos

Fraction No.	cpm/embryo	% inhibition
Control	532.1 $\pm$ 29.4	--
Peak (4 & 5)	36.0	94.0
Rest (1-3 & 6-15)	189.9	63.0

Test: Two way ANOVA
 CD at 5% 38.4

Note: Only fractions corresponding to peak retention time (fraction numbers 4 & 5) were included and all other fractions were pooled in rest fraction. At least 4 replicates each containing at least 20 subject embryos were maintained.

Table 8b: Effect of different HPLC fractions of diffusate of dominant embryos on inhibition in the uptake of labeled sucrose by subject embryos

Fraction No.	cpm/embryo	% inhibition
1-2	372.9 $\pm$ 27.8	11.2
3-4	332.6 $\pm$ 34.8	20.8
5-7	193.3 $\pm$ 31.3	54.0
8-10	163.3 $\pm$ 28.5	60.3
11-15	208.9 $\pm$ 24.5	50.6
Control	420.5 $\pm$ 32.8	--

Test: Two way ANOVA
 Probability value P < 0.001
 CD 36.4

Note: Each value is a mean of three replicates and each replicate had at least 20 subject embryos.

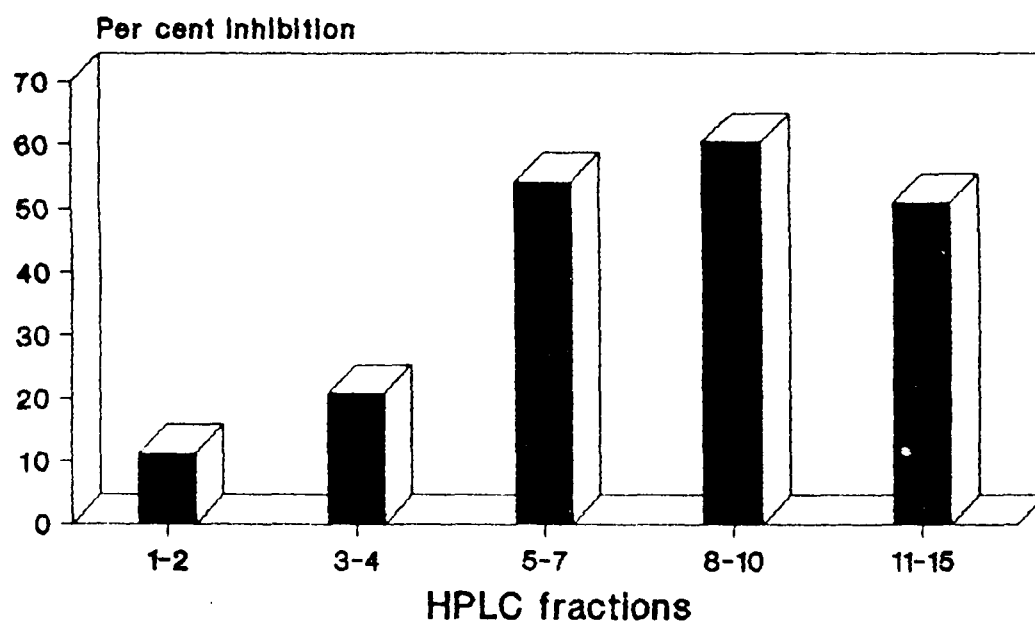


Fig. 8: Inhibition of labeled sucrose uptake by subject embryos by HPLC fractions of diffusates from dominant embryos

may be the probable candidate causing the inhibition in the uptake of labeled sucrose by subject embryos.

Experiment 4.1.8.1: Quantification of indole compounds in the diffusates from different tissues

An attempt was made to quantify the indole compounds in the diffusate of dominant embryos using ELISA technique. The results revealed the presence of indole compounds in the diffusates. The diffusate from the dominant embryos had the highest amount of indole compounds compared to the diffusates from other tissues when expressed on fresh weight basis (table 9). Dominant embryos contained 950 pico moles per mg fresh weight while fruit coat tissue had as high as 445.17 pico moles per mg and aborted embryos had 411.27 pico moles per mg fresh weight.

Experiment 4.1.8.2: Quantification of indole compounds in the diffusates of dominant and aborted embryos with developmental stages

In this experiment the indole compounds were quantified in diffusates derived from dominant and aborted embryos at different stages of development on both fresh and dry weight basis. Based on age (i.e. days after fertilization) dominant and aborted embryos were classified into six stages (see material and methods for details) and indole compounds present in them was quantified. The quantity of indole compounds increased

Table 9: Indole content in different tissues expressed as picomoles per mg tissue (FW basis)

Tissues	Indole content (picomoles)
Dominant embryos	950 $\pm$ 49.2
Completely aborted embryos	411.27 $\pm$ 21.4
Fruit coat tissue	445.17 $\pm$ 37.2
Unfertilised ovules	271.87 $\pm$ 24.3
Leaf tissue	434.4 $\pm$ 36.5

with age (stages) for both dominant and aborted embryos when expressed on per embryo basis (Fig. 9). However, after 4th stage there was decrease in the quantity of indole compounds in both dominant and aborted embryos. A maximum of 229 pico moles per embryo was obtained from the diffusate of 6th stage dominant embryos. In case of aborted embryos, maximum was noticed in the 4th stage with 18.35 pico moles per embryo (table 10). These patterns were however altered when the data was expressed on fresh or dry weight basis. The content increased upto 3rd stage and later started declining in case of dominant embryos while in case of aborted embryos it increased upto 4th stage. Similar pattern was observed when the data was expressed on dry weight basis (fig. 9).

When expressed on fresh weight basis a maximum of 1287.5 pico moles per mg was obtained in 6th stage aborted embryos while for dominant embryos the maximum quantity was observed in the 3rd stage with 1036.4 pico moles per mg (table 10). On the other hand, when expressed on dry weight basis, 3rd stage dominant embryos with indole content of 9422 pico moles per mg and 4th stage aborted embryos with 3060.5 pico moles per mg showed highest indole content in their respective groups. The data suggests the presence of maximum indole compound in 3rd stage dominant embryos.

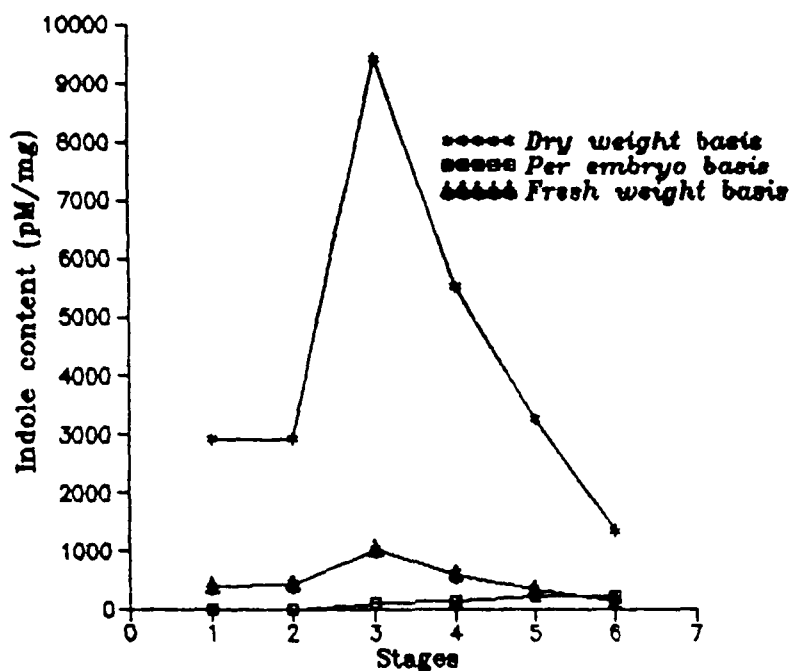


Fig. 9a: Quantity of indole compounds in diffusates from dominant embryos with different stages of development

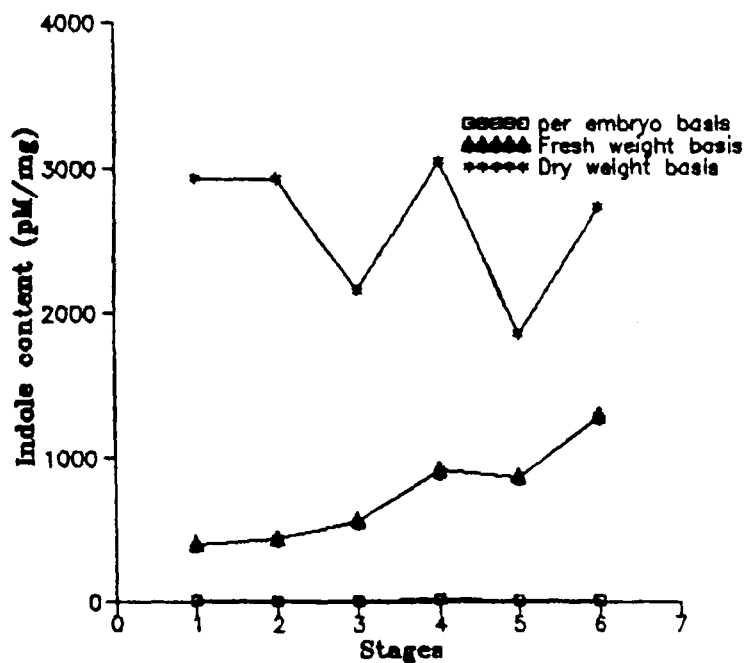


Fig. 9b: Quantity of indole compounds in diffusates from aborted embryos with different stages of development

Table 10: Indole compounds content at different stages of development of dominant and aborted embryos

Stages	Per embryo basis		Fresh weight basis		Dry weight basis	
	Dominant	Aborted	Dominant	Aborted	Dominant	Aborted
1	12.2	12.2	400.85	400.85	2939.0	2939.0
2	14.7	14.7	441.20	441.20	2941.0	2941.0
3	124.7	14.76	1036.40	571.25	9422.0	2166.0
4	151.45	18.35	607.65	918.15	5524.3	3066.5
5	225.4	13.15	358.75	873.45	3270.5	1858.5
6	229.0	15.05	149.7	1287.5	1360.8	2738.5

Test	Two way ANOVA	Two way ANOVA	Two way ANOVA
Probability value	P<0.001	P<0.001	P<0.001
CD Value	4.61	48.1	441

Note: 8 mg embryos were used for diffusate collection. Each value is a mean of two replicates

4.2: Test of predictions of the theory of self organization

Traditionally, two explanations have been forwarded to explain the differential development of seeds within fruits. One of them explains seed abortion as a consequence of competition for resources (Adams, 1967; Lee, 1988) and the other due to chemical interaction among developing seeds. In both the models, it is assumed that the dominant among the embryos outcompete and kill the subordinate embryos. Both these models however, require the development of hierarchy among the developing embryos but do not explain the process of its emergence. Ganeshaiah and Uma Shaanker (1992, 1994) proposed a simple process which can generate such a hierarchy leading to differential development of seeds in fruits independent of any competition for resources or chemical interaction among the developing embryos. Their model is based on a self-organized development of seeds within fruits involving an autocatalytic feedback process of flow of resources into the developing seeds.

One of the predictions of their model is that with increase in sink drawing ability (SDA) of embryos within fruits, seed abortion should also increase. In this section, a test of this prediction is provided by

- a) manipulating the SDA of embryos in a few systems and examining its effect on embryo abortion

- b) evaluating the relationship between the SDA and percent embryo abortion within and across species and
 - c) surveying the literature on the effect of exogenous application of growth regulators on seed abortion
- a) Manipulating the SDA of embryos in a few systems and examining its effect on embryo abortion

In this section an attempt has been made to alter the SDA of embryos of a few selected species and examine the consequence on embryo abortion.

Experiment 4.2.1: Effect of growth regulators on SDA of embryos of *Phaseolus vulgaris* and *Syzygium cumini*

Two day old beans pods were treated with different concentrations of growth regulators, transferred to labeled sucrose solution and incubated over night. Later the embryos were ground in 2 ml of Bray's solution and the radioactivity in the embryos was determined using liquid scintillation system.

Uptake of labeled sucrose increased with growth regulators concentration (Fig. 10). Maximum uptake of 813.4 cpm per mg was obtained in 50:50:5 ppm NAA:GA:BA while in control the uptake was 449.97 cpm per mg. There was significant difference among the treatments with respect to uptake of labeled sucrose (table 11, two way ANOVA, $P < 0.001$).

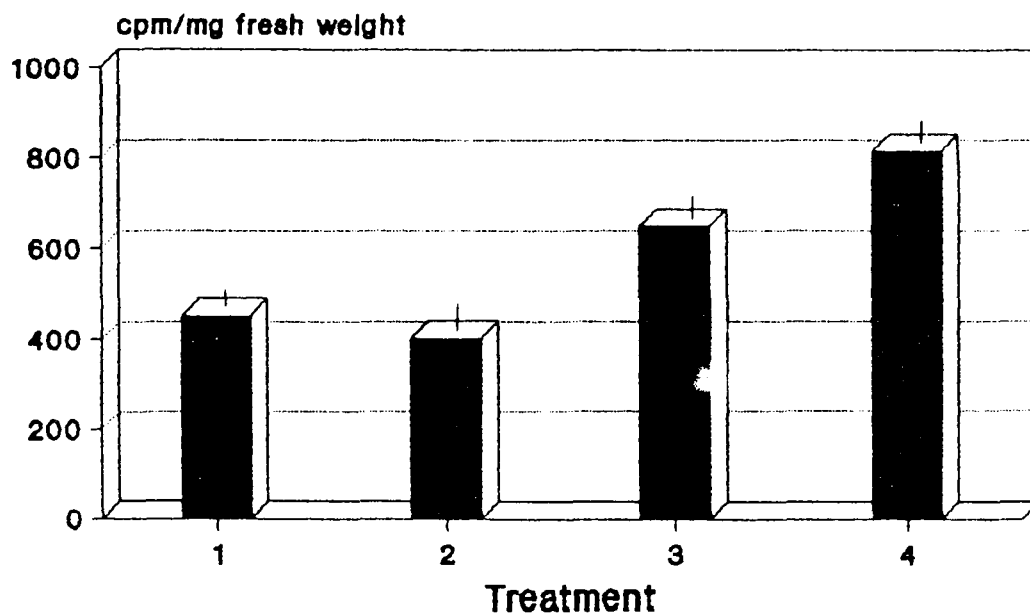


**Fig. 10: Uptake of labeled sucrose by beans embryos
as influenced by growth regulators**

Treatments: 1 = control, 2 = 10:10:0.1 ppm, 3 = 25:25:0.5 ppm and 4 = 50:50:5 ppm ppm of GA:NAA:BA respectively.
Vertical line above bars indicate standard error

Table 11: Effect of different concentrations of growth regulators on uptake of labeled sucrose by beans embryos

GA:NAA:BA (ppm)	cpm/mg
Control	449.97 $\pm$ 38.4
10 : 10 : 0.1	401.7 $\pm$ 41.2
25 : 25 : 1	647.4 $\pm$ 52.4
50 : 50 : 5	813.4 $\pm$ 58.8

Test	Two way ANOVA
Probability value	P = 0.001
CD Value	52.3

Note : Three replicates having at least 20 embryos per replication were used for uptake assay

Embryos of *Syzygium cumini* treated with growth regulators also revealed significant differences in the uptake of labeled sucrose compared to control (fig. 11). Maximum uptake of 422.6 cpm per mg was obtained in 25:25:10 ppm NAA:GA:BA concentration while the least uptake of 288.3 cpm per mg was observed in control (table 12). Thus it is clear from these experiments that application of growth regulators do indeed enhance the SDA of embryos.

Experiment 4.2.2: Effect of exogenous application of growth regulators on embryo abortion

Crotalaria medicaginea and *Solanum seaforthianum*

Extent of embryo abortion with increase in growth regulator concentration was investigated in a few systems. Table 13 shows the comparison between control and growth regulators treatment for the extent of embryo abortion in *Crotalaria medicaginea* and *Solanum seaforthianum*. In both the species significantly higher percentage of embryo abortion was obtained when young developing pods were treated with growth regulators. In *Crotalaria* application of growth regulators resulted in 20.8 % embryo abortion as against 5.81 % in control (table 13; t test, $p < 0.050$) while in *Solanum seaforthianum* it was 12.09 % as against 2.85 % in control (table 13; t test, $p < 0.01$).

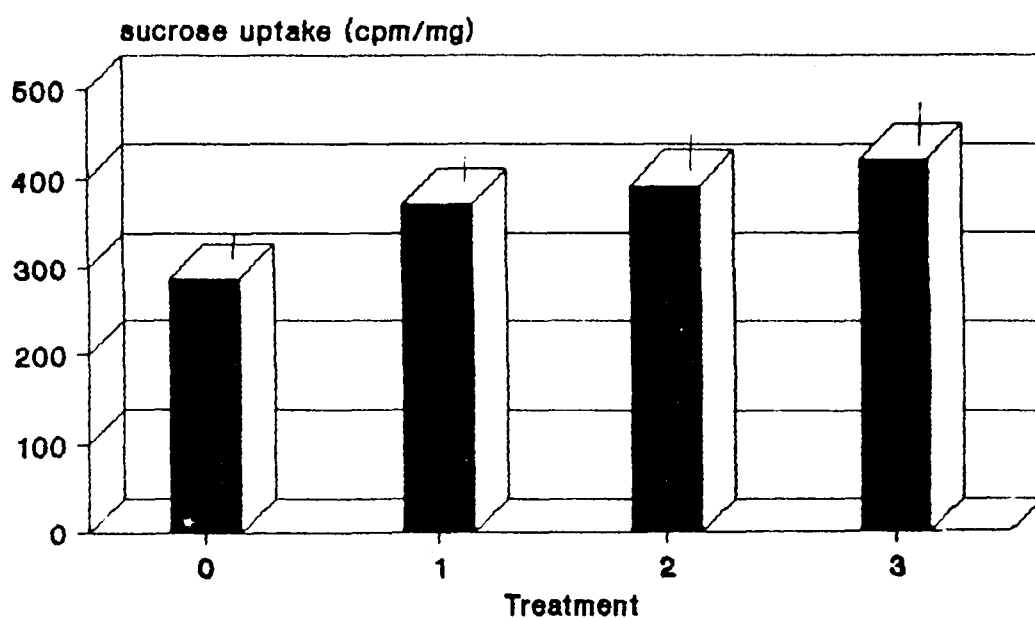


Fig. 11: Uptake of labeled sucrose by *Syzygium* embryos as influenced by growth regulators treatment

Treatments: 0 = control, 1 = 10:10:0.1 ppm, 2 = 25:25:0.5 ppm, 3 = 50:50:5 ppm of GA:NAA:BA respectively. Vertical line above bars indicate standard error

Table 12: Effect of different concentrations of growth regulators on uptake of labeled Sucrose by *Syzygium* embryos

BA : GA : NAA (ppm)	Labeled sucrose uptake (cpm/ovule)	SE (cpm/ovule)
Control	288.3	21.3
0.1 : 10 : 10	372.5	20.2
0.5 : 25 : 25	393.4	15.1
5 : 50 : 50	422.6	9.3

Test	Two way ANOVA
Probability value	P < 0.01
CD at 5%	64.6

Note : Each value is a mean of 4 replicates each containing at least 20 subject embryos

Table 13: Embryo abortion percentage as affected by growth regulator treatments in *Crotolaria medicagenia* Lam and *Solanum seaforthianum*

	Treatment	mean seed abortion (%)	SE (%)	Test	Probability value
<i>Crotolaria medicagenia</i>					
	Control	5.81	1.7		
				t test	P < 0.05
	100:100:10 ppm NAA:GA:BA	20.8	1.8		
<i>Solanum seaforthianum</i>					
	Control	2.85	3.44		
				t test	P < 0.01
	100:100:10 ppm NAA:GA:BA	12.09	12.27		

Note : Three replications having at least 8 pods per replication were included in the analysis

Vigna unguiculata and *Phaseolus vulgaris*

In *Phaseolus vulgaris* and *Vigna unguiculata*, embryo abortion increased with the growth regulators concentration (Fig. 12 a & b). NAA:GA:BA at 100:100:10 ppm resulted in maximum abortion in both cowpea and beans (31.4 and 27.6 respectively as against 12.86 and 3.03 in control, table 14a).

Similar results were obtained in yet another experiment in *Phaseolus*. NAA:GA:BA at 100:100:10 ppm caused a maximum of 34 per cent embryo abortion as against 8.6 per cent in control (table 14b).

Experiment 4.2.3: Effect of exogenous application of growth regulators on embryo abortion and final seed weight in *Phaseolus vulgaris*

In this experiment the influence of exogenous application of growth regulators on embryo abortion and seed weight was investigated. Embryo abortion increased with the concentration of growth regulators (table 15). The mean seed weight did not vary much among treatments when seed weight of aborted seeds were not considered. However, when seed weight of aborted seeds were considered, mean seed weight decreased with growth regulator concentration. The coefficient of variation for seed weight didnot vary much between control and growth regulators treatment when seed weights of aborted embryos were not included in the analysis. However when the seed

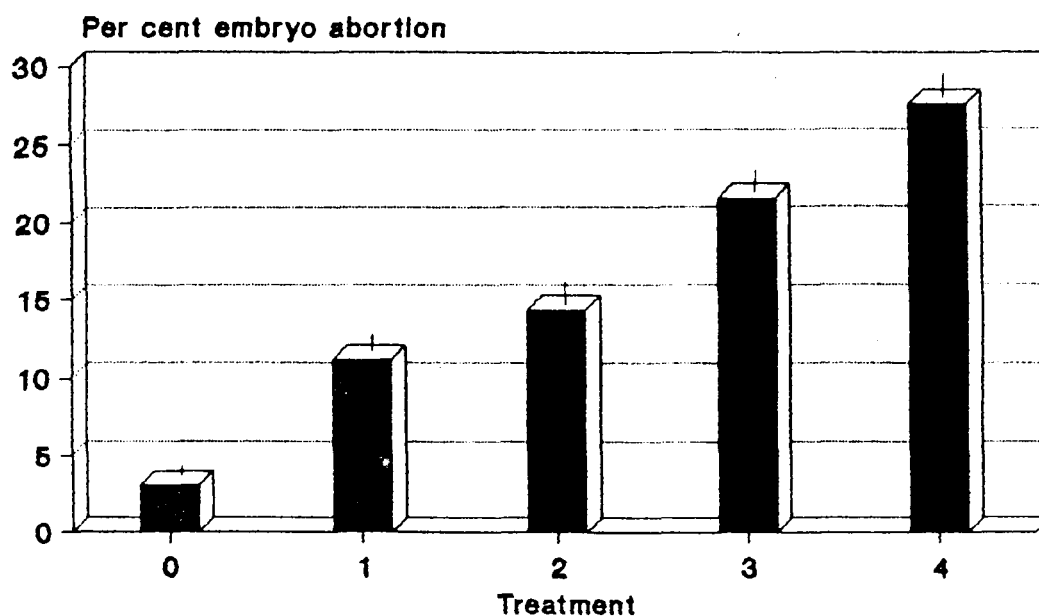


Fig 12a: Embryo abortion as influenced by growth regulators concentration in beans

Treatments: 0 = control, 1 = 10:10:0.1 ppm, 2 = 25:25:0.5 ppm, 3 = 50:50:5 ppm and 4 = 100:100:10 ppm of GA:NAA:BA respectively. Vertical line above bars indicate standard error

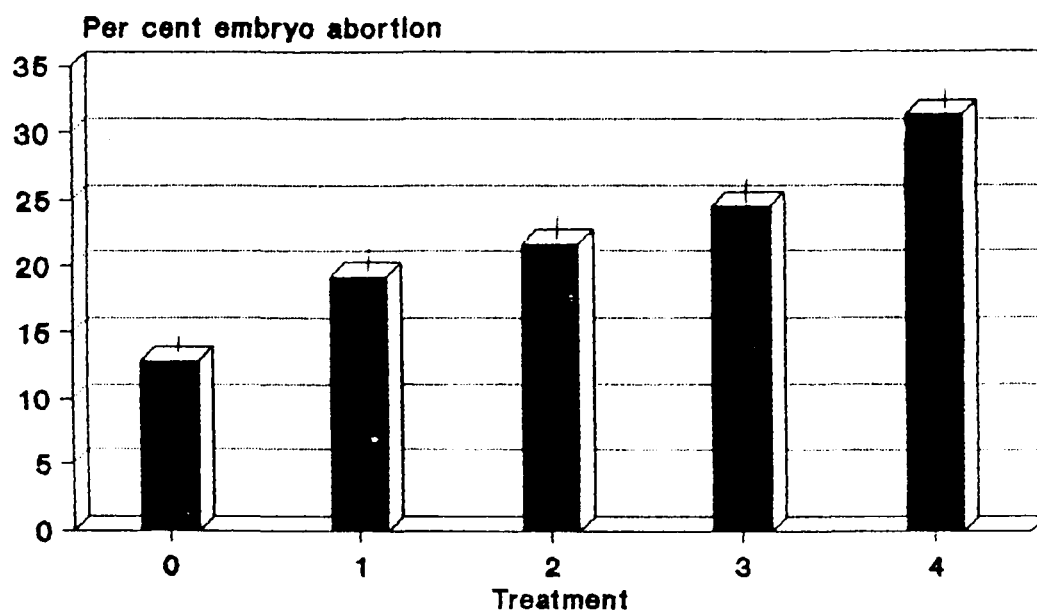


Fig. 12b: Embryo abortion as influenced by growth regulators concentration in cow pea

Treatments: 0 = control, 1 = 10:10:0.1 ppm, 2 = 25:25:0.5 ppm, 3 = 50:50:5 ppm and 4 = 100:100:10 ppm of GA:NAA:BA respectively. Vertical line above bars indicate standard error

Table 14a: Effect of growth regulators on embryo abortion in cowpea and beans

GA :NAA : BA	Mean seed abortion	
	Cowpea	Beans
Control	12.86 ± 1.8	3.03 ± 0.7
10 : 10 : 0.1	19.2 ± 1.6	11.12 ± 1.4
25 : 25 : 1	21.6 ± 2.1	14.30 ± 1.7
50 : 50 : 5	24.5 ± 2.4	21.5 ± 2.0
100 : 100 : 10	31.4 ± 3.5	27.6 ± 1.9
Test	two way ANOVA	Two way ANOVA
CD at 5%	2.4	1.8

Note : At least 3 replicates each containing 6 pods were maintained in each treatment

Table 14b: Effect of different concentrations of growth regulators on embryo abortion in beans

GA:NAA:BA (ppm)	Mean seed abortion (%)
Control	8.6 ± 1.8
10 : 10 : 0.1	13.73 ± 2.9
25 : 25 : 1	15.93 ± 2.3
50 : 50 : 5	17.3 ± 2.8
100 : 100 : 10	34 ± 3.4

Test
Probability value
CD Value

Two way ANOVA
0.001
2.4

Table 15: Effect of growth regulators on mean seed weight, coefficient of variation for seed weight and embryo abortion in beans.

Treatment	n	Mean seed wt SE	Std. deviation for seed wt.	Coefficient of variation (%) for seed wt.	% embryo abortion
Control (a) (b)	31 32	703.1 $\pm$ 27.86 681.9 $\pm$ 34.75	152.6 193.5	21.7 28.4	3.03
10 : 10 : 0.1 ppm GA:NAA:BA (a) (b)	24 28	699.8 $\pm$ 29.57 600.8 $\pm$ 53.85	141.8 279.8	20.26 46.57	11.5
25 : 25 : 0.5 ppm GA:NAA:BA (a) (b)	23 26	722.9 $\pm$ 29.04 639.8 $\pm$ 50.3	136.2 251.5	18.3 39.3	14.3
50 : 50 : 5 ppm GA:NAA:BA (a) (b)	26 32	607.6 $\pm$ 33.18 480.9 $\pm$ 50.92	165.9 283.5	27.3 58.9	22
100 : 100 : 10 ppm GA:NAA:BA (a) (b)	21 29	687.7 $\pm$ 34.86 499.9 $\pm$ 63.58	155.9 336.4	22.6 67.4	27.6 27.6

a = Without inclusion of aborted embryos weight

b = With inclusion of aborted embryos weight

weight of aborted embryos were also included in the analysis, the mean seed weight decreased with concentration of growth regulators (fig. 13a) Further, the coefficient of variation for seed weight increased with embryo abortion(fig. 13b).

Effect of decreasing SDA on embryo abortion in *Syzygium cumini*

Earlier experiments in this section have shown that application of growth promoters (IAA, GA, BA) increase the SDA of embryos and consequently lead to increased abortion of seeds within fruits. In the following experiments an attempt has been made to decrease the SDA of embryos through certain inhibitors and evaluate its effect on seed abortion. It is predicted that decrease in SDA of embryos would lead to a reduction in the extent of seed abortion.

Experiment 4.2.4: Effect of exogenous application of TIBA on the uptake of labeled sucrose by *Syzygium cumini* embryos

Two day old syzygium embryos were pre-treated with TIBA, an auxin transport inhibitor at varying concentrations and the ability of the embryos to take up labeled sucrose was assayed. As seen in fig. 14, the uptake decreased with TIBA concentrations. Control embryos showed maximum uptake of 35.43 cpm per embryo while those treated with 10^{-4} TIBA showed an uptake of only 19.87 cpm per embryo (table 16). Significant

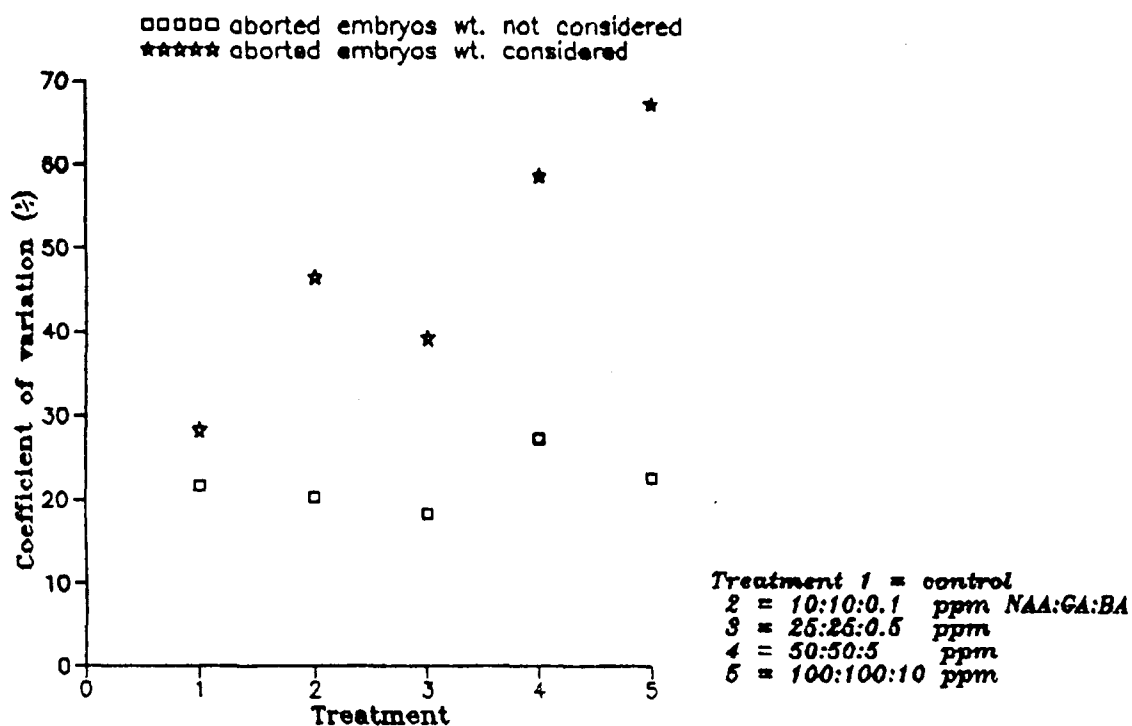


Fig. 13a: Effect of application of various concentrations of growth regulators on the coefficient of variation for seed weight in beans

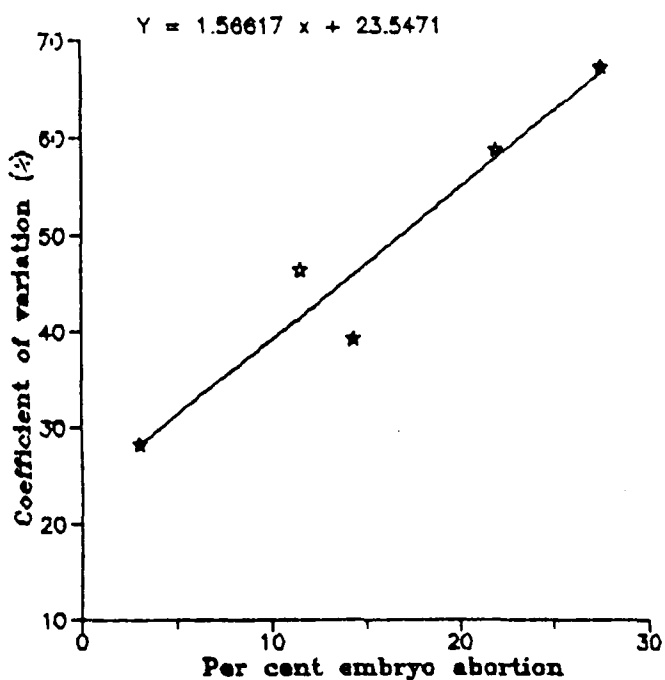


Fig. 13b: Relation between per cent embryo abortion and coefficient of variation for seed weight in beans

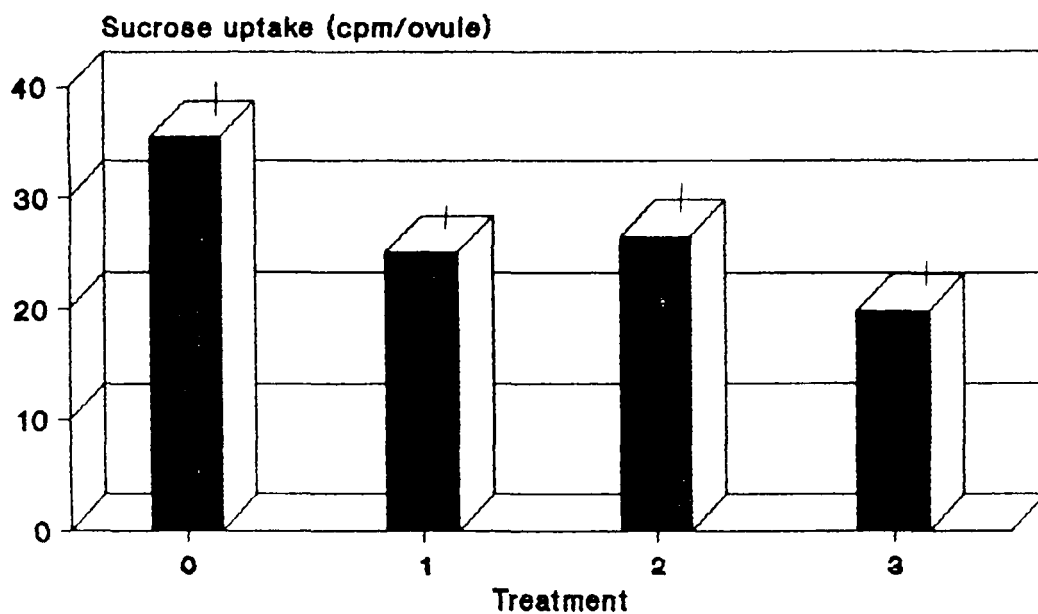


**Fig. 14: Uptake of labeled sucrose by Syzygium embryos
as influenced by TIBA treatment**

Treatments: 0 = control, 1 = 10^{-6} Molar, 2 = 10^{-5} Molar
and 3 = 10^{-4} Molar Tri-iodo benzoic acid treatments
respectively

**Table 16: Effect of Tri iodo benzoic acid (TIBA)
on uptake of labeled sucrose by
*Syzygium*embryos**

TIBA conc.	Mean cpm/ovule
Control	35.43 $\pm$ 2.1
10 ⁻⁶ M	25.1 $\pm$ 1.1
10 ⁻⁵ M	26.5 $\pm$ 1.4
10 ⁻⁴ M	19.87 $\pm$ 1.5

Test	Two way ANOVA
Probability value	P < 0.001
CD Value	3.4

differences were obtained among the treatments. Thus TIBA could serve as a candidate to reduce the SDA of embryos.

Experiment 4.2.5: Effect of exogenous application of TIBA on the development of embryos in *Syzygium cumini*

The objective of this experiment was to examine if application of TIBA can increase the number of developing embryos in *Syzygium cumini* fruits where normally only one of 30 ovules develops to maturity. Young syzygium fruits were implanted on agar medium. 10 ul of treatment solution was added into the flower cavities and observation on the number of small and medium developing embryos were recorded three and six days after the commencement of treatment. The results of the experiment are given in table 17 (a and b). TIBA had no effect on embryo development when fruits were treated for only 3 days. No significant differences were observed between expected and observed frequencies in the number of embryos developing in small and medium categories between the treatments (table 17a; χ^2 -test, $p=0.915$). However, TIBA had significant effect on embryo development when fruits were treated for 6 days. TIBA treatment increased the number of medium sized embryos developing in the fruit (table 17b). Number of medium sized embryos observed were more than expected and the number of small developing embryos observed were less than expected irrespective of TIBA concentration while in control the number of medium

Table 17 a : Effect of different concentrations of TIBA on the number of medium and small developing embryos within *Syzygium* fruits 3 days after treatment

Embryo size	Control	10^{-5} M TIBA	10^{-4} M TIBA	10^{-3} M TIBA
Small	300 [300.5]	296 [298.5]	299 [300.5]	380 [375.2]
Medium	70 [69.5]	72.0 [69.2]	71.0 [69.5]	82.0 [86.8]

Test Chi square
 Probability value P = 0.915

Note : Values in parenthesis indicate expected frequencies , Embryos from at least 30 fruits were pooled in each treatment and observed for the size differences

Table 17b: Effect of different concentrations of TIBA on the number of medium and small developing embryos within *Syzygium* fruits 6 days after treatment

Embryo size	Control	10^{-5} M TIBA	10^{-4} M TIBA	10^{-3} M TIBA
Small	240 (219.24)	238 (250.66)	263 (271.86)	277 (276.24)
Medium	160 (80.76)	105 (92.34)	109 (100.14)	101 (100.76)

Test Chi square
Probability value P < 0.01

Note : Values in parenthesis indicate expected frequencies. Embryos from at least 30 fruits were pooled in each treatment and observed for the size differences

sized developing embryos were less than expected. These results indicate that application of TIBA may enhance the probability of development of embryos probably by reducing the average SDA of the embryos.

In another experiment, developing *Syzygium* fruits were sprayed with TIBA for 9 days and observations were recorded on the number of big and medium (dominant and sub-dominant) and small developing embryos on 13th day. Results are presented in table 18. TIBA treatment significantly increased the number of big and medium sized developing embryos (table 18, χ^2 -test, $p < 0.01$). The number of dominant embryos observed were eight as against an expected value of six and medium sized embryos observed were 44 as against an expected value of 33.5. The number of small developing embryos decreased in TIBA treatment. 548 small developing embryos were observed as against an expected value of 560.5. This supports the results of the earlier experiment that TIBA might enhance the probability of development of seeds.

b) Evaluating the relationship between the SDA and the extent of embryo abortion within and across species

Earlier studies have shown wide variation in the extent of embryo abortion both within and across taxa. In this study, the relationship between embryo abortion and the respective SDA was examined.

Table 18: Effect of TIBA on the number of dominant, sub dominant and small embryos developing within *Syzygium* fruits

Embryo size	Dominant embryos	Sub dominant embryos	Small embryos
Control	4 [6]	23 [33.5]	573 [560.5]
0.1 nM TIBA	8 [6]	44 [33.5]	548 [560.5]

Test Chi square
Probability value P < 0.01

Values in parenthesis indicate expected frequencies . Embryos from at least 30 fruits were pooled and obsevation were made

Experiment 4.2.6: Relationship between SDA and embryo abortion across 15 species

SDA of embryos of 15 species with varying levels of embryo abortion was estimated. The seed abortion across the 15 species was found to be significantly positively correlated with their respective SDA (fig 15; $r = 0.52$, $p < 0.047$). In other words, species with intrinsically low SDA exhibit low rates of embryo abortion than those with high SDA. This relation emerged despite differences among species in their ovule numbers, seed weights and duration of pod filling.

Experiment 4.2.7: Relationship between SDA and embryo abortion in *Derris indica*

Pods of *Derris indica* predominantly contain only two ovules per ovary. Following a week after fertilization, however one of the two embryos gain dominance and the other remain small and eventually aborts. Trees differ markedly in the extent to which their pods exhibit such asymmetrical development of the two embryos within the pod. Such asymmetry within a given tree was estimated and referred as asymmetry ratio. Table 19 presents the data on asymmetry ratio for a set of randomly selected trees of *Derris indica*. Trees differ significantly with respect to the percentage of pods exhibiting asymmetry in the embryo growth. For instance, the asymmetry ratio ranged from 67 to 93 per cent with a mean of 81 per cent. There was a significant positive correlation between asymmetry ratio

Fig. 15: Relation between sink drawing ability of ovules and percent seed abortion. (Cr-Crotalaria sp., Ca-Cajanus cajan, Hi-Hibiscus sabdarifa, Ar-Arigira sp., Da-Dalbergia sissoo, Pe-Peltophorum ferrugineum, Ph-Phaseolus vulgaris, Csp-Cassia spectabilis, Vi-Vigna unguiculata, Bu-Butea monosperma, Le-Leucaena leucocephala, De-Derris indica, Sy-Syzygium cumini, Gl1-Glyricidia spl., Gl2-Glyricidia spl.).

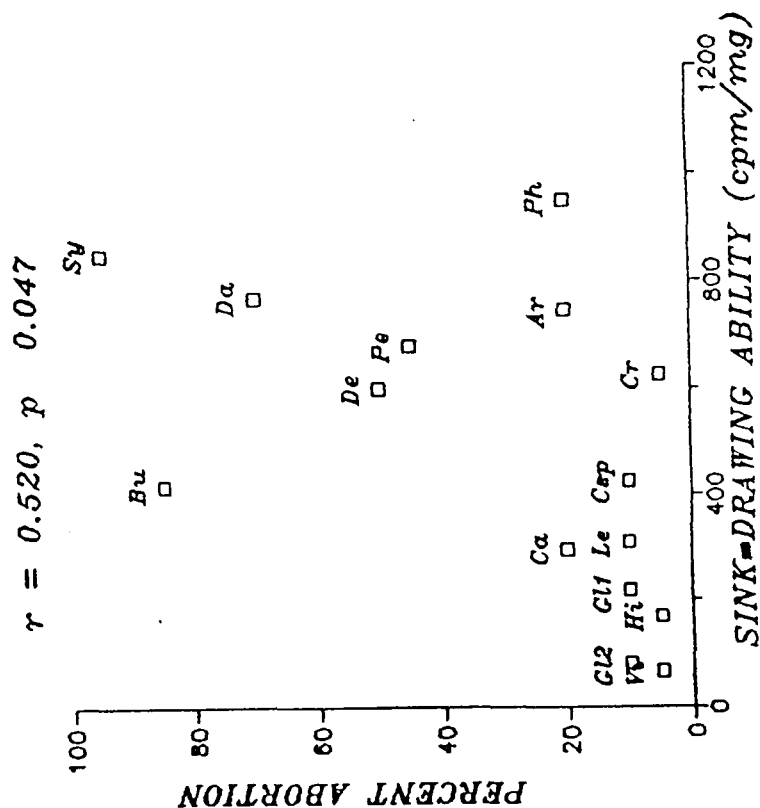


Table 19: Asymmetrical development of seeds with in fruits in *Derris indica* trees

Tree No	no. of pods [C]	No. of pods bearing equal sized embryos [A]	No. of pods bearing unequal sized embryos [B]	Asymmetry ratio $\frac{[B \times 100]}{C}$
1	30	10	20	67
2	30	7	23	77
3	30	8	22	72.5
4	30	5	27	90
5	30	4	26	86
6	30	5	25	82.5
7	30	9	21	70
8	30	7	23	77
9	30	6	24	79.2
10	30	7	23	77
11	30	5	25	82.5
12	30	2	28	93

Test
Probability value
CD Value

Two way ANOVA
P<0.02
5.9

and SDA (fig. 16; $r = 0.50$, $p < 0.010$) supporting the prediction that individuals with high SDA exhibit enhanced asymmetry in the development of seeds within fruits.

Experiment 4.2.8: Relationship between SDA of embryos and the extent of embryo abortion in a few accessions of *Cajanus cajan*

Genotypes of red gram have been reported to exhibit differences in the extent of embryo abortion. A few genotypes that exhibited very low embryo abortion and a few with high abortion rates were selected. SDA of embryos from these genotypes was determined and its correlation with their respective embryo abortion assessed. In two sets of experiments, ACC. No. 7896 and 10903 which had relatively high percentage of embryo abortion (36.5 and 45 % respectively) showed a high SDA while ACC. No. 7332 which had only 16 % abortion had the least SDA. In both experiments the genotypes differed significantly in their SDA (table 20; two way ANOVA, $p < 0.001$).

Genotypes were grouped into high and low abortion groups and examined if they differ with respect to the mean SDA of embryos. Results revealed the existence of significant differences between the two groups for their sink drawing ability (table 21); low abortion group had significantly low SDA than the high abortion group.

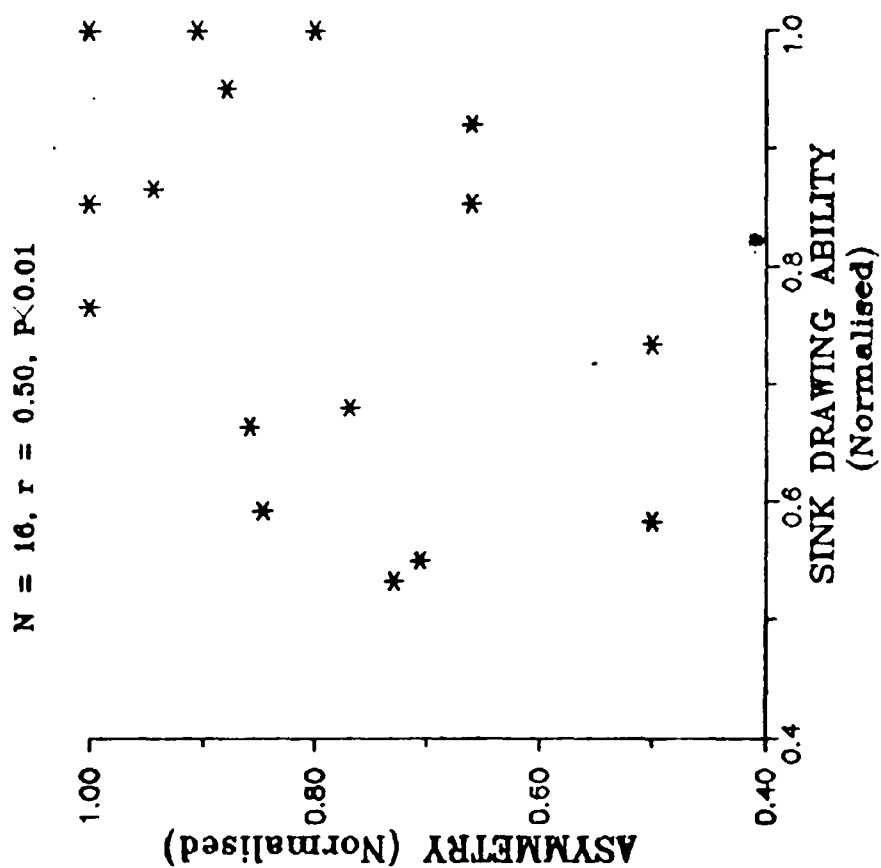


Fig. 16: Relationship between sink drawing ability and asymmetrical development of seeds in *Derris indica*

Table 20: Embryo abortion percentage and mean sink drawing ability of embryos in four redgram accessions

Acc. No	Abortion percentage	Mean sink drawing ability [cpm/mg]	
		Expt I	Expt II
7896	36.5	2433.3 $\pm$ 481.3	2233.0 $\pm$ 411.3
10903	45.0	22647 $\pm$ 484.4	2254.3 $\pm$ 315.4
11145	18.5	2193.0 $\pm$ 581.3	1540.7 $\pm$ 298.8
7332	16.0	1735.0 $\pm$ 394.3	831.3 $\pm$ 249.5

Test

Two way ANOVA

Two way ANOVA

Probability value

P<0.001

P<0.001

CD Value

58.3

82.1

Note : Each value is a mean of three replicates and each replicate had at least 20 embryos

Table 21: Relationship between embryo abortion and mean sink drawing ability of embryos in redgram.

Acc No	percent embryo abortion	mean sink drawing ability (cpm/mg)
Low abortion group (Acc No 11145 & 7332)	17.3	1186.6 $\pm$ 424.9
High abortion group (Acc No. 7896 & 10903)	39.9	2243.0 $\pm$ 7.7

Test
Probability value

t test
P<0.05

Experiment 4.2.9: Surveying literature on the effect of exogenous application of growth regulators on seed abortion

Literature survey was conducted to examine if there exists any relation between the exogenous application of growth regulators and hence increased SDA of embryos and the extent of seed abortion. Literature survey suggests that 83 per cent of trials involving various treatments such as varieties/trials/concentrations/time of/number of sprays of growth regulators that are generally known to increase the SDA increased embryo/seed abortion in the treated compared to control fruits (Table 22a). Further, among those studies for which the treatments differed significantly from control (n=46), 96 % of the trials showed reduced seed set while only 4 % showed increased seed set (Table 22b).

Literature survey regarding the effect of TIBA that reduces the SDA revealed that its application enhanced seed set in plants. Of 8 studies, 6 reported increased seed yield and 2 reported no change following TIBA application (table 22c).

In summary, the results revealed that use of growth regulators which enhance SDA results in reduced seed set and those which decrease SDA results in increased seed set supporting the predictions of self organization model of seed abortion.

Table 22a: References showing the effect of growth regulator sprays on seed set in different crops.

Reference	Crop	Growth regulator	No. of trials/Expts/Conc. / treatments showing			
			reduced seed set	Pe	increased seed set	Pe
Luckwill (1953)	Apple	NAA	3	NT	1	NT
Manivel & Sunderraj (1968)	Grapes	GA	6	NT		
Sunderrajan et. al (1968)	Custard apple	GA	6	NT		
		NAA	6	NT		
Schneider & Lasheen (1973)	Apple	NAA	1	*	1	*
Unrath (1974)	Apple	GA 4-7 + BA	2	NS		
			1	*		
Feinstein et. al (1975)	Mandarin	GA	3	*	1	NS
		NAA	4	*		
		GA + NAA	8	*		
Lewin & Monselise (1976)	Mandarin	NAA	3	NS		
			7	*		
Green & Lord (1985)	Apple	BA + GA 4+7	4	NS		
			1	*		
		BA+GA+NAA	1	NS		
			2	*		
		NAA	1	NS		
			2	*		
Rangaswamy (1986)	Okra	IAA+GA+BA	5	NT	4	NT
		IAA	2	NT	4	NT
Singh et al (1986)	Grape	Auxin	3	NS		
Green (1989)	Apple	GA 4+7	4	**		
Green et al (1989)	Apple	BA	5	*	4	NS
			1	NS		
Mamatha (1989)	<i>Gloriosa superba</i>	NAA+GA+BA	1	NT	1	NT
Prive et al (1989)	Apple	BA			1	NS
		GA 4+7	0		0	
Green & Autio (1990)	Apple	BA	2	**		
Bound et al (1991)	Apple	NAA	1	*		
Fellman et al (1991)	Grapes	GA3	3	*		
Total			88		18	

e: Significance level ; *: Significant at 5% ; **: Significant at 1% ;
 0 : No change; NS: Not significant ; NT: Not tested.

Table 22 b: Contingency table of the number of studies for which the test of significance is available

Response	Reduced seed set	Increased seed set
Result of the test		
Significant (at 5%)	44 (96)	2 (4)
Non significant	15 (75)	6 (25)

Note: Values in each cell refer to the number of trials/experiments/treatments/ concentrations tried by various workers listed in Table 22a. Values in parenthesis indicate the percentage trials across each row

Table 22 c: Effect of TIBA on seed set (Literature survey)

System	Concentration	Response	Reference
Soybean	20,40,60 g/ha	increase in seed yield	Bauer et al (1969)
Soybean	56 g/ha	increase in seed yield in 4 cultivars	Tauner and Ahmed, (1974)
Sunflower	120 ppm 240 ppm	increase in seed yield	Prasad et al. (1977)
Soybean	150 or 300 ppm	no change in seed yield	Abutiate, (1979)
Soybean	15 ppm	increase in seed yield	Phanophat and Phronsattha (1984)
Cowpea		marginal increase in seed yield	Jallow, (1985)
Mungbean	25 ppm	38% increase in seed yield	Reddy and Singh (1985)
Soybean	50 ppm	increase in seed yield	Ravichandran and Ramaswami, (1991)

Note: The studies have reported data on increased seed yield but not seed set. It is likely that increase in seed yield in these cases may be due to increase in fruit/pod set and also seed set.

DISCUSSION

V. DISCUSSION

5.1.1 Mechanism of embryo abortion in the animal dispersed species, *Syzygium cumini*

In *Syzygium cumini*, invariably only one out of the 30 ovules in an ovary develops to maturity. The pattern of abortion is consistent among trees and across seasons. Arathi (1990) showed that lack of sufficient pollen grains on the stigma/lack of fertilization of ovules do not completely explain the single seededness of the fruits. Based on earlier arguments of Ganeshaiah and Uma Shaanker (1988) and Uma Shaanker et. al. (1988) she hypothesised that single seededness is probably a consequence of intra-fruit rivalry among the developing seeds to be the lone survivor and thus to enhance their individual dispersal advantage. Examining the mechanism underlying such rivalry she showed that aqueous extracts of dominant embryos (destined to mature into seeds) cause the abortion of the subject embryos while extracts of control tissues such as fruit wall (maternal tissue) and aborted embryos did not. She proposed that single seededness in *Syzygium cumini* is probably a consequence of a chemically mediated killing, wherein the dominant among the embryos produce a chemical which diffuses out and kills the remaining embryos. This section extends the above hypothesis and attempts to address the nature of the chemical involved in the abortion.

Studies by Arathi (1990) on the effect of extracts of dominant embryos on the subject embryos (test embryos) were based on visual scoring for browning and sclerotization of the ovules. In this study a new assay system was developed based on the inhibition in the uptake of labeled sucrose by subject embryos. Extracts of dominant embryos and other control tissues were evaluated based on the extent to which they inhibited the uptake of labeled sucrose. The assumption underlying the assay is that either directly or indirectly the chemicals contained in the extract of dominant embryos inhibit the mobilization (uptake) of resources by the subject embryos leading to their starvation and abortion. In general, the order of inhibition in the labeled sucrose uptake by subject embryos was dominant embryos > aborted embryos > fruit coat tissue > control. Interestingly, the extract of unfertilized ovules caused less inhibition in the uptake of labeled sucrose by the subject embryos, indicating that the factors responsible for inducing abortion are triggered following fertilization. This inhibition assay response corresponded well with that obtained based on visual scoring from the present study as well as that by Arathi (1990). Because of its more precise nature and ease of quantification, experiments in this study employed largely the inhibition assay only.

The hypothesis that abortion is due to chemically mediated killing of the sub-ordinate embryos by the

dominant embryo requires the production of a chemical by the dominant embryos that is easily diffusible across the embryos within the developing fruits. Several earlier workers have suggested that the abortion of young developing fruits in an inflorescence by early formed dominant fruits might occur through the active diffusion of chemicals by the latter (Cornforth et. al., 1966; Tamas et. al., 1979; Van Steveninck, 1959). In the present study, diffusates from various tissues were compared for their effect on the uptake of labeled sucrose by the subject embryos. Diffusate from dominant embryos resulted in significantly higher inhibition in the uptake of labeled sucrose compared to control tissues (maternal tissues such as fruit wall and others such as aborted embryos). These results suggest the possibility of the production of certain diffusible chemicals by the dominant embryos. Because of its rapid diffusibility the chemical may be a low molecular weight compound. Extracts of dominant embryos digested with trypsin or subject to heat hydrolysis didnot differ in the extent of abortion caused compared to extracts not digested with trypsin or not heat hydrolysed. Further, the diffusates of dominant embryos that were either heat hydrolysed or digested with trypsin also yielded similar extent of inhibition in the uptake of labeled sucrose by the subject embryos as those of control diffusates. This result suggests that the chemicals contained in the extracts and diffusates responsible in

causing the inhibition in the uptake of labeled sucrose might be heat stable and non-proteinaceous.

5.1.2 Chemically mediated abortion through the production of indole compounds.

Of late, two hypotheses based on the production of auxins have been proposed to explain the monocarpic senescence of vegetative organs and the differential development and abortion of sinks in plants.

Engvild (1987, 1989) proposed that senescence of older leaves during fruiting in monocarpic plants may be initiated by the production and diffusion of certain chemicals by the developing sinks. This process was argued to facilitate the mobilization of resources from the senescent tissues to the sinks. This hypothesis gained support by the discovery of the hormone, 3-chloro-indoleacetic acid in developing pea seeds. At very low concentrations, the hormone is shown to induce the abortion of tissues to which it is applied. This hormone is now referred to as 'death hormone'.

Bangerth (1989) proposed the theory of primigenic dominance to explain the development of dominance hierarchy among fruits and thence abortion of fruits in an inflorescence. According to this hypothesis, transport of diffusible auxin basipetally by the first-formed fruits prevent the diffusion of auxin from the remaining later-formed fruits. Such inhibition in the movement of

diffusible auxins from the later-formed fruits have two consequences;

- 1) Poor differentiation of the sieve elements connecting these fruits resulting, in poor nourishment and in extreme cases, the death of the fruits (Jacobs and Gilbert, 1983).
- 2) Build-up of endogenous auxin levels in the fruits which indirectly may lead to arrest of growth and death, of the fruit, through production of ethylene (Burg and Burg, 1968; Russel and Thiman, 1988).

While the first hypothesis suggests an active production and mobilization of indole compound (3-chloro indoleacetic acid) into the developing seeds for abortion to occur, the second merely requires inhibition of the movement of auxin basipetally from developing sinks for their abortion relative to those that develop. In either case, it is intriguing, that differential development of sinks can arise through the production of indole compounds.

An attempt was made in this study to examine if the inhibition of uptake of labeled sucrose by subject embryos by the diffusates of dominant embryos is due to indole compounds. The following results suggest the possibility of involvement of indole compounds in the inhibition.

- a) The absorption spectra of the diffusate from dominant embryos coincide with that of indole compounds such as IAA and tryptophan.

- b) Thin layer chromatography (TLC) of the diffusate from dominant embryo revealed the presence of indole compounds including tryptophan and indoleacetic acid. An unidentified highly diffusible compound staining positive for indole was also recovered in the diffusate.
- c) Organic solvent partitioning especially with butanol, in which indole compounds are highly soluble, also indicated the possibility of the presence of indole compounds in the diffusate.
- d) HPLC analysis of the diffusate from dominant embryos indicated the presence of a single compound with a peak retention time of 3.55 minutes. While this was different from the peak retention time of 4.7 minutes exhibited by standard IAA, there is considerable overlap in their retention times suggesting the possibility of the presence of indole or indole derivatives in the diffusate.
- e) Indirect ELISA confirmed the presence of indole compounds in the diffusates from dominant embryos. Dominant embryos had higher amount of indole compounds at all stages of development per mg dry weight and highest amount at the stage at which dominant embryos cause the abortion of the sub-ordinate embryos.

The above results indicate the possible presence of indole compounds (or their derivatives) in the diffusates. However a test of whether these compounds are responsible

in abortion requires the demonstration that they cause the inhibition in the uptake of labeled sucrose by the subject embryos. HPLC fractions of the diffusates from dominant embryos collected at different retention time intervals revealed that those corresponding to the peak retention time caused the maximum percentage inhibition in the uptake of labeled sucrose. While the exact nature of the indole compound is not yet known and needs further study, results from the present study as well as those by others (Ganeshaiah and Uma Shaanker, 1988; Arathi, 1990; Mohan Raju, 1993) further indicate the possibility of the involvement of auxin or indole compounds in the abortion.

In the wind dispersed species, *Dalbergia sissoo*, of the 4 ovules in an ovary, predominantly only one develops to maturity (Ganeshaiah and Uma Shaanker, 1988; Mohan Raju 1993). Using a similar assay system, they showed that the extracts and diffusates from the dominant stigmatic embryo significantly aborted, and inhibited the uptake of labeled sucrose by, subject embryos. Analysing the chemical nature of the extracts and diffusates, Mohan Raju (1993) found that the chemical is heat stable and non-proteinaceous. HPLC analysis of the diffusate from dominant embryos revealed that the chemical has a peak retention time of 3.4 min with a significant overlap with that of IAA. He concluded that the chemical may be an indole or its derivative. In fact, involvement of diffusible IAA in the dominance phenomena in fruits of

apple and tomato has been shown by Gruber and Bangerth (1990).

Application of triiodo benzoic acid (TIBA), an auxin transport inhibitor, was found to significantly reduce the differential development of embryos and reduce seed abortion in *Syzygium cumini*. Developing fruits treated with TIBA had a more equitable development of embryos. These results are compatible with the predictions of primigenic dominance theory (Bangerth, 1989). By inhibiting the differential flow of auxin basipetally from developing sinks, a more equitable development of sinks and reduced abortion is expected. Increase in seed yield and seed set in several systems (table 22c) subsequent to application of TIBA may constitute further evidence in this regard.

Yet another evidence suggesting the involvement of auxins (or indole compounds) in abortion comes from the use of ethylene action inhibitors. Arathi (1990) showed that the extracts of dominant embryos pretreated with an ethylene action inhibitor, Silver thiosulphate (STS), significantly reduced the extent of abortion of subject embryos compared to that by untreated extract. She hypothesised that the chemical contained in the extract either directly or indirectly results in the production of ethylene leading to the abortion of embryos. These results are also supported from field experiments wherein

inflorescence of *Syzygium* sprayed with STS showed delayed abortion and in few cases resulted in two seeded fruits.

Kondo (1990) reported that application of ethephon in apples increased seed abortion while application of amino vinyl glycine (AVG), an inhibitor of ethylene action, decreased the rate of seed abortion. In *Dalbergia sissoo*, subject embryos pretreated with STS were affected much less in their ability to take up labeled sucrose in the presence of extract of dominant embryos compared to subject embryos not treated with STS (Mohan Raju, 1993).

It is well known in the literature that high levels of endogenous auxins in tissues result in the production of ethylene (Burg and Burg, 1968; Russel and Thiman, 1988). In this context, it is likely that the abortion induced by the extract/diffusate of dominant embryos is because of a) either high levels of IAA or certain indole derivatives in them which leads to the production of ethylene in subject embryos or b) because of an inhibition in the basipetal movement of auxin from the subject ovules due to primigenic dominance, auxin or indole derivatives accumulate in the subject embryos leading to once again the production of ethylene. While the latter might not operate in the *in vitro* assay system it is likely to contribute to the abortion of embryos *in situ*.

5.1.3 Conclusions and implications

The results of the present study suggest that the differential development of seeds within fruits often resulting in their abortion may be mediated by production and diffusion of indole compounds by the most dominant embryos within the fruit (fig. 17). Dominance might arise due to temporal differences in fertilization of ovules.

Though the exact nature of chemical is yet to be ascertained, the existing data indicate that it could be an indole based compound, probably an auxin. As discussed earlier, a number of experiments both from this study and else where demonstrate the presence of these compounds in the extract/diffusate from the dominant embryos as well as from other tissues. Clearly the compound is not specific only to the embryos. It however should be noticed that on per unit weight basis, it is nearly three fold more in the dominant embryos compared to other tissues. Abortion mediated by the indole compounds as discussed here combines the roles of maintaining dominance as well as leading to subordination among the embryos. For instance, among a set of simultaneously developing embryos because of the temporal differences in fertilization, one or more embryos develop a head start and become dominant. These produce growth hormones such as IAA which facilitate mobilization of resources into them. However at the same time, the later fertilized embryos both because of the

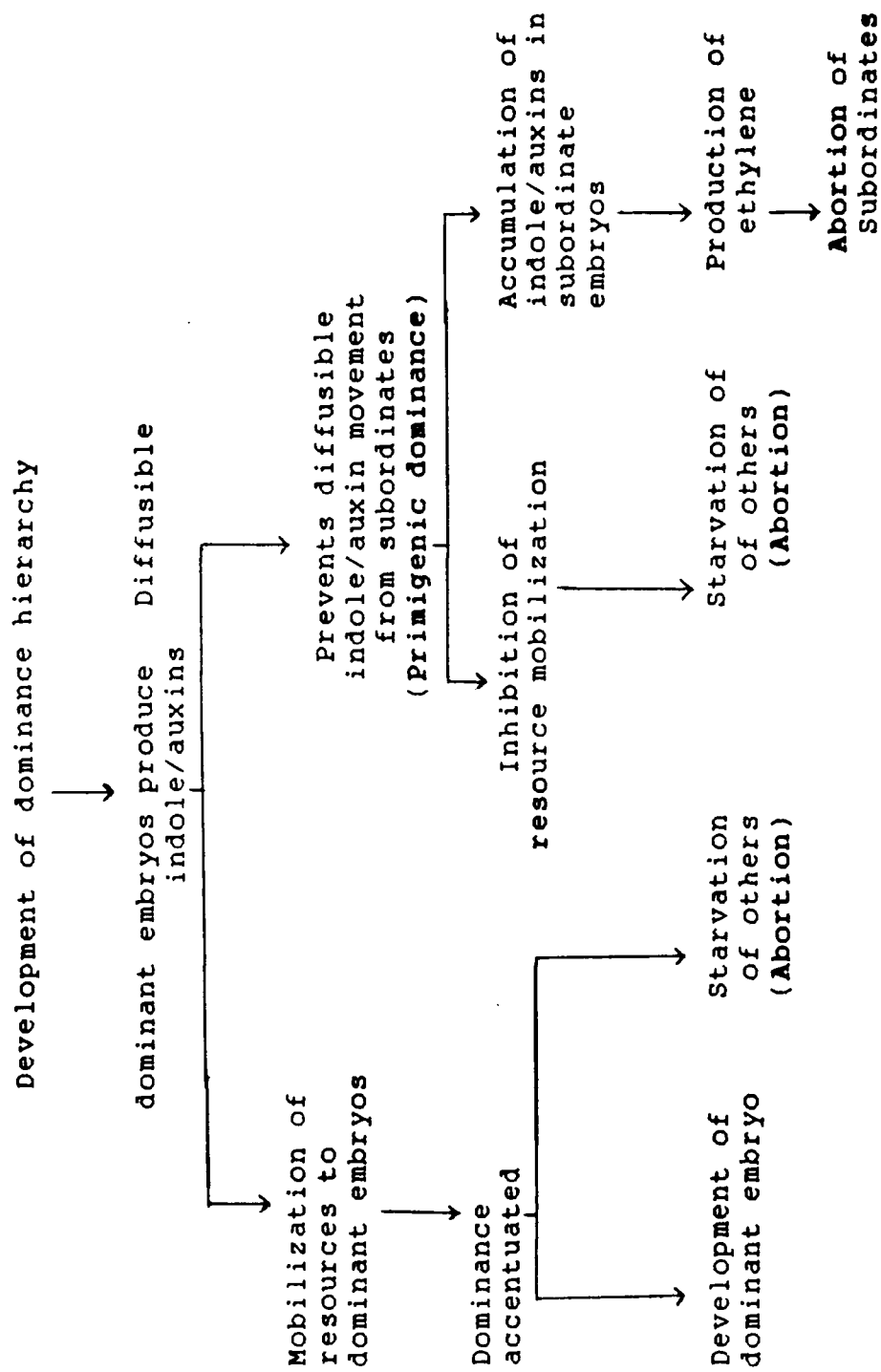


Fig. 17: Schematic representation of the possible mechanism of embryo abortion in *Syzygium cumini*

competing, early formed dominant sinks, as well as the inhibition of the diffusible auxin movement due to primigenic dominance, are poorly developed and are often aborted. Thus it is clear that both development and death of embryos could be brought about by a single compound like auxins. Evidence that the abortion may result from the production of a 'death hormone', 3-chloro-indole acetic acid by the dominant embryo is not forthcoming from these studies though it may also constitute a likely candidate. The mechanism appears to be similar in at least two systems examined namely in the wind dispersed tree *Dalbergia sissoo* (Ganeshaiah and Uma Shaanker, 1988; Mohan Raju, 1993) and in the animal dispersed tree *Syzygium cumini* (Arathi, 1990 and in the present study). In both the species, production of fewer seeds impart a dispersal advantage to the fruits and thence to the surviving seeds within fruits because of decreased wing loading and increased pulp to seed weight ratios respectively. It would be interesting to examine the possibility of such a mechanism in several other systems which exhibit a consistently high seed abortion such as in species where the entire fruit is dispersed as an unit either through wind, water or animal.

5.2 Test of predictions of the theory of Self organization

Existing hypothesis such as chemically mediated and resource limitation hypotheses explaining the differential development of seeds within a fruit, assume the existence

of an initial hierarchy among the developing ovules but do not explain the process by which such hierarchy is achieved among the seeds of a fruit. In some species, the hierarchy might be built up from intrinsic factors such as position of ovules within a fruit (Harris, 1915; Rocha and Stephenson, 1990; Radha, 1990 and Radha *et. al.*, 1993a). For example, in legumes where ovules are arranged linearly along the ovary, those near the stigma are likely to gain a head-start as they might be fertilized before other ovules while those at the base may derive an advantage from being proximal to the resource supply point (Horovitz *et. al.*, 1976; Bawa and Buckley, 1989; Radha, 1990; Radha *et. al.*, 1993a). But these resource and fertilization gradients leading to a dominance hierarchy are not found in all species; in species belonging to families such as Solanaceae, Myrtaceae etc., ovules are placed radially around the stigma (style) and resource supply point (pedicel). In these species, fertilization and resource gradients cannot contribute to the generation of initial hierarchies among ovules and thus cannot explain the differential development and abortion of seeds.

Ganeshaiah and Uma Shaanker (1992 and 1994) and Uma Shaanker *et. al.* (1995) proposed a simple mechanism that can generate such a hierarchy leading to seed abortion without requiring any gradients or limitations of resource and pollen or chemical mediation of abortion. Their model suggests a self organised development of seeds within

fruits involving an autocatalytic feedback process of resource flow into the developing seeds. Their model assumes a simple rule that the probability of any sink getting resource molecule is a function of the SDA of each unit of the tissue and the amount of resource molecule already moved to that sink. Accordingly, any resource molecule moving into a sink, autocatalytically increases the probability of the latter getting further resources. Thus, starting from a set of identical ovules all with equal SDA, they showed that the differential development of sinks and thence their abortion can occur purely by a) a random drift of resource resulting in initial asymmetry among the developing sinks and b) amplification of this initial asymmetry into a dominance hierarchy among the developing sinks due to an autocatalytic feedback process. In other words, their model implies that the history of an ovule influences its fate. Ovules that are favoured randomly during initial stages are further favoured in getting resources. The extent to which the random drift of resources and its feedback leads to dominance is a function of the SDA of the tissue. The SDA is defined as the ability of each unit weight of the tissue to draw resources. A major prediction emerging from their model is that species or individuals with high SDA would have a higher proportion of their ovules aborted.

In this study an attempt has been made to provide a test of this prediction by

- a) manipulating the SDA of embryos in a few systems and examining its effect on embryo abortion,
- b) evaluating the relationship between the SDA and per cent embryo abortion within and across species and
- c) surveying literature on the effect of exogenous application of growth regulators on seed abortion

5.2.1 Manipulating the SDA of embryos in a few systems and examining its effect on embryo abortion

5.2.1.1 Effects of increasing SDA on seed abortion

Application of several growth regulators such as NAA, GA, BA etc. to developing sinks is known to increase the SDA (Goodwin, et. al., 1978; Lis and Autozewski, 1982; Ho, 1988). Accordingly, it may be predicted that application of these growth regulators to developing sinks should result in higher rates of seed abortion compared to sinks without this treatment. Indeed in all the four species examined, namely *Vigna unguiculata*, *Phaseolus vulgaris*, *Crotalaria medicaginea* and *Solanum seaforthianum* the per cent seed abortion increased with growth regulators concentration. Further, in *Phaseolus vulgaris*, application of growth regulators also increased the variance for seed weight within fruits, i.e., increased SDA induced by growth regulator application resulted in an enhanced asymmetry in development of seeds. The positive association between SDA and rates of abortion of ovules is difficult to explain as a consequence of increasing

competition for resources since resource status of the fruits is not altered with the treatments. The relationship can however be explained assuming the development of an intense dominance hierarchy among developing ovules at high levels of SDA. In other words, though all the ovules are equally capable of drawing resources, under high SDA (due to the application of growth regulators), the random drift in the resource flow to certain ovules offers a greater dominance advantage to them leading to their complete development and suppression of the others.

The self organization model assumes that there are no innate differences among the developing ovules but the fruits of *Phaseolus*, *Vigna* and *Crotalaria* where ovules are arranged linearly in the ovary might have two kinds of gradients. First, those near the pedicel could have an advantage of better resource flow than those at the stigmatic end and second, those at the stigmatic end may have an head-start in establishing themselves as strong sinks as they are probably early to be fertilized (Bawa and Buckley, 1988; Radha, 1990; Radha et. al., 1993). While, the process of self organization might not alter these gradients, the rate at which asymmetry is built up among ovules following the process of self-organization become more stronger and positionally predictable with these gradients. For instance, if the fertilization gradient is strong, self organization model predicts a

greater bias in the development of seeds towards the stigmatic end and abortion at the peduncular end in species with higher than those with lower SDA of embryos. But such positional bias in development of seeds were not recovered in these species, suggesting that the inherent differences among ovules arising due to the fertilization and resource gradients might not be strong.

Finally, to prevent such intrinsic gradients from confounding the results, the prediction of the theory of self organization was tested using *Solanum seaforthianum* where the ovules are arranged radially around the stigma (style) and the resource supply point. In this species also, fruits treated with growth regulators exhibited increased abortion compared to control fruits. In fact, a number of earlier studies on systems with radially arranged ovules have also reported increased abortion when sprayed with growth regulators (table 22a).

Thus the prediction of the self-organisation model that abortion increases with the SDA of ovules appears to hold irrespective of fruit type.

5.2.1.2 Effect of decreasing SDA on seed abortion

As a corollary to the prediction made above, decreasing SDA of developing sinks should result in reduced seed abortion. Results of the present study show that the application of TIBA, an auxin transport inhibitor to the embryos, reduces their SDA. Further, as predicted

its application to developing fruits of *Syzygium* was found to result in significantly greater number of developing embryos compared to fruits not treated with TIBA. Application of TIBA *in situ* on developing fruits resulted in reduced asymmetry among developing embryos.

Several other workers have also reported an increase in seed set consequent to TIBA application (table 22c). However again as in the chemical thinning of fruits, the underlying mechanism has not been clear. The process of self organization offers a rationale on which these results can be explained. More recently, Muller-Robert et. al. (1991) developed an antisense mRNA against sucrose synthase gene which is a key enzyme in the unloading of sucrose into developing seeds. Inhibition of the activity of the enzyme through this approach reduced the SDA of the organs. They showed that potato plants that were transformed containing the antisense mRNA for sucrose synthase had increased number of tubers with a relatively more symmetrical development compared to untransformed plants which had few tubers with a high degree of asymmetry in their development. In other words, as predicted by the self organization model, decreased SDA resulted in decreased seed abortion.

5.2.2 Evaluating the relationship between the SDA and per cent embryo abortion within and across species

The natural rates of seed abortion across 15 species

was found to be significantly correlated to their SDA ($r = 0.52$, $p < 0.047$). This relation emerged despite differences among species in their ovule numbers, seed weights, duration of pod filling etc. Similar relation prevailed between SDA and seed abortion among a set of *Cajanus* genotypes examined. Genotypes that had low SDA exhibited low seed abortion and conversely those with high SDA exhibited high abortion. Such relation was also borne out among individuals of *Derris indica* where the degree of asymmetrical development of sinks within a pod was positively correlated to their SDA. These results further support the predictions of the theory of self organisation that the SDA is the key determinant of the rate of seed abortion in plants and that differences among the genotypes and species in seed abortion would be explained by the differences in the SDA of their ovules. The results of this study show that this prediction is true both within and between taxa. Earlier, Ganeshaiah and Uma Shaanker (1988) showed that the pattern of increase in seed abortion with SDA would not be different for the species that differed in their ovule number per flower and in seed mass. It should however be realised that species-specific rates of seed abortion could also be explained by specific and local explanations. However the emergence of the above relation across taxonomic groups suggests that seed abortion may be regulated by a common mechanism based on the process of self organization, where, by merely

altering the values of a single parameter, SDA, differential rates of seed abortion could be obtained.

5.2.3 Surveying literature on the effect of exogenous application of growth regulators on seed abortion

The major prediction of the model that the extent of seed abortion is proportional to its SDA of ovule appears antithetical to the general notion regarding the regulation of seed development in plants which assumes that enhanced SDA should result in higher seed set. However a review of literature on the effect of application of growth regulators on seed set in fruits over the last 50 years is very revealing. Nearly 96 per cent of the studies/experiments using various growth regulators such as NAA, IAA, GA and BA or various mixtures of these following fertilization, have shown to result in significant decrease in seed set. Many of these studies also demonstrate a concentration dependent decrease in seed set. However very few of these studies provide information on increase in the SDA following application of growth regulators along with the seed abortion rates. For instance, Rangaswamy (1986) showed that application of mixture of growth regulators (IAA, GA and BA) in Okra, increased the ^{14}C assimilate transport into the fruit with a concomittant reduction in seed number per fruit.

It is a common commercial practice to use growth regulators to thin-out fruits and as indicated in table

22a, it has frequently resulted in the reduced seediness of fruits. It is ironical that despite the extensive use of growth regulators, the underlying mechanism has not been understood. Tubner and Murneek (1935) in a paper entitled "Embryo abortion and mechanism of hormone thinning of fruits" state that "factors affecting the embryo and or endosperm abortion are not clearly understood when associated with the use of NAA or similar growth controlling substances". Nearly four decades later, there still appears to be no clear explanation for the underlying mechanism.

More recently, Weinbaum and Simmons (1974) suggested that the abortion of seeds on application of NAA result from the decreased transfer of carbohydrates to the developing seeds or alternatively through the failure to synthesise starch. Ebert and Bangerth (1982), investigating the possible mode of action of three apple thinning agents, speculated that abortion could result from either inhibition in the total diffusible auxin from the fruit or by altering the resource mobilizing ability of fruits in relation to other sinks. However, viewed in the context of the process of self organization, it is clear that "NAA or similar growth controlling substances" might result in the asymmetrical development of seeds within fruits by merely increasing the SDA of ovules in the manner predicted by the process of self-organisation.

Conclusions and implications

In this section it is shown that seed abortion in plants could emerge purely by a simple process of self organised flow of resources into the developing sinks. The process depends upon two simple steps; generation of initial asymmetry among the sinks purely due to random drift in resources and amplification or reinforcement of this initial asymmetry into a dominance hierarchy by an autocatalytic feedback process. In other words, it simply states that the resource history of an ovule determines its fate. The model is sensitive to the SDA of the developing sinks. As defined, the rate of build up of dominance hierarchy increases with the SDA. Thus, keeping other things constant, it predicts that system or individuals with high SDA would exhibit high rates of seed abortion. The model is independent of any active interaction among developing seeds within fruits. It also is independent of the resource status of the plant. However, these two namely, alterations in the resource status and active interactions if present at all in the system, would only aggravate the rate of asymmetry in the development. The results presented in this study have provided evidence in support of the predictions of the theory of self organization. It is important to note that both across and within taxa the prediction is upheld. The model offers a mechanistic basis for the wide range of seed abortion observed in plants based on minimal and

realistic assumptions. Finally the model also provides a rationale for the use of growth regulators to manipulate seed development in plants.

SUMMARY

VI. SUMMARY

Intra-fruit seed abortion in plants is a widespread phenomenon. Though several proximate and ultimate reasons for the consistent patterns of abortion has been proposed by earlier workers, there has been few concerted efforts in understanding the immediate mechanistic basis of seed abortion in plants.

The present study was carried out with two main objectives. 1) To investigate the mechanism of embryo abortion in the animal dispersed species *Syzygium cumini* and 2) to provide a test of prediction of the theory of self organization as applied to the differential development of seeds within fruits.

Mechanism of embryo abortion in the animal dispersed species, *Syzygium cumini*

In the animal dispersed species, *Syzygium cumini*, of the 30 ovules in an ovary only one develops to maturity. Arathi (1990) hypothesised that the abortion of the majority of embryos could be due to the production and diffusion of a chemical killing agent by the dominant embryo. This study extends this hypothesis.

Aqueous extract of dominant embryos caused a significantly greater abortion of test (subject) embryos compared to extract of control tissues such as fruit coat, aborted embryos and unfertilised ovules. A more precise assay, based on the per cent inhibition of uptake of

labeled sucrose by subject embryos also showed that extracts from dominant embryos caused significantly higher inhibition in the uptake of labeled sucrose by subject embryos. Further, it was found that aqueous diffusates from dominant embryos also caused significantly higher inhibition in the uptake of labeled sucrose by subject embryos compared to other control tissues. In general, the order of inhibition in the labeled sucrose uptake by subject embryos was dominant embryos > aborted embryos > fruit coat tissue > control. These results suggest the possibility that abortion of embryos in *Syzygium cumini* could be due to the production of a chemical killing agent by the dominant embryo. The chemical appears to be heat stable and non-proteinaceous. Further, because of its diffusible property, it is likely that it is a low molecular weight compound.

The absorption spectra of the diffusate from dominant embryos coincided with that of indole compounds such as IAA and tryptophan. Thin layer chromatography (TLC) of the diffusate from dominant embryos showed the presence of indole compounds including tryptophan and indoleacetic acid. An unidentified highly diffusible compound staining positive for indole was also recovered in the diffusate.

HPLC analysis of the diffusate from dominant embryos indicated the presence of a single compound with a peak retention time of 3.55 minutes. While this was different

from the peak retention time of 4.7 minutes exhibited by standard IAA, there was considerable overlap in the retention time suggesting the possibility of the presence of indole or indole derivatives in the diffusate.

Indirect ELISA confirmed the presence of indole compounds in the diffusates from dominant embryos. Dominant embryos had higher amount of indole compounds at all stages of development per mg dry weight and highest amount at the stage at which dominant embryos cause the abortion of sub-ordinates.

These results suggest the possibility of indole compounds or their derivatives in the dominant embryos causing inhibition in the uptake of labeled sucrose by subject embryos. HPLC fractions of the diffusate from dominant embryos collected at different retention time intervals revealed that the fraction corresponding to the peak retention time caused the maximum percentage inhibition in the uptake of labeled sucrose suggesting that these compounds may be responsible in the inhibition of the uptake of labeled sucrose by subject embryos. Though the exact nature of the chemical is yet to be ascertained, the existing data indicate that it could be an indole based compound.

Testing the predictions of the theory of self organization

Ganeshaiah and Uma Shaanker (1992 and 1994) developed a model based on the theory of self organization to

explain the differential development of seeds within fruits. Their model suggests a self organized development of seeds within fruits involving an autocatalytic feed back process of resource flow into the developing seeds. The process involves movement of resource units in proportion to the existing resource status of the ovule. Ovules that have received more resource units would be autocatalytically favoured and hence dominate over others at a rate defined by their sink drawing ability (SDA) of the ovules of a species. Such a process leads to varying levels of seed abortion that are independent of resource or pollen limitation and any active interaction among the developing embryos. A major prediction of their model is that species/individuals with high SDA would have a higher proportion of their embryos aborted.

The present study attempts to provide a test of this prediction by evaluating the relationship between SDA and seed abortion. The evidences are drawn by examining the relationship in naturally occurring systems and by manipulating the SDA in a few species. Evidence from the literature is also provided.

Application of mixture of growth regulators containing NAA, GA, BA that increase the SDA, in few systems such as *Vigna unguiculata*, *Phaseolus vulgaris*, *Crotalaria medicaginea* and *Solanum seaforthianum* increased seed abortion rates. Further, in *Phaseolus* the

application of growth regulators also increased the coefficient of variation for seed weight indicating the generation of greater asymmetry in the development of sinks. Such relation can be explained assuming strong dominance hierarchy among developing ovules generated at high levels of SDA due to growth regulator application. Under high SDA, the random drift in the resource flow to certain ovules offers a greater dominance advantage to them leading to their complete development and suppression of the others.

The natural rates of seed abortion across 15 species was found to be strongly positively correlated to their SDA ($r = 0.52$, $p < 0.047$). Similar relation prevailed between SDA and seed abortion among a set of *Cajanus* genotypes examined. Genotypes that had low SDA exhibited low seed abortion and conversely those with high SDA exhibited high abortion. Such relation was also borne-out among individuals of *Derris indica* where it was seen that the degree of asymmetry of sinks within a pod was positively correlated to their SDA. These results indicate that the prediction regarding the positive relationship between SDA and seed abortion is true across taxa as well as within taxa. Emergence of above relation suggests that seed abortion across species may be regulated by a common mechanism based on the process of self organization where by merely altering the SDA, differential rates of seed abortion could be obtained.

Review of literature on the effect of application of growth regulators on seed set in fruits over the last 50 years reveals that nearly 96 % of the studies/experiments using various growth regulators such as NAA, IAA, GA and BA or various mixtures of these following fertilization resulted in significant decrease in seed set. Many of these studies also demonstrate a concentration dependent decrease in seed set.

As a corollary to the above, it is predicted that application of growth regulators that reduce the SDA should reduce the rate of abortion. Application of Triiodo benzoic acid (TIBA), an auxin transport inhibitor to developing embryos of *Syzygium cumini* reduced the sink drawing ability and reduced seed abortion. Its application both *in vitro* and *in vivo* to *Syzygium* fruits resulted in increased number of big and medium sized embryos. Increased seed set consequent to the application of TIBA has also been shown by several workers.

These results provide evidence in support of the prediction of the theory of self organization as applied to differential development of seeds within fruits. The model provides a basis to explain the wide range of seed abortion prevailing in plants based on minimal and realistic assumptions and without any active interaction among the developing sinks (seeds).

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VII. REFERENCES

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