



JEB™

ISSN: 0254-8704 (Print)
ISSN: 2394-0379 (Online)
CODEN: JEBIDP

Studies on the reproductive strategies of sapota (*Manilkara zapota*)

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Key words

Floral chamber,
Floral traits,
M. zapota,
Pollination syndrome,
Thrips

Publication Info

Paper received : 06.02.2016
Revised received : 09.07.2016
Accepted : 06.10.2016

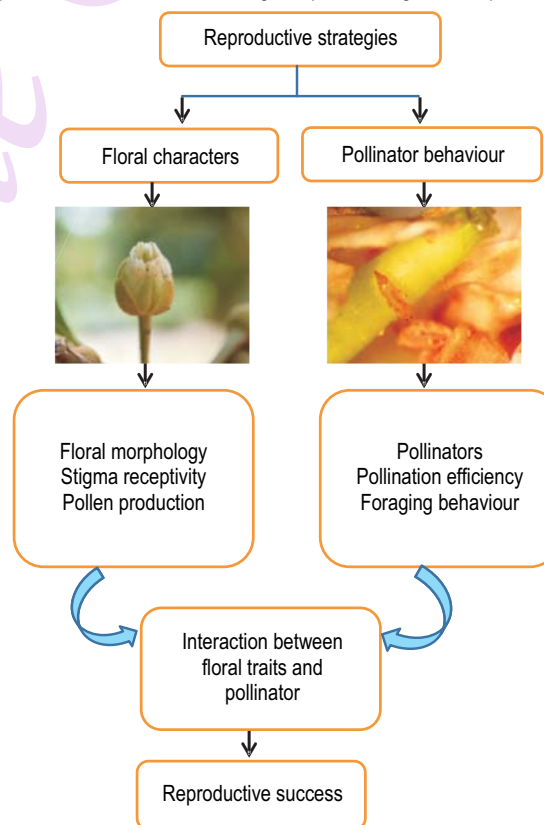
Abstract

Aim: *M. zapota* is inadequately known with respect to its reproductive strategy and functional specialization which are crucial aspects in determining its reproductive success. The present investigation was conducted to study the role of floral traits in pollinator recruitment, pollinators' involvement in selection of floral traits and strategies of plants to ensure pollination services.

Methodology: Fifteen-year-old trees of sapota (var. Kalipatti) were taken as an experimental material. Floral characters like floral morphology, anthesis, pollen dehiscence, stigma receptivity, pollen production were studied by tagging twenty flowering branches distributed across ten plants. Pollination efficiency was also studied. The relation between parameters was worked out through simple linear regression equation.

Results: Flowers were characterized by small aperture, floral chamber, long spell of stigma receptivity (48 hrs), imperceptible nectar secretion, high pollen ovule ratio (980:1) and self-compatibility. The peak stigma receptivity was between 07:00 – 10:00 hr on the day of anthesis. On the basis of visitation rate (3.0-5.5/flower) and pollination efficiency (13.85 – 17.87%) thrips were considered as primary pollinators and *Silvanopsis* sp. (beetle) as a co-pollinator. Presence of narrow aperture, floral chamber and imperceptible secretion of nectar make the flowers of sapota functionally specialized.

Interpretation: Floral traits like narrow flower aperture played a crucial role in filtering specific visitors (thrips and beetle) to access floral rewards. Moreover, the morphological adaptation like floral chamber facilitated better pollen services by providing shelter and breeding place for pollinators.



Introduction

Manilkara zapota (L.) P. Royan (Sapotaceae) is an important tropical fruit of India cultivated between the latitudes of 12–25° N and longitudes of 72–85° E. It is commonly known as 'Sapota' or 'Chiku'. Under tropical climatic condition, sapota bears flowers in different flushes *i.e.*, June–July, September–October and March–April. However, fruit set shows temporal variation. Low fruit set has been a major limitation in sapota which could be due to the dependence of plant on relatively inefficient pollinator. Pollination primarily depends on the efficiency of pollinator in delivering quality pollen onto the receptive stigma. It has been observed that the interaction between flower visitors and floral traits (rewards) demonstrates intricate network of pollination mechanism including mutualism which is considered as one of the driving forces in the evolution of angiosperm (Grimaldi, 1999). Traits like fragrance, nectar, petal colour and structural modification have been evolved to attract pollinators to ensure pollination services (Thien *et al.*, 2000). Most plants show moderate to substantial generalization in their pollination system and in fact visited by diverse assemblages of flower visitors (allophilic) that could be equally or more effective pollinators. On the other hand, some flowers are functionally specialized and attract specific visitors (euphilic). The exhibition of functional specialization by euphilic flowers is considered as 'pollination syndrome', which is a suite of floral traits, including rewards, associated with the attraction and utilization of specific 'functional group' of pollinators (Ollerton *et al.*, 2009; Fenster *et al.*, 2004). Moreover, syndrome also describes the pattern of floral adaptation in plant groups to their pollinators (Johnson and Steiner, 2003). It has been reported that in spite of the recruitment of less-effective pollinators, plant usually exhibits efficient pollination strategies like high pollen ovule ratio, extended stigmatic receptivity etc., to ensure its reproductive success (Galloni *et al.*, 2007; Garcia *et al.*, 2014).

M. zapota is inadequately known with respect to its reproductive strategy and functional specialization which are crucial aspects in determining its reproductive success. The present investigation was carried out aiming to study the pollination biology of sapota in order to unravel its reproductive strategy to answer following questions like role of floral traits in pollinator recruitment, pollinators involvement in selection of floral traits, and strategies of plants to ensure pollination services.

Materials and Methods

Studies were conducted at the Central Horticultural Experiment Station, Bhubaneswar (20° 27' N, 85° 82' E) during 2014–2015. The climate was tropical hot and humid with the average temperature range of 22.2 to 33.7 °C, annual precipitation of 1650 mm and relative humidity of 70.5–76.8%. Fifteen-years-old trees of sapota (var. Kalipatti) were taken as experimental material. Flower phenology (floral morphology,

anthesis and pollen dehiscence) was studied by tagging twenty flowering branches distributed across ten plants. Total number of newly opened flowers was recorded daily at 06:00hr for 15 consecutive days in June and September (peak flowering) and consequently, the time of anthesis was established by ascertaining the number of newly opened flowers. Similarly, the time of pollen dehiscence was established when more than 50% flowers dehisced their pollen. Pollen production per flower was quantified by hemocytometric method. Pollen of ten flowers were counted and average number of pollen was calculated. The number of ovules/flower was counted under stereo microscope (Leica) by observing transverse section of ovary. Pollen: ovule ratio (P/O) was worked out by dividing the number of pollens and ovules. To work out the degree of autogamy, 200 buds were bagged and fruit set was recorded after 15 days. For self-compatibility studies, 200 bagged buds were emasculated and pollinated with the pollens of same plant on the day of anthesis and fruit set was recorded. Duration of stigma receptivity was worked out by pollinating emasculated flowers (n = 300) at different intervals and on the basis of fruit set in fertilized flowers, receptivity was confirmed. The duration of peak stigma receptivity was determined on the basis of maximum percentage of fruit set in pollinated flowers. In order to identify pollinators, flower visitors were captured and identified morphologically under microscope. To ascertain visitation frequency of visitors, fifty flowers from five different plants were collected at an interval of three hours (07:00, 10:00, 13:00, 16:00 hrs) on the day of anthesis and the number of thrips and beetles were counted using magnifying glass (10X). Pollination efficiency of thrips and beetle was worked out on the basis of number of pollinated stigmas in thrips visited flowers (n = 200) and beetle visited flowers (n = 200), respectively. To test for differences in visitation frequency and pollination efficiency one way analysis of variance (ANOVA) was performed followed by mean separation using honestly significant difference (HSD) test. The relation between parameters was worked out through simple linear regression equation following least squares method.

Results and Discussion

Bisexual flowers of sapota were characterized by restricted opening of petals, presence of small aperture (<2.5mm), floral chamber at the base approach herkogamy (placement of stigma beyond stamen) (Fig. 1). Anthesis took place in early morning (05:00 - 06:00 hrs) which was followed by anther dehiscence (07:00 – 09:00 hrs). Stigma was receptive a day before anthesis and remained receptive a day after anthesis. Nectar was not observed to be an important reward for visitors as imperceptible amount of nectar was observed even on the day of anthesis. The highest percentage of fruit set was recorded when pollination occurred between 07:00–10:00 hr on the day of anthesis indicated the peak stigma receptivity. It was also observed that stigmatic exudate acted as a trap for flower visitors. Flowers had moderately high number of pollens but less number of ovules which in turn culminated in high pollen: ovule ratio (Table 1).

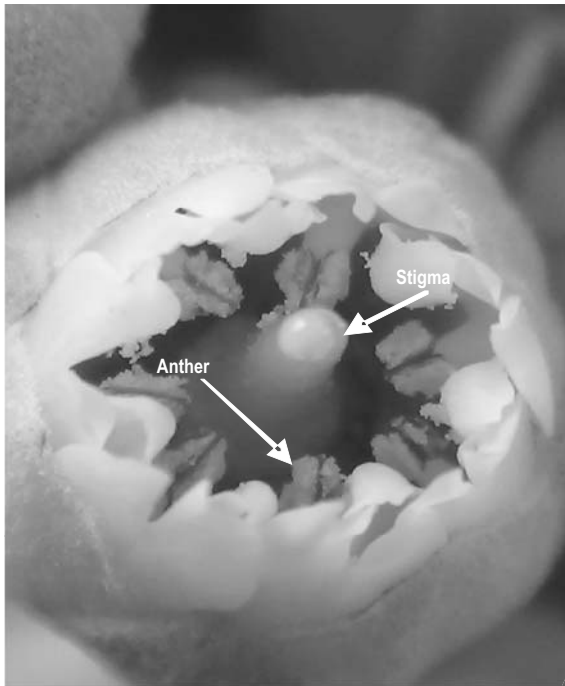


Fig. 1 : *M. zapota* exhibiting approach herkogamy and restricted anthesis

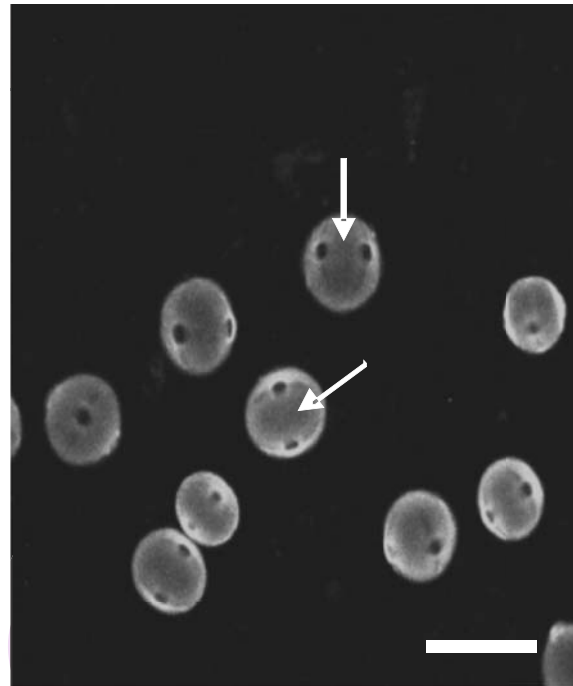


Fig. 2 : Pollen dehiscence at two-celled stage Bar - 50 μ m

Table 1 : Floral traits and pollination behaviour of *M. zapota*

Sex arrangement	Approach herkogamy (Stigma placed above anthers)
Flower aperture (diameter)	2.3 \pm 0.1 mm
Period of anthesis	0600-0700 h
Period of anther dehiscence	07:00 - 09:00 hrs on the day of anthesis
Pollen/flower	9723 \pm 157
Ovule/flower	9.0 \pm 1.2
Pollen: ovule ratio (P/O)	980.6:1
Duration of peak stigma receptivity	07:00-10:00 hrs on the day of anthesis
Nectar content	Imperceptible
Fruit set in autogamous flowers	Nil
Fruit set in open pollinated flowers (%)	14.5
Fruit set in self-compatible flowers (%) (hand pollination)	27.5

Sapota was observed to be highly self-compatible, as the fruit set percentage was substantially high (>25%) when flowers were pollinated with the pollens of same plant. Pollen of *M. zapota* dehiscid at 2-celled stage i.e., vegetative and generative cells (Fig. 2), which gave an indication that division of generative cell might have taken place inside pollen tube.

Floral traits not only facilitate pollination but also protect pollen resource from other visitors (Thomson, 2003). It is evident in sapota that floral trait, especially restricted flower opening might have prevented other visitors possibly from wasting pollens. Moreover, the presence of floral chamber is an indication

of thripophily and cantharophily (beetle pollination) as exhibited in *Annona squamosa* (Kishore *et al.*, 2012). Occurrence of narrow entrance and floral chamber might have increased plant's fitness to thrips and beetles by excluding other visitors who could have acted as pollen thieves (Endress, 1994). In some of the plants, width of corolla aperture is most critical in restricting pollinators and imposes an adaptive trade off that favours high degree of specialization (Muchhala, 2006). Presence of floral chamber in sapota could be an important adaptive character to facilitate pollination. The chamber not only initiates the process of floral thermogenesis which provides direct energy reward to pollinator but also creates barrier for predators (Thien *et al.*, 2000).

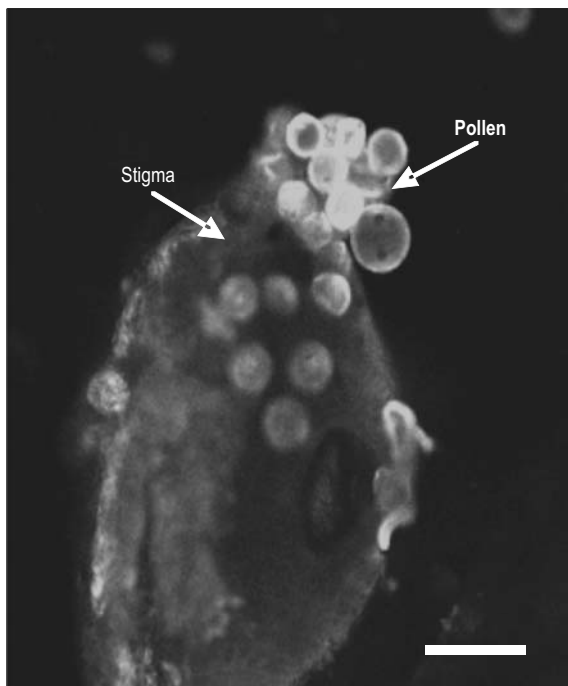


Fig. 3 : Pollinated stigma of thrips visited flowers. Bar - 50 μ m



Fig. 4 : Thrips inside the flowers of *M. zapota*



Fig. 5 : *Silvanopsis sp.* inside the flowers of *M. zapota*

Moreover, the chamber mostly serves to select the flower visiting spectrum and to retain the small insect inside the flower which in

turn increases the probability of pollination. The relation between visitors and floral chamber in sapota is an example of 'brood-site pollination mutualism' as plant's provide safe place to visitors for stay and breeding, and in return received pollination services (Sakai, 2002). *Thrips sp.* and *Silvanopsis sp.* usually take shelter in the floral chamber of sapota and live primarily on the pollen grains (pollenivorous). The continuous availability of resources inside the flower might have facilitated multiplication of visitors throughout the flowering period which in turn increased the pollination services. Floral traits of *M. zapota* seem to be functionally specialized primarily due to restricted opening of flower and presence of floral chamber. Such type of functional specialization may be called pollination syndrome which restricts many and allows a few by offering particular reward system to visitors. *M. zapota* offered pollen and site for brooding as rewards which helped in recruiting specific visitors like thrips and beetle.

Moderately high P/O ratio and long duration of stigma receptivity in sapota indicate poor pollen delivery system which was further substantiated by the poor pollination capacity of relatively less effective pollinators. On the other hand, low P/O ratio is an indication of efficient pollen delivery system (Stefan and Walter, 2009; Wang *et al.*, 2004). It may be presumed that plants with high P/O ratio waste more pollen due to the recruitment of less efficient pollinators. Furthermore, pollen shedding at 2-celled stage in *M. zapota* implied that generative cell of pollen grain divides into male gametes after landing on stigma or in the pollen

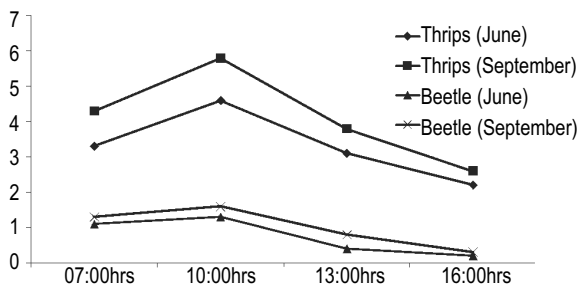


Fig. 6 : Temporal variation in visitation frequency of floral visitors of *M. zapota*

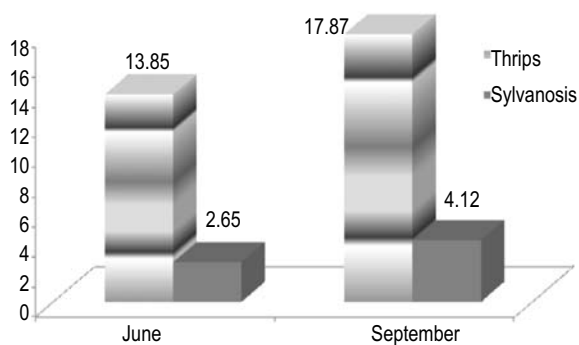


Fig. 7: Temporal variation in pollination efficiency of flower visitors

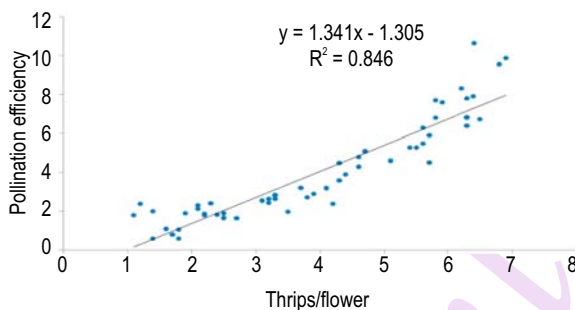


Fig. 8 : Linear relation between number of thrips/flower and pollination efficiency

tube. In such condition, pollen are in temporary dormancy condition, and the division of generative cell is postponed till pollen germination (Bhojwani and Bhatnagar, 2008). It may be presumed that shedding of pollen at 2-celled stage could be the strategy of plants to ensure reproductive success by giving more opportunity to less effective pollinators (thrips and beetle) to carry pollen and deliver on receptive stigma.

Thrips sp. (Fig. 4) and *Silvanopsis* sp. (Fig. 5) were identified as major flower visitors. However, thrips were more abundant which was substantiated by their visit frequency (Fig. 6). During peak stigma receptivity, there were more than 4

thrips per flower, whereas the number of beetle was relatively less (1.5). Thrips are generally considered to be ineffective pollinators on account of body size, pollen load and directed flight, which are deemed essential to be an effective pollinator (Kirk, 1997). But these traits are not always true nor are these traits always limited for thrips. Larvae and adults of thrips feed largely on pollen grain; subsequent transportation of pollens to stigma was more likely as thrips were highly mobile, whereas *Silvanopsis* sp. were less mobile and acted as co-pollinator.

The visit frequency of flower visitors showed significant temporal variation ($p < 0.05$) as the occurrence of visitors was significantly high in September (>5). The high rate of visit in September may be due to the prevalence of favourable temperature ($T_{max} - 31.6\text{ }^{\circ}\text{C}$) and relative humidity (75 -80%). Reddy *et al.* (2015) also indicated the influence of maximum temperature on foraging behavior and visit frequency of pollinator. Thrips and beetle started visiting flowers in the morning on the day of anthesis and continued till 18:00 hrs. However, high visitation rate was recorded between 07:00 – 10:00 hrs on the day of anthesis (Fig. 6). Foraging behaviour of pollinators indicated that adult thrips stayed for relatively longer period (>2 hrs) inside the flower, whereas beetle stayed for short spell (data not shown). Moreover, the larvae of thrips were present inside the floral chamber even after the cessation of stigma receptivity. It was also observed that thrips took shelter and multiplied in the flower chamber and fed mainly on pollens and stigmatic exudate. Moreover, the presence of floral chamber might have acted as a shelter and breeding site for thrips as in some of the plants, pollinators are likely to use the enclosed chambers as breeding sites before and during flower anthesis (Ishida *et al.*, 2009). When pollen delivery onto stigma (Fig. 3) and pollination efficiency were taken into account, thrips were found to be effective by virtue of pollinating more number of stigmas (Fig. 7). On the other hand, *Silvanopsis* sp. was relatively less effective in pollinating stigma possibly due to low pollination efficiency. The temporal variation in pollination efficiency indicated that September was relatively conducive period due to the prevalence of moderate temperature ($T_{mean} - 28.3\text{ }^{\circ}\text{C}$) and relative humidity (75 -80%) for visitors to effect pollination services. Linear relation between number of thrips and pollination efficiency (Fig. 8) indicated that pollinator's abundance had significant effect on pollination efficiency of sapota ($R^2 = 0.846$ $P < 0.01$).

The recruitment of specific group of pollinator might have the strategy of sapota to avoid wastage of pollen - the most valuable resource for reproduction. In sapota, thrips and beetles were the functional groups that increased plant's fitness by being pollinators not necessarily most effective. Moreover the overall fitness of a plant is actually a function of all its pollinators, not just the most effective one. As a result, it may be possible to rely on less effective pollinator like thrips and beetle in the absence of most effective one as evident in sapota. The recruitment of only *Thrips* sp. and *Silvanopsis* sp. as visitors in sapota gives a clear

evidence of pollination syndrome. The pollination of strictly out-breeders leads to reproductive failure if particular pollinator, on which they rely, is lost or absent, whereas the chance of losing pollination services in strictly out-breeders is reduced if their pollinators are generalist (Ashman *et al.*, 2004; Kremen *et al.*, 2007).

Studies indicate that floral traits like narrow flower aperture play a crucial role in filtering specific visitors (thrips and beetle) to access floral rewards (Fenster *et al.*, 2004). Moreover, the morphological adaptations like floral chamber facilitated better pollen services by providing shelter and breeding place for pollinators. Occurrence of thripophily (thrip pollination) and cantharophily (beetle pollination) in *M. zapota* due to morphological adaptation is an explicit example of pollination syndrome which may be an important model for convergent adaptation for specific group of pollinators. Moreover, pollen dehiscence at 2-celled stage, high pollen/ovule ratio and long duration of stigma receptivity are important adaptive features of functionally specialized flower of *M. zapota* to counter the consequences of pollen wastage and delay in pollen delivery by relatively less effective pollinators. It may be presumed that the chance of reproductive failure in *M. zapota* is less as plant depends on generalist pollinators (thrips and beetle), which are common in different agro-ecosystems. However, thrips may prove to be of much greater importance in sapota due to higher pollination efficiency and visit rate. In two lines state relevance of this study.

Acknowledgments

Financial assistance from Indian Council of Agricultural Research, New Delhi is gratefully acknowledged. Authors are thankful to the anonymous reviewers for their valuable suggestions and comments.

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