The International Journal of Plant Reproductive Biology 13(2) July, 2021, pp.122-126
DOI 10.14787/ijprb.2021132122126

Floral biology, pollination mechanism and breeding system in *Milletia pinnata* (L.) Panigrahi (Fabaceae)

Alpna Johri

J. D. B. Govt. Girls College, Kota-324001, India
e-mail-alpnajohri@gmail.com

Received; 20.02.2021; Revised: 04. 05. 2021; Accepted and published online: 01. 06. 2021

ABSTRACT

Milletia pinnata syn. Pongamia pinnata (L.) is one of the few nitrogen fixing trees commercially known as Karanj. The seeds of the plant produce 30-35 % oil, is called Karanj oil or Honge oil. It is being considered as an alternative source for biodiesel. Its root, bark, leaf and flower of the tree have medicinal properties. It is an ornamental perennial tree. Its flowers are pentamerous, hermaphrodite and complete. Phenological studies indicate that it flowers twice in a year, in late spring to early summer and in autumn. First flush of flowers occurs in March-April and extend up to May and a second flush of flowers occurs in September to November. Anthesis occurs between 07:00 h-07:30 h. Papilionaceous structure of flower facilitates self-pollination but white tinged with pink or purple flowers displayed in pendulous raceme and sweet fragrance attract several visitors. P/O ratio is 25,625. High P/O ratio in this plant indicates that species is xenogamous. Fruits are hard, thick, almond coloured indehiscent pod which remain attached to the trees and fall down in April to July of the next year.

Keywords : Karanj, Anthesis, Biodiesel, Hermaphrodite, Phenology.

A variety of approaches and techniques have been proposed and implemented for conservation of plant resources. These techniques would not be successful without information about the reproductive features of plant. The understanding of various aspects of reproductive biology including pollination, breeding system of flowering plants is important for biodiversity conservation (Moza and Bhatnagar 2007, Chauhan and Chauhan 2013). Reproductive biology has been largely studies in herbaceous crops and trees have not received much attention due to their large size, long life cycles, and inaccessible flowers in conducting researches (Tandon *et al*. 2005). The techniques of conservation of biodiversity include *in situ* and *ex-situ* conservation. In vitro conservation techniques include tissue culture. Many of the micro propagation protocols that produce positive results in laboratories fail to take off in the field because of lack of information about the reproductive features. It has been observed that reproductive Biology is very important to determine the seed and fruit set, conservation and for understanding pollination and breeding systems that regulate the genetic structure of populations.

Milletia pinnata (L) Panigrahi. *syn. Pongamia pinnata* (L.) Pierre (family-Fabaceae) is native to eastern and tropical Asia, Australia and Pacific islands, almost found along sandy beds of streams and the sea coast. *M. pinnata* originated from India (Sujatha *et al.* 2008) and spread across Asia into the Pacific Scott *et.al*. 2008).This ornamental flowering tree is commonly called as Karanj, Pongam, Honge tree or Indian beach tree and it is cultivated as a road side tree. It is medicinally important and its root bark, leaves and flowers are used to cure skin diseases, rheumatism, whooping cough, malaria and ulcers. Aqueous extract of seed, flower and pericarp show significant antipyretic, anti-inflamatory, anticancer, analgesic, anti-diabetic activities (El-shabrawy *et al*. 2007). The wood is used as fuel and agricultural implements etc. It is preferred species for controlling soil erosion. Seeds of Karanj contains 30- 35% oil and its physical and chemical properties are almost similar to the diesel . Pollination and reproductive biology of *Pongamia pinnata* L. (Fabaceae) have earlier been studied by and Kukade and Tidke, (2013) and Veereshkumar *et al.* (2021).

The present study deals with the floral biology, pollination mechanism and breeding system in *Milletia pinnata* (L) Panigrahi growing as an avenue tree in Kota (Rajasthan).

MATERIAL AND METHODS

Study site — The study site is Chhatra Bilas Garden, located in Kota city. Kota is one of the eastern district of Rajasthan. It is situated between 75º37′ to 77º26′ east longitude and 24º25′ to 25º51′north latitude. There are about 150 *M. pinnata* trees in garden.

Phenology and floral biology — Ten marked trees (P1–P10) were selected for study. Phenological events like flowering, fruiting, leaves shedding, emergence of fresh leaves, fruit dispersal were recorded over a period of two flowering seasons. Observations were made every day during the flowering time. Fruit maturation and dispersal were recorded once a week.

Ten inflorescence from each tree were tagged at the time of initiation of flowering and were observed till fruit maturation. Average size of flower, average number of flowers borne on an inflorescence was recorded from tagged flowering branches, bud development and their opening at various hours of the day were noted from 06:00h to 18:00h.

Average number of pollen grains produced in a flower and pollen grain viability at the time of anther dehiscence was estimated. Mature undehisced anthers were squashed in 25% glycerol (v/v) + 1% acetocarmine to estimate the number of average pollen grains. They were examined under microscope. Pollen production per flowers was calculated by counting the number of pollen grains per anther and then multiplying this figure by total number of anthers per flower (Cruden 1977).

Pollen viability — It was observed by method after Hauser and Morrison (1964) using 0.25 TTC (2, 3, 5 triphenyl tetrazolium chloride) solution at 5.8 pH using 0.15 M tris-HCL buffer.

Stigma receptivity — Stigma receptivity and post pollination events were recorded. Number of ovules was determined by taking the longitudinal sections of the ovary and then counting the number of ovules. Stigma receptivity was determined by using α-naphthyl acetate test as described by Shivanna and Rangaswami (1992).

Anthesis — Time of anthesis and measurements of floral parts were studied in detail.

Pollination — Floral visitors, their mode of foraging and pollination were noted.

Breeding System — By randomly tagging the flowering branches (n=30) on 20 trees, the total number of flower buds borne and the number of fruits that developed were computed.

Fruit dispersal — Distance of fruit dispersal was computed by marking five isolated trees at different locations within the population. A red dot was placed on the mature fruit (n = 50) when they are still attached to the tree. The distance travelled by fruits after dispersal was measured from the base of trunk of respective trees.

Pod setting percentage and Seed setting percentage were also calculated adopting the following formulae:

$$\text{Pod setting percentage} = \frac{\text{Number of pods formed} \times 100}{\text{Total number of flowers per inflorescence}}$$

$$\text{Seed setting percentage} = \frac{\text{Number of seeds formed} \times 100}{\text{Total number of flowers per inflorescence}}$$

Data analysis — Mean and standard errors of floral traits and other data were calculated using Microsoft Excel.

RESULT AND DISCUSSION

Plant is diploid (2n = 22) showing high pollen fertility and good fruit and seed setting. Various aspects like morphology, phenology, floral biology, floral structure, anthesis, anther dehiscence, stigma receptivity, floral visitors, pollen characteristics and pollination are presented in the following paragraphs.

Morphology — *Milletia pinnata* (L.) Panigrahi is a perennial 6-8 meters tall tree with erect, woody stem and unipinnately compound leaves (Fig 1a). The inflorescence is axillary pendulous raceme (Fig. 1 a, b). The flowers are pedicellate, pentamerous, complete, zygomorphic, hermaphrodite, papilionaceous and pink or purple with a pleasant fragrance. Similar morphological characteristics have earlier been reported by Maheshwari (1963).

Phenology — The tree is deciduous and leaves turn yellow and brown in the first week of February and finally abscise in the first week of March to make the tree completely leafless. Young dark green leaves start appearing by the end of March (Fig. 1a). The tree flowers twice a year. The first flush of flowers appears in the third week of March and extends up to May (Fig. 1a). The number of inflorescences per plant is very high and the number of flowers per inflorescence is 41.8±2.27. Fruiting starts at the end of April (Fig. 1c). The percentage of flowers which set and develop into a mature fruit is very small, ranging from 0.1 to 3%.

The fruit-set percentage is low and the number of fruits per inflorescence is 3.4±0.56. Young fruits are pale-green in colour in May and turn pale almond coloured, hard and woody (Fig. 1d). The second flush of flowers occurs in last week of September and continues till November. Peak flowering time was mid September to early November.

Shripad *et al.* (1985) reported that *P. pinnata* is deciduous, moderate tree with flowering between January to May and profuse flowering occur in February. Kawakita and Kato (2002) reported that flowering and fruiting season of *Pongamia* is May to November in the subtropical forest of Southern Japan. Srimathi *et al.* (2013) observed that initiation of flower buds is between mid-April to mid-May and on an average of 35.7 flowers develop per inflorescence. Kukade and Tidke (2013) reported that the plants start blooming from the first week of March and peak flowering in May. Buds open between 07:00h -09:30 h.

Floral Biology — Inflorescence of *M. pinnata* is axillary pendulous raceme, with 41.8 ± 2.27 buds and flowers (Fig.1a, b). Length of inflorescence is 11.08± 0.57cm. The youngest visible bud was 0.1 × 0.1 cm, green, smooth, oval shaped. Sepals develop on 14th day the terminal end of bud before 07:00h and petals appear on 15th day and complete flower open on 16th day from the day of initiation of bud. The flower is hermaphrodite and papilionaceous. Structure of flower

facilitates self-pollination. Flower colour is white tinged with pink or purple with a pleasant fragrance. Size of flower is 2.34±0.14.cm x 1.12±0.05 cm. at the time of anthesis. Sepals are brown, campanulate nearly truncate. The sepals are 0.4±0.03 cm long. Flower is papilionaceous. Standard petal is broad, orbicular with hook like structure with the nectar at the base. The size of standard is 1.44±0.02 cm x 1.08±0.06 cm. Wing is 1.4±0.04 cm x 0.42±0.02 cm and keel is 1.22±0.02 x 0.42±0.02 cm in size (Fig. 1 C). There are 10 monadelphous stamens as the filaments of all the ten stamens are fused (Fig. 1 D). The posterior stamen is smallest, while the average size of stamen is 1.14±0.02 cm. Anthers are dithecous, dorsifixed, introrse, dehisce by longitudinal slit at the time of anthesis between 07:30h to 08:00h although flower completely open at 10:00h.. Pollen grains are 26.97±1.10 µ x 25.97±1.25µ in size. About 98.6% pollen grains are viable at the time of anthesis during first phase of flowering, while in second phase there is reduction in pollen viability (87.7%). The size of carpel is 1.56±0.15 cm x 0.23±0.03 cm. There are 2.1±0.1 ovules per ovary. Anther and stigma are enclosed in the boat shaped keel petals. The stigma is knob-like papillate and wet. The style is long filiform solid. The receptivity of stigma lasts between

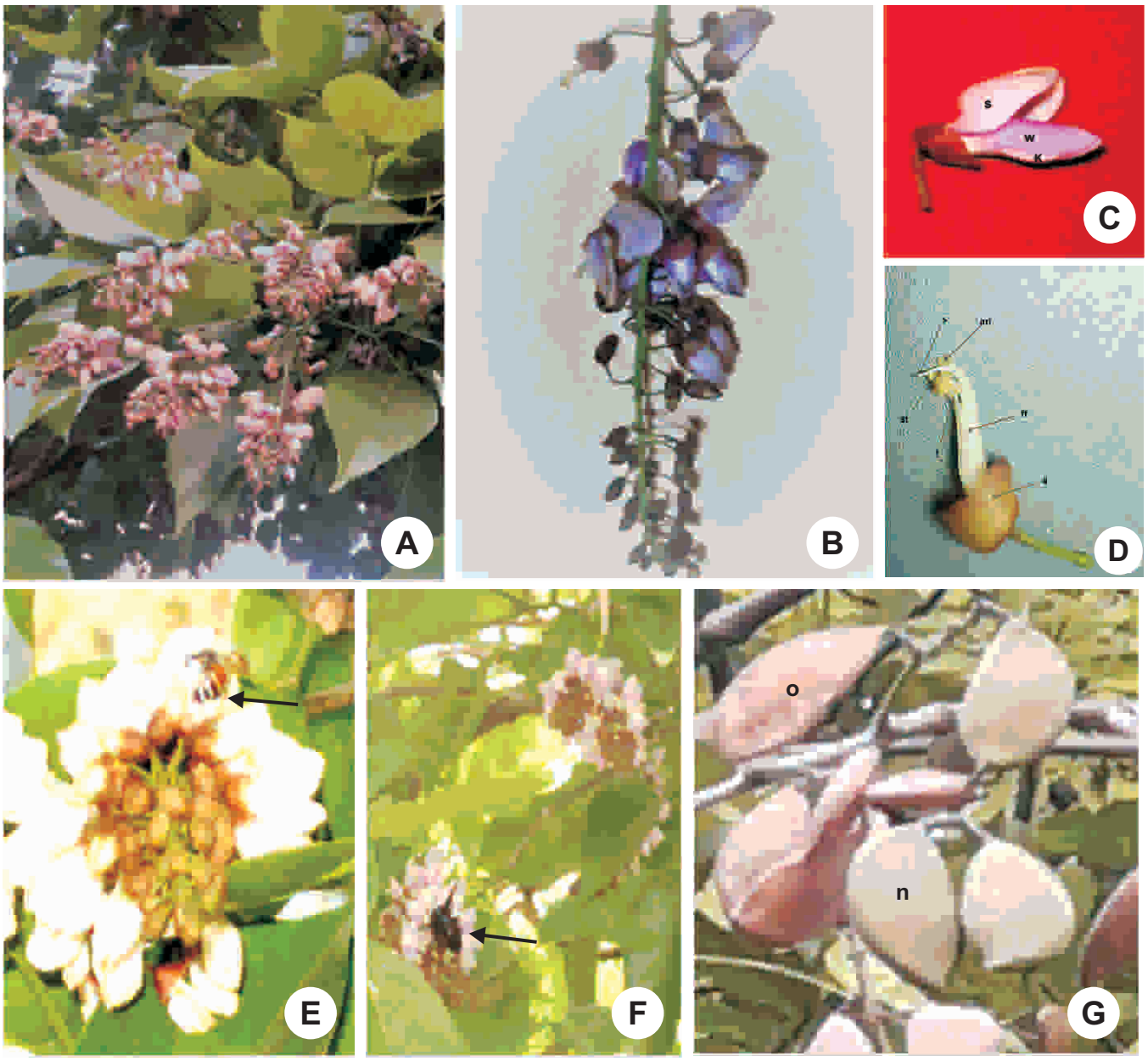

Fig. 1 A– G. *Milletia pinnata* (L.) Panigrahi. A. Flowering branch, B. Inflorescence, C. Flower (s: Standard, w: wings, k: keel), D. Reproductive structures (an: anthers, f: filaments, ff: fused filaments, s: stigma and st: style, k : calyx), E. *Micrapis florae* on flowers (arrow), F. *Scolia soror* on flowers (arrow), G. Young (n) and mature fruits (o).

06:00 to 11:30 h on the day of antheis and is lost prior to closure of the flower. According to Soloman Raju and Rao (2006) that the stigma attains receptivity one hour after anther dehiscence but the stigma receptivity lasts during 09:00h to 16:00h in *P. pinnata*, Kukade and Tidke (2013) reported that the stigma became receptive at 06.00h- 11:30h on the flower opening day and losses receptivity shortly before the closure of flower. Veereshkumar (2021) reported that stigma remains receptive for 8 h after anthesis, the maximum receptivity is noticed 2 h after anthesis.

Anthesis — Anthesis occurs on 16th day from the day of initiation of buds. Petals start opening between 07:00h to 07:30h and they are completely open at 10:00h. Petals start to come close after 17:00h and fail to open on 17th day as the flower longevity is just one day. Petals dry on 19th day and young fruits are seen on 20th day. The fruits are green flat, elongated pod which become pale green after 6 months and almond coloured after 10 months (Fig. 1 G). Solomon Raju and Rao (2006) observed that buds in *P. pinnata* open between 07:00h to 10:00h with anthesis at 08:00h. Veereshkumar *et al.* (2021) observed anthesis in *P. pinnata* between 08:00 and 10:00 h with peak flower opening between 08:00 and 08:30 h with one day flower longevity. The environmental factors (temperature, relative humidity and rainfall) influences the time of anthesis and delay in flower opening and anther dehiscence is due to weather conditions (Tidke and Dharamkar 2003, Tidke 2005).

Pollination biology — Pink flowers with white ting in the long pendulous raceme with sweet fragrance attract several visitors. Pollen and nectar are reward for visitors. More frequent activity was found during the forenoon hours and maximum visitation occurred between 08:30h-09:30 h. Most frequent visitors were honey bees (*Micrapis florea* (Fig 1E), *Apis dorsata, Trigona* spp., Carpenter bee (*Xylocopa* spp.) and *Scolia soror* (1F). Some unidentified insects including wasps also visit flowers. While searching for nectar, landing and feeding pollen, some pollen grains stick to their body parts and wings of these insects transfer pollen on the stigma of other flowers. Kukade and Tidke (2013) reported that *Apis dorsata, A.cerana, A. florea, Xylocopa latipes* are frequent flower visitors and pollinators in *P. pinnata*. Veereshkumar *et al.* (2021) reported that *Megachile* species are more common visitor as compared to *Apis*, *Xylocopa* species.

Both self and cross pollination occur. Structure of flower facilitates in self-pollination. In open flowers the stigma is fully loaded with indicating self -pollination**.** Honey bees move inside between standard and keel from top and sometimes from lateral side of flower for the nectar. *Trigona* spp. and *Xylocopa* spp. enter from terminal end of keel which is slightly open. While searching for nectar pollen grains stick to their body parts including wings. These insects leaden with pollen transfer the pollen to stigma of other flower .The stigma and anthers come into contact with their month parts, wings, legs etc. Cross pollination occur when the stigma comes in contact with the pollen covered body parts of insects. Thus showing entomophilous pollination which is a critical factor for seed or fruit set in most plants including *M. pinnata* (Solomon Raju and Rao 2006, Dhillon *et al.* 2009).

Breeding system — The tree is both self and cross pollinated. Self-pollination is favoured by papilionaceous shape of corolla and insects help in cross pollination. Number of pollen per flower was 51250 and P/O ratio was 25625. According to Cruden (1977) the pollen ovule (P/O) ratio is the general indicator of breeding system of the species. It is a facultative xenogamous species with high P/O ratio.

Fruit — Fruits are hard, thick, woody, indehiscent pod (Fig. 1G). Fruiting commence in the month of April. Young fruit is flat, green in colour. They increase in size in 5 months **(**up to September**)** and become pale green after 6 months (up to November) and reach to maturity after 10 months and become hard, woody and almond coloured. The size of matured fruit is 5.38±0.04 cm x 2.0± 0.07 cm. The number of fruits per inflorescence is 3.4±0.56. The indehiscent fruits fall between April to July of the coming year. They release seeds due to mechanical pressure. The fruits and seeds disperse under the tree or its nearby area between only half meter to 4 meters from the tree trunk. Seeds are nearly kidney shaped, brown, 2.1 ± 0.09 cm x 1.33 ± 0.02 cm in size. The number of seeds per fruit is 1 but sometimes one more poorly developed seed may be present as the seed production is limited. During present investigation, both pod setting percentage and seed setting percentage are 8.13 i.e. same because number of seed is always one in each pod.

In *Pongam**ia*** trees, the pod and seed set percentage obtained was only 3.0 % and 2.4 % respectively. Formation of flowers, fruits or seed setting characters are highly variable in trees from locality to locality the variation may be related with genetic effect of the tree (Nelsonnavamaniraj 2005). Srimathi *et al.* (2013) studied floral phenology, fruit and seed maturation and harvest index based on fruit colour in *Pongamia pinnata*.

Acknowledgement— Sincere thanks are due to Prof. S. K. Chaturvedi, Former Professor & Head, Department of Botany, Nagaland University, Mokokchung, Nagaland for the identification of insects.

REFERENCES

Chauhan SVS and Chauhan Seema 2013. Role of reproductive biology in biodiversity conservation. In: Anita M and Chauhan SVS (eds.). *Proceedings of I world Biodiversity Congress,* Global Science Research Foundation and The Society of Plant Reproductive Biologists. Pp. 51-56.

Cruden RW 1977. Pollen ovule ratio: a conservative indicator of breeding system in flowering plants. *Evolution***31**32-46.

Dhillon RS, Hooda MS, Ahlawat KS and Kumari S 2009. Floral biology and breeding behavior in Karanj (*Pongamia pinnata* L.Pierre). *Indian Forester* **135** 618-628.

El-Shabrawy AO, El-Gohary HM, Youssef MM, El. Gedally RA and Sleem AA 2007. Phytochemical and Biological studies of the flowers and fruits of *Pongamia pinnata* L. Pierre growing in Egypt. *Egyptian J. Biomedical Sci*. **23** (1)1-4.

Hauser EJP and Morrison JH 1964. The Cytochemical reduction of nitro blue tetrazolium as an index of pollen viability. *Am. J. Bot.* **51**(7) 748-752.

Kawakita A and Kato M 2002. Floral biology and Unique pollination system of root holoparasites, *Balanophorakuroiwai* and *B. tobiracola (*Balanophoraceae). *Am. J. Bot*. **89** 1164-1170.

Kukade SA and Tidke JA 2013. Studies on pollination and reproductive biology of *Pongamia pinnata* L. (Fabaceae). *Indian J. Fundamental and Applied Life Sci.* **3**(1) 149 – 155.

Maheshwari JK 1963. *The Flora of Delhi*. Council of Scientific and Industrial Research. New Delhi.

Moza MK and Bhatnagar AK 2007. Plant reproductive biology studies crucial for conservation. *Curr. Sci*. **89** 243 244.

Nelsonnavamaniraj K 2005. Studies on Phenology, seed collection and post-harvest seed management techniques for production of quality planting stock in *Bixa Orellana*. Ph.D. Thesis, Tamil Nadu Agriculture University, Coimbatore, India

Shivanna KR and Rangaswami NS 1992. *Pollen Biology: A laboratory Manual*. Spinger-Verlag, New York.

Shripad NA, Nagalakshamma KV and Gowda PG 1985. Pollen flora of Lalbagh Botanical garden, Bangalore -I. *Indian J. Bot.* **8** (1) 49-66.

Solomon Raju AJ and Rao SP 2006. Explosive pollen release and pollination as a function of nectar feeding activity of certain bees in the biodiesel plant *Pongamia pinnata* (L.) Pierre (Fabaceae). *Curr. Sci.* **90**(7) 960-967.

Srimathi P, Marriappan N, Sundaramoorthy L and Sudhakar K 2013. Studies on floral phenology, fruit and seed maturation and harvest index based on fruit colour in *Pongamia pinnata* (L.) Pierre. *Afri. J. Pl. Sci*. **7(**11) 513-520.

Sujatha K, Panda BM and Hazra S 2008. De novo organogenesis and plant regeneration in *Pongamia pinnata* oil producing tree legume. *Trees* **22** 711-716.

Tandon R, Gupta P, Sunnichan VG, Shivanna KR and Mohan Ram HY 2005. Reproductive biology of some Indian trees of economic importance. In: Chaturvedi SN and Singh KP (eds.) *Plant Reproductive and Molecular Biology*. Aaavishkar Publishers, Distributors, Jaipur. Pp.10-29.

Tidke JA 2005. Pollination ecology of *Crotalaria sericea* Retz. *J. Ind. Bot. Soc.* **84** 80- 84.

Tidke JA and Dharmakar RO 2003. Flowering phenology, pollen production and insect behavior in some ornamentals. *J. Physiology Res.* **16 (**1)73-76.

Veereshkumar, Kaushik SK, Rajarajan K, Kumaranag KM, Uthappa AR, Sridhar KB, Badre Alam B and Handa AK 2021. Pollination biology of *Pongamia pinnata* (L.) Pierre: a potential biodiesel plant. *Genetic Resources and Crop Evolution*. **68** 59–67.