Int. J. Adv. Res. Sci. Technol. Volume 5, Issue 1, 2016, pp.544-552.

IJARST

International Journal of Advanced Research in Science and Technology

journal homepage: www.ijarst.com

ISSN 2319 – 1783 (Print)

ISSN 2320 – 1126 (Online)

Fruiting pattern in Delonix regia (Boj. Ex Hook) Raf. (Family: Caesalpiniaceae).

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ARTICLEINFO

Article history:
Received : 19 Feb. 2016
Accepted : 28 Mar. 2016
Available online : 30 Mar. 2016

Keywords:
Breeding systems,
Fruit set,
Fruiting Flower,
Pod length,
Seed number and weight.

ABSTRACT

The various parameters included in the study are blooming phenology, breeding systems, assessment of temporal depletion and deposition of pollen under flower – visitors activity, natural fruit set, fruiting flower and pod length and seed number, weight at inflorescence level. The trees of Delonixregia bloomed after leaf shedding. It flowers during March – September. The period of blooming extended over 128.5 (R = 97 - 158) days. The flowers per raceme were 11-14. Flowers are bisexual and compatible for geitonogamous and xenogamous pollination. Flowers were compatible for geitono and xeno pollen. In observation for fruit set at inflorescence level by hand-pollination, most of the odd numbered flowers developed into fruit. There were no seed abortions. The number of fruits per inflorescence were being commonly 1 - 2. Mostly the third, fifth and seventh flowers transformed into mature fruits. Hand-pollination of geitono and xeno pollen resulted in fruit set. There is no fruit set through autogamy. The number of fruits in inflorescence was being commonly 2 – 4. Mostly the odd numbered flowers were transformed into fruits. Flower and fruit abortions occurred in Delonixregia. The percentage of flower and fruit drop were more at initial and final seasons. The flower and fruit abortions were discussed in the context of the resource limitation and sexual selection hypothesis.

PAPER-QR CODE

IJARST Volume 5, Issue 1

Citation: *Sujan Chandar.et. al., Fruiting pattern in Delonix regia (Boj. Ex Hook) Raf. (Family: Caesalpiniaceae),Int. J. Adv. Res. Sci. Technol. Volume 5, Issue 1, 2016, pp.544-552.*

Introduction:

There has been a growing concern and commitment for the preservation, improvement and management of biodiversity to ensure the health and stability of the planet earth, and wealth and sustainability of human societies. It is also being increasingly realised that a complete knowledge of sexual and pollinating system is of vital importance in the *in situ* and *ex situ* conservation of plant biodiversity.

The flowering plants display a variety of sexual or breeding systems and each system regulates gene flow in varying degrees. In most studies of tropical tree sexual systems, hermaphroditism predominates, followed by dioecious and monoecious. Self-incompatibility occurs in majority of hermaphrodites. In such flowers, vector-mediated pollination may result in autogamy, geitonogamy and xenogamy. Both autogamy and geitonogamy are genetically equivalent and do not contribute to genetic diversity. In such flowers, it is expected that some mechanism must be there to produce out crossed off springs, self-incompatibility, and other devices such as diochogamy, herkogamy, or temporal dioecism, may promote outcrossing.

The trees are naturally long-lived and accumulate genetic load. Therefore, of necessity, such trees should achieve out crossing and maintain heterozygosity to avoid deleterious effects of genetic load. Tropical trees often have only few of the flowers ending in fruit. The lack of sufficient pollinators is one of the several causes for such low fruit-flower ratios. Spatial variations in environment are more pronounced in the tropics and

therefore it is suggested that, even if the pollination requirements of a species are known on the basis of study carried out at a particular area, the requirements may differ depending on a change in the environment at a different place and thus merits a study. Also it is possible that widespread species may be serviced by different pollinators in different parts of their geographical range.

Delonixregia(Boj. ex Hook.) Raf. (Syn. *Poinciana regia*) of the family Caesalpiniaceae is exotic - grown as an ornamental in India for its large red flowers. It is native to Madagascar. In India it has been successfully used in protecting channels and riverbanks.

Keeping the above important aspects of biological conservation in view, study on sexual system of *Delonixregia* of Caesalpiniaceae was undertaken. The study documents the reproductive successive of *D. regia* based on the flowering behaviour, insect-plant interaction leading to pollination, fruit set and seed formation. The knowledge on the reproductive ecology of *Delonixregia* will help to suggest appropriate strategies for getting high rate of fruit production thus seed production increase.

Methodology:

The present study on fruiting pattern of species *Delonix*Rafin. i.e. *D.regia* (Boj. ex Hook.) Rafin., belonging to family *Caesalpiniaceae* was carried out during 2006 -2009.

For the study of *Delonixregia* present at different places of Visakhapatnam (17º41'N latitude and 82º18'E longitude) and Narasannapeta, Srikakulam district (18º20'N latitude and 83º50'E longitude), were utilized. These areas receive rainfall in the two monsoons – the North-East monsoon (December–February) and the South-West monsoon (June-September). The months of October and November receive the retreating monsoon rains and unpredictable cyclones. Temperature is highest during May, day temperature ranging between 30-40º C and night temperature between 20-28º C. Rainfall ranges between 100-150 cm per annum. In *D. regia* utilized for major part of the study while for breeding experiments the trees present at Narasannapeta were utilized.

A brief description of the general distribution of study sites and economic importance of species is given in the following. *Delonix*Rafin. is a genus of flowering plants in the family *Caesalpiniaceae.* It containsmoderate sized trees native to Madagascar and East Africa. Among the different species of *Delonix*the best known species is*Delonixregia*(Boj. ex Hook.) Rafin. Its common names include gold mohur, peacock flower, royal Poinciana etc. derived from its large flame red flowers. It is now wide spread in most tropical and sub-tropical areas of the world and is exotic in India. The plant mainly valued as a decorative tree, often being planted in avenues and gardens. It can also be planted as live fence posts and as shade tree in dairy forms, Tea plantations and compounds. The large pods as well as the wood are used as fuel. The tree yields gum and the seeds contain gum, that may be useful in textile and food industries. Bark has medicinal properties.

Assessment of Reproductive Behavior:

Mature buds on different plants were tagged and enclosed in paper bags pierced with a needle to maintain equilibrium in weather inside and outside. The bagged flowers were tested for different modes of reproduction. Fifty flowers were used for each of the following tests performed.

1. Mature flower buds were emasculated prior to anthesis and bagged todetect apomixis.
2. (a) The stigma of flower was pollinated with pollen of the same flowerand bagged to observe the manipulated autogamy.

   (b) The flowers were bagged as they were in order to detect spontaneous autogamy.
3. The emasculated flowers were hand –pollinated with pollen of adifferent flower on the same plant and bagged to test geitonogamy.
4. The emasculated flowers were pollinated with the pollen of a differentconspecific plant and bagged to assess the operation of xenogamy.

The bagged flowers were followed for about 10 days to observe fruit set. Then, the percentage of fruit and seed set for each mode of pollinating system were calculated.

Determination of Natural Fruit Set:

The flowers that set fruit under open-pollinations were examined for the percentage of mature fruit and seed.

Assessment of Temporal Depletion and Deposition of Pollen under Flower –Visitors Activity:

The number of pollen per flower was determined at the time of anther dehiscence. As soon as the foraging activity of the flower visitor began, a fixed number of foraged flowers were removed at 4 h intervals, and the pollen remained in the collected anthers was counted. Subtracting this number from the previous number gave the number of pollen removed or depleted during the said interval. At the same time, stigmas were collected, pressed between two microscope slides, stained with cotton-blue, and examined for pollen under light microscope. The number of pollen deposited on the stigmas was then recorded. Ten such counts were made separately for pollen depletion from anther and pollen deposition on stigmas, from the counts thus obtained, the mean number of pollen grains depleted and that deposited at 4 h interval was assessed.

Pod Length and Seed Number:

The matured and dried pods of the previous year were collected, and then length was determined. Keeping them in water for few days and drying facilitated their dehiscence. The number of seeds were counted, and weighted. Seed abortion was also noted by examining the dehisced pod.

Results:

Blooming Phenology:

Delonixregia tree is deciduous. It bloomed after shedding foliage. Leaf-fall occurred in general during the last week of February and the second week of March. In the following month, leaf initiation and simultaneous flowering began. In some cases leaf shedding and flowering were not uniform in all the branches of a tree. Thus in these cases some branches were in foliage and some were in flower. In general blooming began during the last week of March and first week of April and progressed to a peak between the third week of May and second week of July and began to terminate during the third week of July and first week of September. Thus on the whole, the blooming season was compact with recognizable initial, peak and final phases. The length of flowering period varied from tree to tree (Table-1). On average the flowering period of 25 trees studied at different sites was 128.5 days and the range was 97 – 158 days.

Breeding Systems:

There was no fruit formation through apomixis. Also no fruit was seen with flowers prevented from insect visitors thereby indicating that automatic self-pollination does not occur. Hand-pollination gave some fruit formation in autogamy tests, but the fruit did not stand up to maturity.

Geitonogamous hand-pollinations gave enough fruit formation in the initial (56%), peak (64%) and final (44%) phases of flowering seasons but with more fruit during the peak phase. Xenogamous pollinations were not as effective as geitonogamous pollination. The fruit set was considerably less: initial 34%, peak 26%, and final 22% (Table-2).

Natural fruit set in 610 flowers distributed in 50 inflorescences on different trees was 21.14%. This is rather close to the percentage of fruit set from xenogamy and very less compared to the fruit set in geitonogamy.

The Fruiting Flower:

The natural fruit set observations indicated that each and every flower in an inflorescence did not end in fruit. Out of the 100 inflorescences observed for the number of fruits they produced, it was found that six inflorescences (12%) had no fruit. Fifteen inflorescences (30%) each had a single fruit, 23 inflorescences (46%) each two fruits, four inflorescences (8%) each three fruits, and two inflorescences (4%) each four fruits. The relative frequency of the flowers in sequence forming fruit is as follows: 1st flower zero, 2nd flower 8, 3rd flower 58, 4th flower 6, 5th flower 36, 6th flower 12, 7th flower 26, 8th flower 4, 9th flower 10, 10th flower zero, and 11th flower two only (Fig-1). Thus the frequency of 3rd flower yielding fruit was maximum (58%), followed by the 5th flower (36%), 7th flower (26%), 6th flower (12%), 9th flower (10%) etc.

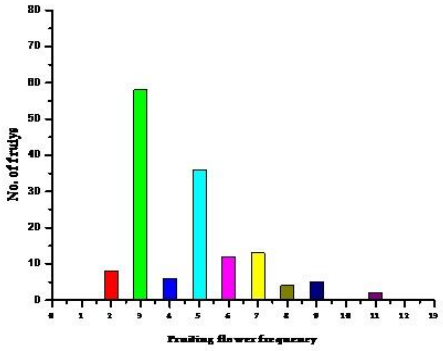

Figure: 1. Inflorescence fruiting frequency (n= 100), Fruit set under natural observation in *Delonixregia*

The geitonogamous and xenogamous hand pollinations revealed that odd number flowers set as fruits than even numbers. The hand pollinations of geitonogamous results revealed that 3rd number flower sets as fruit as highest (37.2%) followed by 5th flower (25%), 7th flower (18.75%), 4th& 9th flower for each (2.08%) (Fig-2). Xenogamous hand pollinations also showed the same pattern, but fruit set was more than geitonogamous hand pollination , 3rd & 5th flower set as fruit for each (33.01%), followed by 7th flower (30.18%), 9th flower (25%), 11th flower (17.92%) and 13th flower as (4.71%) (Fig-3). Geitonogamous hand pollination 118 fruits were formed out of 100 inflorescences. In that 3rd flower set as fruit for 44 times followed by 5th flower 36 times, 7th flower 27 times, 9 the flower 3 times, 2nd , 4th and 6th flower for each 2 time. Xenogamous pollination, 212 fruits were formed out of 100 inflorescences . In that the occurrence 3rd and 5th flower set as fruit for each 70 times followed by 7th flower 64 times, 9th flower 53 times, 11th flower 38 times,13th flower 10 times, 6th and8th flower each for 3 times, and 4th flower set as one fruit.

Seeds in a Fruit (Plate- I):

Depending on the pod length, the number of seeds contained in a pod varied. The length of 125 pods averaged 40.56 cm, and the average number of seeds per pod approximated to 20, and the weight of seed averaged 7.42 grams (Table-3). Each of the 125 pods was found to be full with well-developed seeds and there was no seed abortion.

Table: 1. Flowering phenology of *Delonixregia*

Study site	No. of plants	Initial	Peak	Final	Total flowering days
Andhra University – Applied Physics Department	2	09 May to 20 May	21 May – 14 July	15 July – 20 August	103
Lawsons Bay Colony Road	4	28 March to 11 May	12 May – 19 July	20 July – 18 August	140
Andhra University – Instrumentation Department	2	08 May to 20 May	21 May – 16 July	17 July – 04 Sept.	116
Andhra University – Out Gate Opposite Road	3	25 April to 18 May	19 May – 20 July	21 July – 7 Sept.	135
Dabagarden Area	3	10 May to 22 May	23 May – 17 July	18 July – 28 August	110
Old C.B.I.	2	18 April – 16 May	17 May – 22 July	23 July – 07 Sept.	142
Andhra University – Post Office Road	3	05 April – 18 May	19 May – 20 July	21 July – 29 August	146
A.U. GMC R.S. Hostel Surroundings	3	12 May – 27 May	28 May – 12 July	13 July – 16 August	97
Municipal Guest House	1	31 March – 16 May	17 May – 15 July	16 July – 06 Sept.	158
East Point Colony Road	2	17 April – 14 May	15 May – 18 July	19 July – 04 Sept.	141

Flowering period in days: Range 97 – 158; Mean ($\overline{X}$) = 128.5 ± 19.57.

Table: 2. Fruit Abortion and Maturation at Different Phases of Flowering in *Delonixregia*

GEITONOGAMY

Flowering phase	No. of flowers pollinated	No. of flowers set fruit	Fruit set (%)	No. of fruits aborted	Fruit abortion %	Fruit Maturation	Fruit Maturation %
Initial	50	28	56%	10	35.7%	18	64.2%
Peak	50	32	64%	13	40.6%	19	59.3%
Final	50	22	44%	12	54.5%	10	45.4%

XENOGAMY

Flowering phase	No. of flowers pollinated	No. of flowers set fruit	Fruit set (%)	No. of fruit aborted	Fruit abortion %	Fruit Maturation	Fruit Maturation %
Initial	50	17	34%	7	41.1%	10	20%
Peak	50	13	26%	5	38%	8	61.5%
Final	50	11	22%	6	54.5%	5	45.45%

AUTOGAMY

Flowering phase	No. of flowers pollinated	No. of flowers set fruit	Fruit set (%)	No. of fruit abortion	Fruit abortion %
Initial	20	4	20%	4	100%
Peak	20	3	15%	3	100%
Final	20	2	10%	2	100%

Table: 3. Fruit (pod) Average Length, Seeds Average Number and Weight in *Delonixregia*

Sl. No.	Study site	No. of Plants	Average length of POD in cm.	Average No. of seeds	Average weight of seeds in gram
1	Andhra University – Applied Physics Department	2	40.065	19.995	7.15
2	Lawsons Bay Colony Road	4	44.3	20.232	8.09
3	Andhra University – Instrumentation Department	2	39.57	21	7.64
4	Andhra University – Out Gate Opposite Road	3	39.17	18.55	7.4
5	Dabagarden Area	3	45.42	30.4	11.59
6	Old C.B.I.	2	36.47	23.85	14.55
7	Andhra University– Post Office Road	3	41.2	17.01	6.78
8	A.U. GMC R.S. Hostel Surroundings	3	40.54	19.33	7.25
9	Municipal Guest House	1	41.03	11.66	4.95
10	East Point Colony Road	2	37.875	16	6.41

Length of the pod: Range = 36.47 – 45.42 Mean $\overline{X}$ = 40.56 ± 4.96

No. of seeds: Range = 11.66 – 30.4 Mean $\overline{X}$ = 20.39 ± 6.54

Weight of seeds: Range = 4.95 – 11.59 Mean $\overline{X}$ = 7.42 ± 2.56

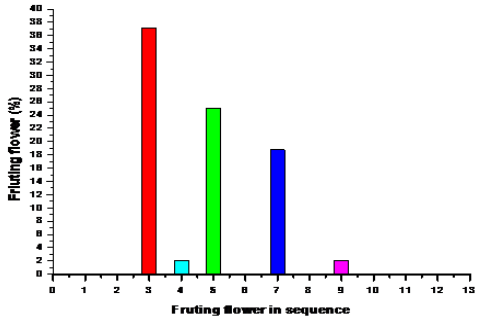

Figure: 2. Inflorescence fruiting frequency(n= 100), Fruit set under Geitanogamous hand-pollination in *Delonixregia*

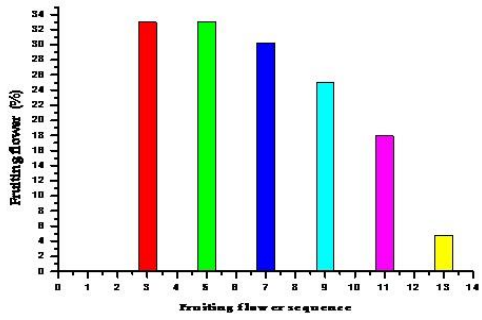

Figure: 3 Inflorescence fruiting frequency (n= 100), Fruit set under Xenogamous hand-pollination in *Delonixregia*

Discussion:

The results obtained and the observations made in respect of *Delonixregia*under study was discussed below in the light of available literature and established concepts of sexual systems.

*Delonixregia*produce bisexual or hermaphordite flowers with both sexual organs functional. Plants with hermaphrodite flowers can contribute genes to the next generation through both male and female function, pollen and seeds respectively. The flowers are nearly homogamous with slightly protogynous nature. As such pollination can be expected to occur by any of the recognized modes of pollination. However, there is no spontaneous autogamy and the manipulated autogamy also is totally ineffective in producing fruit. Substantial fruit was obtained through manipulated geitonogamy and xenogamy. In *Delonixregia*Lepidopteran activity at the flowers may bring about both these effective pollinations. Geitonogamy involves transfer of pollen between flowers on the same plant and requires same pollination mechanism as cross-pollination, consequently it has ecological properties of cross-fertilization and genetic properties of self-fertilization (30). A certain amount of geitonogamy in the natural pollination of *Delonixregia is* inevitable because the plants are self-compatible and the trees being large may produce many flowers at anthesis at the same time.

However, the foraging movements of pollinators at a given time often curtailed after a few visits. (30) noted that geitonogamy would be far more common were if not for the fortunate and still largely un explained habit shared by virtually all flower visitors of visiting only a

fraction of the available flowers on a plant before moving to the next plant.

The flowers produce more pollen and pollen viable for one and half day of four days of flower life span (44). They showed stigma receptivity for 24 hours. Plants could avoid exposure to infectious agents by minimizing stigmatic receptivity (21). (8) predicted that P/O ratio is indicator of breeding systems. The P/O ratio found in *D.regia* is predicted for facultative xenogamy. (8) reported that this mating system facilitates out-crossing when pollinators are available and facilitates selfing in their absence. The high P/O ratio seems to be imperative to compensate the pollen loss associated with the pollen collecting behaviour of foragers.

According to (23), pollinators visiting steady-state individuals are presumed to be low in number and in species diversity. They shift to new plants after a shorter foraging period and make longer distance flights between species. Thus it appears that the steady state flowering pattern is an ecological adaptation to enforce pollinator movement between conspecifics to ensure cross-pollination. Further, spreading presentation of pollen in time creates an opportunity for the visitors to pick up pollen on more occasions, thus increases the chances for the occurrence of out-crossing (31),(36) and (38). Also the strategy of prolonged delivery of pollen reduces geitonogamous pollen discounting (19,18). Lower fruit production in natural condition (21.14%) in *Delonixregia* respectively compared to very high flower production suggest that these species have not evolved a strategy to increase fruit production through geitonogamy. Probably the compatibility of geitonogamous pollination provides reproductive assurance in the event of failure of out-crossing and also in trees isolated by large distance from conspecifics.

In the classical botanical view, self-fertilization is not an intrinsic characteristic of angiosperms. (26) argues that long-lived species such as *Delonixregia*accumulate larger mutation loads and are obliged to maintain obligate outcrossing. Based on this argument, (2) expressed that outcrossing should in general be strongly favoured in long-lived plant species independently of pollinator availability. In species with hermaphrodite flowers having compatibility for both selfing and outcrossing, functional self-incompatibility termed as cryptic self-incompatibility, sensus (3) is presumed to operate as predicted by (46), (6), (22) and (10). Functional self-incompatibility is expressed in competitive situations such as when both self and foreign pollen are deposited on the receptive stigma. There is mounting evidence that plants can distinguish different pollen grains of different origin and genetic relatedness (33).

Such cryptic incompatibility may be operating in *Delonixregia*thus contributing to heterozygocity. In the species thus possess a balanced breeding system, balancing the benefits of out-crossing against the need to geitonogamousselfing when foreign pollen is not available. It is often believed that the actual sexual system represents a compromise determined by evolutionary history and evolutionary potential of population as well as requirement of present environment, and must allow permanent survival of population. Viewed in this context, condition of mixed inbreeding and out breeding constitutes the most ideal sexual system. Most angiosperms have developed such a mixed strategy (7); a mixed breeding system of *Delonix*conforms to this viewpoint. A mixture of crossing and selfing can keep the evolutionary pot boiling nearly as well as outcrossing alone as expressed by (7). Being ornamental plants *Delonixregia* was cultivated in different tropical environments and the possession of flexible sexual system is advantageous in changing environments. But interestingly, *Delonixregia* retained specialized pollination system utilizing the papilionidaebutterflies and hawkmoths (43).

Hand-pollinations demonstrated that sexual system of *Delonixregia* preclude autogamy, proceeds geitonogamy and xenogamy. The plant produces seed-bearing fruit only in geitonogamy and xenogamy. Through the hand-pollination of random position of flowers at inflorescence level with three stages (Initial, Peak and Final) of blooming reveal that fruit formation was set with the autogamy, geitonogamy and xenogamy. But in autogamy fruits did not attain maturation. Caesalpinioid legume trees, like many other tree species, are often self-incompatible or at least preferentially allogamous (14),(29) and(1). Thus in *D. regia*geitonogamy hand-pollination brought about 54% fruit set and 31% fruit attained to maturation. In xenogamy it is about 28% fruit set and 16% attained for maturation. Thus in this species both self-pollination and Cross-pollination operate strongly. This pollination necessitates the involvement of some agent to move pollen from flower to flower within a plant or plant to plant. Bees move mostly at intra plant level bring geitonogamous pollination. Many caesalpinioid legumes are pollinated by single bees, and social bees, especially *Apis*spp., are often predominant among floral visitors (13). Because visits by social bees often result in a high proportion of geitonogamous pollinations, especially on trees, which bear large numbers of flowers, the availability of cross pollen may often limit fruit set (29) and (1).

The proportion of flowers that produce fruit is highly relevant to a plants reproductive success. In recent years low fruit set levels have been documented in numerous hermaphroditic plants (15). In *Delonixregia*there was only 12.46% of the flowers that produced fruits under natural conditions. Various mutually non-exclusive hypotheses have been proposed to explain the excess flower production (11). The fruit in *D. regia*takes a long time up to 10-11 months for fruit development. Though it is green for nearly 4-5 months performing photosynthesis, it might require more resources.

The development of a low number of fruits in relation to the number of flowers produced is common in allogamous species (Stephenson, 1981; Sutherland, 1986), especially in those with high fruit-production costs (11) and (37). The flower surplus increases attractiveness for pollinators (49) and (1), constitutes an ovary pool against unforeseeable loss of flowers by extrinsic causes (11),(12),(15),(16) and (17), and enables the plant to have a measure of control over the quality of its progeny by selective abortion of developing fruits (24),(25),(27) and (40). If the resources are limited and there is no pollen restriction, sexual selection may operate since the female would exercise mate choice, selectively aborting lower quality fruits and seeds (23),(47) and (48).*Ceratoniasiliqua*(Leguminosae: Caesalpinioideae) is a species that produces a large number of racemes with numerous flowers (1). This species presents a low percentage of fruiting per raceme in wild populations (1) and cultivated ones (5).

The pattern of fruit production within the raceme of *D. regia*, nearly 76% of the sampled racemes had 1-2 fruits, against an average of 13 flowers per raceme. The order of flower opening is from base to apex of the raceme. Normally the order of flower opening determines the order of pollination. Pollen deposition on stigma initiates ovary growth. The seeds developing within the ovary produce hormones that mobilise resources into the fruit. Thus the fruits that begin to develop first are usually those with the highest probability of completing their development (15)and(16). Flowers can also have different reproductive potential, so that the probability of their producing fruits varies from one to another (4) and (9). Within the inflorescence level the flower position also determines the fruit set. In *D.regia* species most of odd position flowers are transforming into fruits. Interestingly basal zone odd position flowers set fruit compared with intermediate zone. The production of fruits and seeds often decreases from base to apex of the inflorescence (28),(34) and (35). The basipetal trend in fruit distribution is usually explained as, on one hand, earlier pollination of the basal flowers, whose transformation into fruit would limit later fruit set, and on the other, the more favourable position of basal fruits with respect to resources (39),(28) and (9).

However, different patterns of fruit production have been recorded in different species: fruit production decreases from base to apex of inflorescence (28), (34) and (12), a higher fruit production may occur in the central zone of the raceme (4) or lastly there may be no constituent fruiting pattern relatable with position within the inflorescence (50). In *ceratoniasiliqua* (Caesalpiniaceae) the apical flowers of raceme have higher probability of being converted into fruit (1). Observing fruit set of early, intermediate and late flowers within the inflorescence of *Pancratiummaritimum*(*Amaryllidaceae*), (32) found that the fruit set of early flowers is significantly higher than that of intermediate and late flowers; the fruit set of intermediate flowers is significantly higher than that of late flowers. In *Agave mckellveyana*, (42) found that most basal flowers aborted regardless of weather or not supplemental pollen were supplied. In *Lathyrusvernus*, (1) found that fruit set of late flowers was not improved by supplemental pollination.

In species with high fruit production costs, each inflorescence may function as an independent unit with regard to resources use (16) and (17). This may be happening in *D. regia* so that the limitation of resources would operate at the inflorescence level, giving rise to almost similar pattern of fruit production within the raceme. In many legumes, fruit production is higher at base of the inflorescence (9). Considering the full package of fruit with seeds, it can be said that those flowers with complete pollination would only transform into fruits, of course, the number of fruits depended on the available resources to the inflorescence. The extra flowers may be functioning only as pollen donors, and/or may serve as reserve ovaries (11).

PLATE - I

Plate- I : Seeds in a Fruit

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