

Assessment of changes in leaf biochemical constituents of palas (*Butea monosperma*) during different stages of lac insect (*Kerria lacca* Kerr) life cycle

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Abstract

Lac insect (*Kerria lacca* Kerr) is an important source of natural lac resin. Its cultivation has been strengthening the economy of farmers who depends on the forest resources for their livelihood. Palas (*Butea monosperma*) is a major host plant for lac insect cultivation. Being a phloem sap feeder, lac insect exerts stress on the host plant; therefore, to cope up with stress host plant undergoes a variety of biochemical and physiological changes. The present study was attempted to analyze the important biochemical changes in the host plant palas during different stages of rangeeni lac insect life cycle (baisakhi summer crop). The study revealed that lowest level of total sugar, soluble protein, free phenol and proline were found after lac insect mating stage (fertilization). The period after lac insect mating till maturity is critical period due to high demand of phloem sap for lac insect development. Therefore, it is necessary to maintain nutritional demands of host plants at critical period of lac insect development for achieving high lac production.

Introduction

Lac insect (*Kerria lacca* Kerr) is a commercially important insect species of family Tachardiidae (Kerriidae). It is cultivated mostly in the tropical forest region of South, Southeast and East Asian countries for non-wood forest products (NWFP) like lac resin, wax and dye which has commercial value (Ramani et al., 2007; Ranjan et al., 2011). India is a leading lac producer in the world and its cultivation is carried out mainly in Jharkhand, Chhattisgarh, Madhya Pradesh, West Bengal, and Odisha states. Lac insect completes its life cycle only on specific host plants and Palas is one of the leading host plants for lac culture along with other two i.e. *Kusum* (*Schleichera oleosa*) and *Ber* (*Ziziphus mauritiana*). It is medium size; deciduous tree (which sheds leaves) belongs to fabaceae family and widely distributed in tropical and subtropical area of India, Burma and Sri Lanka. It gives several valuable products which have commercial and medicinal importance-like gum, water soluble dye, fodder and leaves for platters etc. In addition to this Palas has been widely used for lac cultivation since ancient time (Lohot et al., 2016). The life cycle of lac insect starts with first instar larva also called nymph (crawlers) and they set out for three successive molting and become an adult where, only female survives (male dies after mating) and produces lac (Sharma et al., 2010). Lac insect fulfills its nutritional requirements from the phloem sap of host plants. Phloem sap feeders exert additional stress on host plants thereby, causing biochemical and physiological changes in the host plants (Dicke and Baldwin 2010; Mithofer and Boland 2012). The requirement of phloem sap is varying according to different life cycle stages of lac insect. Hence, host plants undergo various biochemical changes during different stage of lac insect life cycle to maintain themselves.

In recent decade, area under lac host plants rapidly declined due to deforestation etc., which led to concern over the sustainable lac production (Sharma et al., 2010; Mohansundaram et al 2014; Ghosh et al., 2017). Thus, the present study attempts to analyze the influence of lac insect feeding on biochemical response of Palas with respect to different developmental stages of lac insect. The obtained results

would help in elucidating the fundamentals of lac insect-host plant interaction during critical developmental stages of lac insect.

Materials and method

The present study was carried out at Plant Physiology and Biochemistry Laboratory of ICAR-National Institute of Secondary Agriculture, Namkum, Ranchi during 2017-18. Palas (*B. monosperma*) was used as host plant for the study. Seven trees were inoculated with *rangepeni* strain of lac insect (*Kerria lacca kerr*) and same number of trees without lac insect was served as control. The standard cultural/package of practices of lac cultivation were followed.

Sample Collection

Young, healthy and fresh leaves from selected plants were collected around 10 am from Institute Research Farm. Midrib and petiole were removed from leaves before crushing to get true representation of sample.

Biochemical Estimation

Total Sugar was estimated by phenol method (Dubois et al., 1951; Buysse and Merck, 1993). Estimation of total (Free) phenols was carried out by Folin-Ciocalteu method (Bray and Thorpe, 1954). Soluble protein was estimated by Lowry (1951) method. Photosynthetic pigments like total chlorophyll and carotenoid were estimated by DMSO method (Hiscox and Israelstam, 1979) and values were calculated according to Arnon (1949) and Litchenthaler and Wellburn (1983). The absorbance of all the biochemical constituents had recorded using spectrophotometer (Shimadzu-UV-1700 E 23 OCE) at specific wavelength for a particular constituent and comparing them with standard curve prepared by known amounts of that particular constituents. RBD analysis was done with standard statistical packages. The amount of total soluble sugar, soluble protein, and free phenols are presented in mg per gram fresh weight (mg/g fr.wt) whereas, total chlorophyll and carotenoid content was given in μg per gram fresh weight. In addition, percentage increase or decreases in biochemical constituents between lac insect infested and control plants during different stages of lac insect life cycle were also calculated.

Results

The obtained results showed remarkable differences in biochemical constituents in lac insect infested plants during different stages of lac insect life cycle. The amount of total soluble sugar in lac insect infested plants was highest at pre-sexual maturity period (36.4 mg/g fr. wt.) and then decreases after lac insect mating i.e. fertilization (6.7 mg/g fr. wt.) and maturation (8.92 mg/g fr. wt.) stage (Fig.1). Total sugar content of lac insect infested plants was also compared with non-infested plants during the various developmental stages of lac insect. It was found that percent increase in infested plants was more during the pre-sexual maturity period (58.58%) and percent increase drop off after fertilization stage (17.54%) and becomes negative towards maturity stage. When soluble protein was estimated, the

highest amount was observed at pre-sexual maturity stage (83.6 mg/g fr. wt.) and then decreases sharply after fertilization (35.1 mg/g fr. wt.) and maturation (41.41 mg/g fr. wt.) stage (Fig.1). In comparison to control plants, percentage increment of protein in infested plants was highest at pre-sexual maturity period (14.83%) and values are negative in other stages. In case of free phenol, highest level was found during pre-sexual maturity period (30.0 mg/g fr. wt.) and then decreases after fertilization (15.7 mg/g fr. wt.) and maturation stage (16.26 mg/g fr. wt.) (Fig.1). In comparison to control plants, free phenol was 59.16 % higher in infested plants at pre-sexual maturity period than other stages. Significant variations were also observed in plant pigments like total chlorophyll and carotenoid levels in lac insect infested plants. The total chlorophyll content was highest at maturation stage (109.65 µg/g fr. wt.) followed by stage after fertilization (50.51 µg/g fr. wt.) and pre-sexual maturity stage (34.76 µg/g fr. wt.) (Fig.2). Lac insect infested plants showed less amount of total chlorophyll than control plants in all the stages except at maturation stage (Table 2). The highest amount of carotenoid content (16.02 µg. g⁻¹ fr. wt.) was observed at maturation stage followed by stage after fertilization (11.72 µg/g fr. wt) (Fig. 2). In comparison to control plants, lac insect infested plants showed high amount of carotenoid in all the stages except pre-sexual maturity period (Table 2).

Discussion

In this study, lac insect was inoculated to the Palas tree in early November 2017, during the tree's leafy stage as it was preparing for its flowering and fruiting phase, which typically occurs in March and April month of the following year (Lohot et al., 2016). The first sampling was carried out in January 2018, during the pre-sexual maturity stage of lac insect, when the Palas tree was beginning to shed its leaves. Palas tree remained leafless from February to April (2018). Second and third samplings were taken in May and June 2018, when the tree have fresh developed leaves. Once the Palas tree entered its leafy stage, both the lac insect and the tree itself had significant demands for primary metabolites to support growth and development. This led to a noticeable reduction in total sugar, soluble protein, and free phenol levels in the leaves. The higher levels of total sugar and soluble protein observed during the pre-sexual maturity phase of the lac insect suggest that the insect's nutritional needs were relatively low as it was preparing for sexual differentiation. The decrease in these nutrients following post-fertilization stage could be attributed to the increased metabolic demands of the lac insect for embryo development and resin production. Additionally, the Palas tree itself requires metabolites for its own growth and development, which likely contributed to the depletion of these metabolites in the leaves. In a previous study on the bushy host plant *Flemingia semialata* (Ghosh et al, 2017; Lohot and Ghosh, 2018), it was observed that total sugar and soluble protein content increased following lac insect inoculation, with a significant decline as the lac insect approached maturity. This increase was notably rapid after post-fertilization stage (mating of female and male) compared to pre-sexual maturity stage. *F. semialata*, being a bushy host plant, has to meet the phloem sap demands of the lac insect throughout its various developmental stages. Due to its bushy form *F. semialata* stimulates the synthesis of metabolites to satisfy both the lac insect's nutritional requirements and its own growth needs. Furthermore, *Flemingia semialata* does not shed its leaves, allowing it to adjust to the continuous

feeding of lac insect. These findings suggest that host plant type (habit) and its growth developmental phases play a crucial role in the feeding behavior of the lac insect. In this context, lac insect feeding appears to have a considerable impact on the host plant's metabolite production pathways, particularly those related to sugars and proteins, which are diverted to meet the increased demands for resin synthesis by the lac insect.

It is well established that under biotic stress conditions, an increase in free phenol content in infested plants can inhibit larval development and growth by acting as a defense mechanism that deters insect feeding. The role of secondary metabolites, such as free phenols, in plant defense has been widely documented (Howe and Jander, 2008; Stam et al., 2014; Schuman and Baldwin, 2016; Helmi and Mohamed 2016). Phenolic compounds trigger various signaling pathways that enhance the production of toxic secondary metabolites and activate defensive enzymes (Kaur et al., 2017). In the present study, the phenol levels in lac insect-infested plants were initially elevated, but they later stabilized after the fertilization and maturity stages. This pattern suggests that the lac insect is capable of overcoming the host plant's resistance mechanisms to ensure its survival. A similar observation was made in *Flemingia semialata*, where lac insect-infested plants exhibited higher free phenol content compared to control plants (Lohot and Ghosh, 2018).

Leaf age plays a critical role in determining the content of photosynthetic pigments (Bhonwong et al., 2009; Bertamini and Nedunchezian, 2002). In the case of the Palas tree, the pre-sexual maturity and post-fertilization stages of the lac insect coincide with the development of new leaves, which mature as the lac insect reaches its later stages. Younger leaves typically have a less developed chlorophyll system compared to mature leaves. This could explain the lower total chlorophyll content observed during the pre-sexual maturity and post-fertilization stages of the lac insect, in contrast to the maturation stage when the leaves are fully developed. Carotenoids are well known for their ability to detoxify reactive oxygen species (ROS), a process critical in plant defense (Taiz and Zeiger, 1998). For example, in wheat, larvae of the Hessian fly (*Mayetiola destructor*) have been shown to cause a rapid increase in ROS levels (Liu et al., 2010). Similarly, studies by Dubey et al. (2013) reported an elevation in ROS and H₂O₂ concentrations in aphid- and whitefly-infested leaves. In this study, the increase in carotenoid content relative to total chlorophyll in lac insect-infested leaves suggests that carotenoids may play an important role in detoxifying ROS generated during the infestation. In our earlier research on *F. semialata*, we found that total chlorophyll content increased throughout the lac insect's life cycle, except during the maturation stage, which showed a similar pattern to the observations in this study. A comparable trend was also noted for carotenoid content (Lohot and Ghosh, 2018).

Conclusion

The period from post-fertilization (post-mating) to the maturity of the lac insect is a crucial phase in its life cycle. During this time, the female lac insect supports the development of embryos for the next generation while also producing lac resin. As a result, the lac insect has a huge demand for phloem sap to meet its nutritional needs, which in turn significantly impacts the biochemical composition of the host

plant. This stage represents a critical period of interaction between the lac insect and the host, with substantial alterations in the plant's metabolic profile.

Declarations

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Conflict of interest statement

On behalf of all authors, the corresponding author states that there is no conflict of interest.

References

1. Arnon DI (1949) Copper enzymes in isolated chloroplasts, polyphenol oxidase in *Beta vulgaris*. *Plant Physiol* 24:1-15.
2. Bertamini M, Nedunchezian N (2002) Leaf age effects on chlorophyll, Rubisco, photosynthetic electron transport activities and thylakoid membrane protein in field grown grapevine leaves. *J Plant Physiol* 159:799–803.
3. Bhonwong A, Stout MJ, Attajarusit J, Tantasawat P (2009) Defensive role of tomato polyphenol oxidase against cotton bollworm (*Helicoverpa armigera*) and beet armyworm (*Spodoptera exigua*). *J Chem Ecol* 35:28–38.
4. Bray HG, Thorpe WV (1954) Analysis of phenolic compounds of interest in metabolism. *Methods Biochem Anal* 1:27-52.
5. Buysse J, Merckx R (1993) An improved colorimetric method to quantify sugar content of plant tissue. *J Exp Bot* 44:1627-1629.
6. Dicke M, Baldwin IT (2010) The evolutionary context for herbivore-induced plant volatiles: beyond the “cry for help”. *Trends Plant Sci* 15:167-175.
7. Dubey, N.K., Goe, R., Ranjan, A., Idris, A., Sing, S.K., Bag, S.K., Chandrashekar, K., Pandey, K.D., Singh, P.K. and Sawant, S.V. 2013. Comparative transcriptome analysis of *Gossypium hirsutum* L. in response to sap sucking insects: aphid and whitefly. *BMC Genomics*, 14: 241-260.
8. Dubois N, Gilles K, Hamilton JK, Robers PA, Smith F (1951) A colorimetric method for determination of sugars. *Nature* 168-67.
9. Ghosh J, Lohot VD, Singhal V, Sinha NK (2017) Drought resilient *Flemingia semialata* Roxb. for improving lac productivity in drought prone ecologies. *Indian J Genet PI Br* 77:153-159.
10. Helmi A, Mohamed HI (2016) Biochemical and ultrastructural changes of some tomato cultivars after infestation with *Aphis gossypii* Glover (Hemiptera: Aphididae) at Qalyubiyah, Egypt. *Gesunde*

Pflanz 68:41–50.

11. Hiscox JD, Israelstam GF (1979) A method for extraction of chlorophyll from leaf tissue without maceration. *Can J Bot* 57:1332-1334.
12. Howe GA, Jander G (2008) Plant immunity to insect herbivores. *Annu Rev Plant Biol* 59:41-66.
13. Kaur H, Salh P K, Singh B (2017) Role of defense enzymes and phenolics in resistance of wheat crop (*Triticum aestivum* L.) towards aphid complex. *J Plant Interact* 12:304-311.
14. Lichtenthaler HK, Wellburn WR (1983) Determination of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. *Biochem Soc Trans* 11: 591-592.
15. Lohot VD, Ghosh J (2018) Biochemical response of *Flemingia semialata* (roxb.) to sap sucking lac insect *Kerria lacca* kerr. *Indian J Entomol* 80:1672-1677.
16. Lohot VD, Thamilarasi K, Ghosh J, Mohanasundaram A, Sharma KK (2016) 'Monograph on Palas (*Butea monosperma* Lam.) Taub.' ICAR-IINRG, Ranchi (Jharkhand) India 1-11.
17. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. *J Biol Chem* 193:265.
18. Mithofer A, Boland W (2012) Plant defense against herbivores: chemical aspects. *Annu Rev Plant Biol* 63:431-450.
19. Mohanasundaram, A., Monobrullah, Md., Sharma, K.K, Anees, K., Singh, R.K, Meena, S. C. and Verma, Sweta. (2014). Climate change: Effect of weather parameters on production of summer season crop of rangeeni strain of Indian lac insect, *Kerria lacca* (Kerr.) at Ranchi, Jharkhand. *J. Agrometeorol.*,16 (Special Issue-1), 108-113.
20. Ramani R, Baboo B, Goswami DN (2007) Lac- An introduction. Indian Lac Research Institute, Ranchi. 12.
21. Ranjan SK, Mallick CB, Saha D, Vidyarthi AS, Ramani R (2011) Genetic variations among species, races inbred lines of lac insects belonging to the genus *Kerria* (Homoptera, Tachardiidae). *Genet Mol Biol* 34:511-519.
22. Schuman MC, Baldwin IT (2016) The layers of plant responses to insect herbivores. *Annu Rev Entomol* 6:373–94.
23. Sharma KK, Monobrullah Md, Singh JP and Ramani R. 2010. Pre-summer mortality in rangeeni lac-insects. ICAR Newsletter 16(3): 15.
24. Sharma KK, Ramani R (2010). Morphology and Anatomy of Lac Insects. In Recent Advances in Lac Culture. Sharma K.K. and Ramani, R. (eds.). IINRG, Ranchi. pp. 37-45.
25. Stam JM, Kroes A, Li Y, Gols R, Van Loon JJA, Poelman EH, Dicke M (2014) Plant interactions with multiple insect herbivores: from community to genes. *Annu Rev Plant Biol* 65:689-713.
26. Taiz, L. and Zeiger, E. 1998. Photosynthesis: The Light Reactions, Plant Physiology (2nd Ed.). pp. 155-193.

Tables

Table 1. Biochemical constituent's variation in leaves of lac insect infested *B. monosperma* during different developmental stages of lac insect

Stages	Total sugar (mg g ⁻¹ fr. wt.)	Soluble Protein (mg g ⁻¹ fr. wt.)	Free Phenol (mg g ⁻¹ fr. wt.)	Total Chlorophyll (µg g ⁻¹ fr. wt.)	Carotenoid (µg g ⁻¹ fr. wt.)
Pre-Sexual Maturity Period	36.4 ± 1.27	83.6 ± 1.70	30.0 ± 1.19	34.76 ± 4.42	7.34 ± 0.4
After Fertilization	6.7 ± 0.31	35.1 ± 0.94	15.7 ± 0.57	50.51 ± 2.87	11.72 ± 1.04
At maturation	8.92 ± 0.63	41.14 ± 2.36	16.26 ± 0.36	109.65 ± 1.94	16.02 ± 0.55
CD	2.16	5.38	2.93	10.74	2.07

Table 2. Percent increase/decrease of biochemical constituents in leaves of lac insect infested *B. monosperma* during different developmental stages of lac insect over non infested condition

Stages	Total sugar	Soluble protein	Free Phenol	Total Chlorophyll	Carotenoid
Percent increase/decrease					
Pre-Sexual Maturity Period	58.58	14.83	59.16	-70.05	-36.14
After Fertilization	17.54	-14.94	15.06	-38.93	166.69
At maturation	-43.50	-20.51	15.06	3.18	15.36

Figures

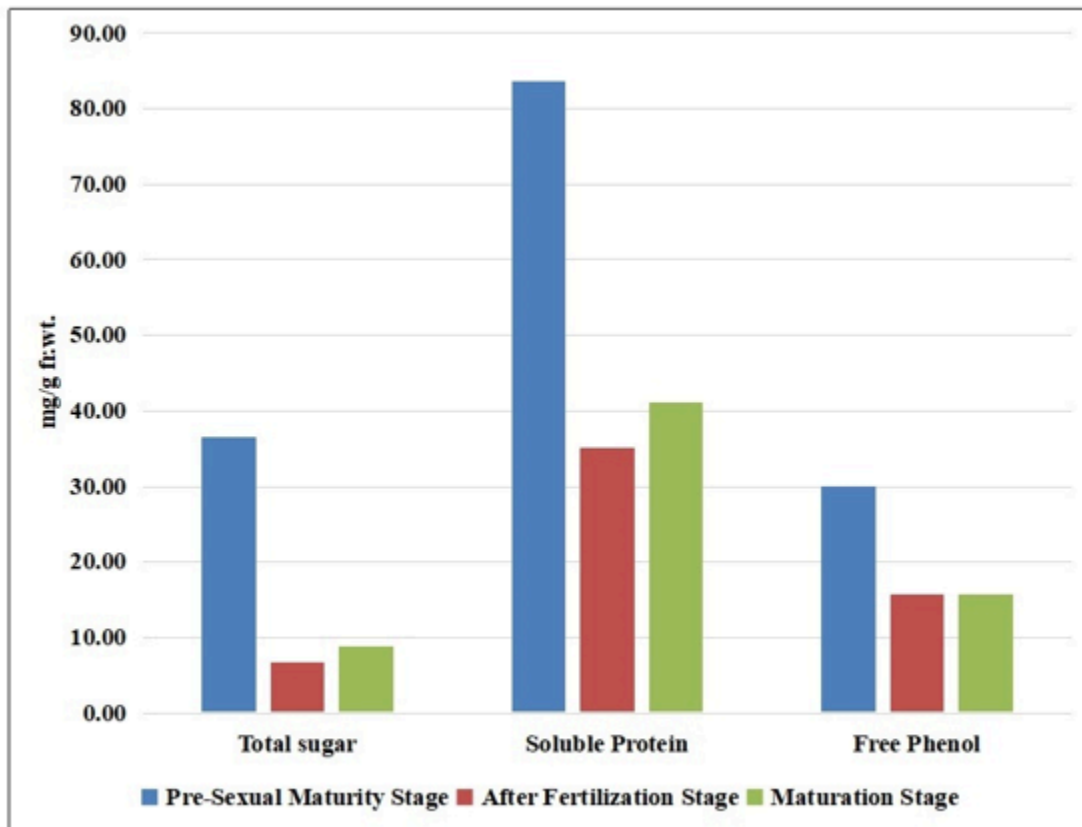


Figure 1

Effect of lac insect infestation on total sugar, proteins and free phenols content in *B. momosperma* leaves

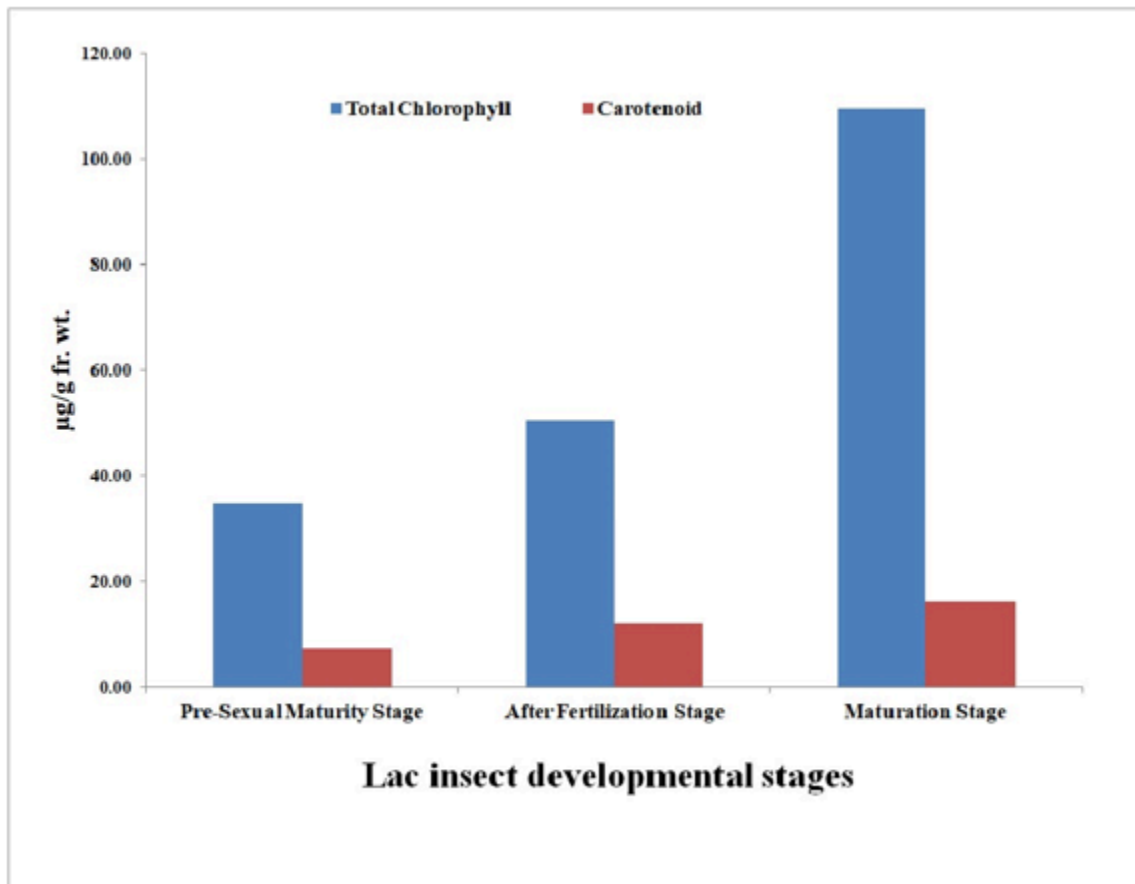


Figure 2

Effect of lac insect infestation on total chlorophyll and carotenoid content in *B. momosperma* leaves