International Research Journal of Natural and Applied Sciences
ISSN: (2349-4077)
Impact Factor 7.032 Volume 9, Issue 04, April 2022
Website- www.aarf.asia, Email : editor@aarf.asia , editoraarf@gmail.com

The Biochemical Studies of Mitragyana Parvifolia (Rubiaceae) (Roxb.) Korth's Reproductive Structure

Dr. Rajesh Kumar (Associate professor Botany)
SNKP Govt. College Neemkathana. District Sikar, Rajasthan (India)

Abstract

Mitragya parvifolia a perennial tree belonging family Rubiaceae is a medicinal plant. This tree commonly known as 'kaim' or 'kadamb'. Reproductive structure of *Mitragyana parvifolia* is stigma and pistil.Stigmatic exudates contain water, phenolic compound, sugar, proline . Phenolic compound are water soluble and most frequently occur combined with sugar as glycosides and play important role in pollen germination, pollen nutrition and promote the attachment of pollen grain on stigma. Proline is the major amino acid in pollen connected with pollen fertility and play important role in sexual reproduction, Proline contents of pistil increases after pollination.

Keywords : Rubiaceae; Proline; Phenolic compound; Reproductive structure

Introduction

Mitragya parvifolia a perennial tree belonging family Rubiaceae. Its a bisexual tree and reproductive structure is stigma and pistil. Stigma is Male Reproductive Unit and Pistil is Female Reproductive unit. Proline is an amino acid or building blocks of protein. Proline synthesis is required for pollen development and fertility and its also associate with fertilization. Phenolic compounds are water soluble they secondary metabolites and found in fruits, vegetables, since they most frequently occur combined with sugars as glycosides and they usually located in the cell vacuole. Among the natural phenolic compounds, of which over thousand structures are known, the flavonoids from the largest group but simple monocyclic phenols, phenylpronoids and phenolic quinones all exist in considerable numbers. They play important role in pollen germination and pollen nutrition inselective promotion of inhibition of pollen grains on the stigma(Martin, 1970; Martin and Rubert,1972; Tara and Namboodari,1974; Sedgley,1975). Fukasawa(1968) have reported an appreciably low amounts of sucrose in the anthers of male sterile plants.Veena (1984) has recorded that the pistil of the plants growing Mysore exhibited higher quantities of total nitrogen, total protein, total phenolics,IAA and GA3 like substance as compared to that shown by fruitless plants of Delhi which were deficient in these substance. These findings indicated the deficience of phenolics in the pistil of fruitless plant. The quantity of free prolines in the pistil was considerably at all stages of development and increased slightly in the pistil of open flower.

(Dhakre and Chauhan,1991). Singh(1993) has recorded that carbohydrate and proteins were present in the stigmatic exudates of *Tecoma stans* during seedless, partially seedbearing periods . Phenilocs and lipids were more or less absent during seedbearing periods. Proline of pollen readily diffuse into pistil tissues and as models experiments with L-Proline –L-Proline-C" have shown, is metabolically rapidly incorporated. Proline contents of pistils increases after pollination. It is believed that proline present in the pollen grains and finally in pollen tubes enhances proline content pistil (Britikov *et al*., 1964). According to Singh (1988), the quantity of proline in pistils of seedbearing plants of *Millingtonia hortensis g*rowing at Jammu increases after pollination a compared to that in pistils of this species at Agra which remain unfertilized.

Material and Methods:

BIOCHEMICAL STUDIES:

Micro Chemical tests: For micro chemical tests of sugar, proteins, phenolics and lipids, stigmas from floral buds of different stages were boiled in distilled water and tests were made in filtrate of stigmatic exudates. Various tests were made following the procedures given by Mann and Saunders(1960).

Sugars:i.Fehling's Test:In 5ml extract of stigmatic exudates, 5ml of fehling's solution was added and boiled. Reduction takes place and red precipitate of cuprous oxide is formed. At first, it might be yellow but becomes red on standing.ii. Benedict's Test:Added 3ml of Benedict's solution of 1 ml of extract and warmed after heating reddish brown precipitate was obtained to indicate the presence of sugar.

Proteins: i.Biuret Test:Added 2ml of alkaline copper sulphate to the stigmatic extract and mixed thoroughly. Reddish violet colour confirmed the presence of proteins. ii.Millions Test:Added a few drops of millions reagent to the filtrate and heated Blue colour confirmed the presence of proteins,

Lipids:Added a few drops of sudan black B solution (0.5% in 70% alcohol) to small quantity of filtrate and shaken well. A red colour confirmed the presence of lipids.

Phenols:To the aqueous extract to stigmatic exudates a drop of ferric chloride solution was added.

i. Resorcinol and full cresol gave violet or blue colour. ii. Catechol and hydroquinone gave green colour and further addition of FeCl3. Yellow solution of p-benzoquinone was obtained.

Quantitative Estimation of Total Phenolies in the Anthers and Pistils: Total phenolic compounds estimated in the stigmas following the methods after Bhatia et al., (1972).1gm of dry material was refluxed with 50ml methyl alcohol for 4 hours in soxhlet. Excess alcohol was evaporated at reduced pressure. The contents then brought to a constant volume of 100ml by distilled water. To suitable aliquot of this extract 0.5ml of folin Dennis reagent was added and tube was shaken well exactly for 3 minutes to this. 1 ml solution of saturated sodium bi carbonate was added. It was again shaken and volume was made to 15ml by adding distilled water. It was kept in dark for 1 hour and was read at 725nm on a calorimeter. Total phenolics were calculated from standard curve-10mg of tannic acid was dissolved in 100ml off distilled water and standard were made.

Quantitative Estimation of Free Proline in the Anthers and Pistils : Free proline in the anthers and pistils was estimated by following methods of Bates *et al.*, (1973) .

1. Fresh anthers (200 mg) at different stages of development were homogenized with 5 ml of 3% sulphosalycylic acid.
2. Centrifuged and supernatant was decanted.
3. In the supernatant 5ml glacial acetic acid and freshly prepared ninhydrin solution (1.25g ninhydrin was added in mixture of 8 ml orthophosphoric acid, 30ml acetic acid and 12ml of distilled water by warming and shaking it was cooled and kept in refrigerator at 4°c) was added.
4. The mixture was boiled for hour in a water bath then cooled at room temp.
5. 10 ml toluene or benzene was added to the mixture in separating funnel for extraction.
6. Chromatographia containing benzene was separated and allowed to stand for sometime fill it become clear.
7. Transmittance was read at 520 mm. Standard curve was prepared by using pus poline (11)

Quantitative Estimation of Reducing Sugar in the Anthers and Pistil: Pipette 0.1ml of the test solution containing 50-150mm of sugar into 1.5ml of water and thoroughly mixed. Added 0.2ml of the barium hydroxide solution the mixture followed by 0.2ml of aqueous Zinc sulphate with thorough shaking and centrifuged, Used Iml of the supernatant for further analysis.1ml of the alkaline copper reagent was added to the sample and mix well. Place a marble on top of each and heat on a boiling water bath for 15min. The tubes were cooled in and arsenomolybdate reagent was added, and left to stand for a minute until the effervescence ceases, Blue colour was diluted with water to a final volume of 100ml (or any other convenient volume) and read the extinction at 510 nm.

Result and Discussion

Micro Chemical Teste. The result of the microchemical test of the stigmatic exudates of*M. Parvifolia*are show the presence of carbohydrate and phenolics. However, proteins and lipids are present in trace(Table 01).

Table (01). Microchemical test of stigmatic exudates of *M. Parvifolia.*

S.No.	Chemicals	Result
1.	Carbohydrate	Present
2.	Proteins	Trace
3.	Phenolics	Present
4.	Lipids	Present

The amount of stigmatic exudates on the stigma and its composition varies m species to species. Lipids, phenolics, carbohydrate, amino acids and proteins are generally present in exudates (Baker *et al*., 1974 and Vasil, 1974) Chauhan *et al.* (1986) have reported absence of sugars, phenolics and lipids on the stigmatic surface of seedless plants *Clerodendron splendens*. Dhakre and Chauhan (1991) have shown the complete absence of phenolics, proteins and lipids on the stigmatic surface at different stage of development in the seedless *Campsis grandiflora* (Bignoniaceae). However, sugars have been found to be present in traces in the stigmatic exudates at early stages of megasporogenesis i.e. at megaspore mother cell stage and 4-8 nucleate embryo sac stage.

Quantitative Estimation of Total Phenolies in Anthers and Pistils :

In anther the quantity of total phenolics of *M. Parvifolia*during early flowering period (May-June)at immature bud stage (11^{th} day after bud initiation) is low (0.025 mg/g), but with the

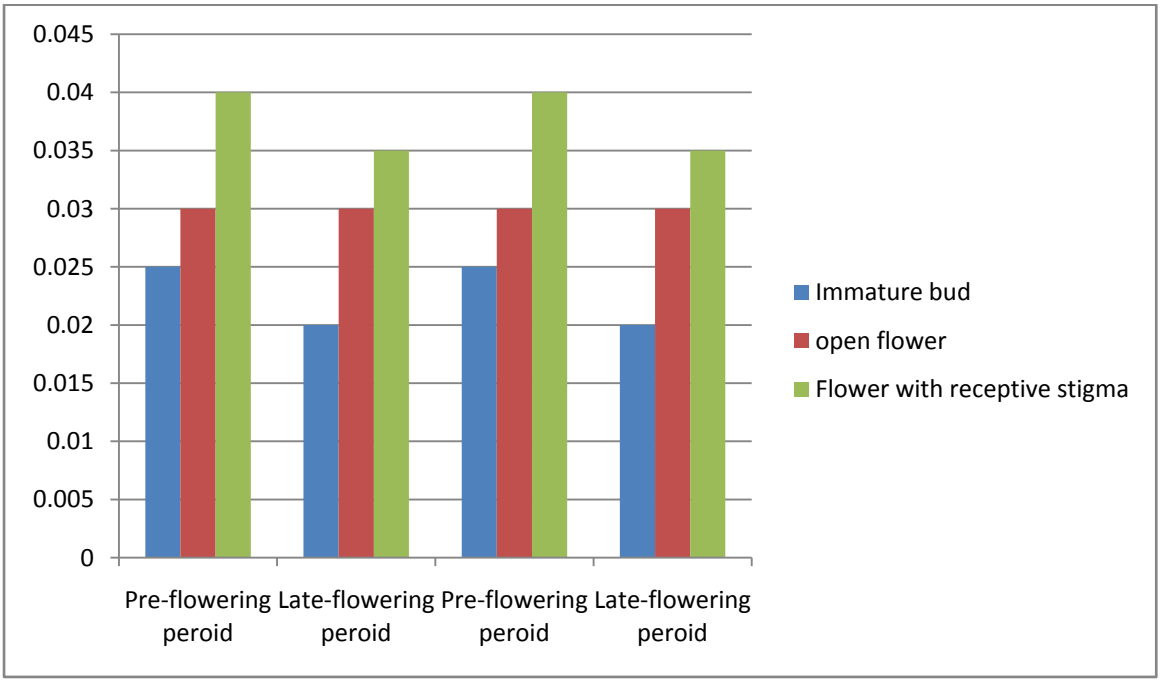
open flowers stage (13th day after bud initiation) and flower with receptive stigma stage (14th day after bud initiation) the values increase gradually. The quantity of total phenolics is higher at open flower stage (0.03 mg/g) and is maximum at the flower with receptive stigma stage (0.04 mg/g). It is interesting to note that the quantity of total phenolics in the anthers during late flowering period (July August), at Immature bud stage is low (0.02mg/g), However, it is 0.03 and 0.035 mg/g at the open flowers stage and flower with receptive stigma stage which is comparatively lower than the early-flowering pod (Fig 01).

In pistils the quantity of total phenolics of *M. Parvifolia* during early flowering period (May-June) at immature bud stage is low (0.02 mg/g), but at the open flowers and flower with receptive stigma stages the value increase gradually. The quantity of total phenolics is higher at open flower stage (0.133 mg/g) and is maximum at the flower with receptive stage(0.034mg/g). It is interesting to note that the quantity of total phenolics in the pistil during late flowering period (July-August), at immature bud stage is low (0.025mg/g). However, it is 0.035 and 0.045 mg/g at the open flowers stage and flower with receptive stigma stage which is comparatively lower than the early-flowering period (Fig 01).

Present observations have clearly indicated that the quantity of total phenolics in the anthers and pistils in Mitragyna parvifolia during early and late flowering periods at all the developing stages showed significant differences During early flowering period, the quantity of total phenolics is low in pre-anthesis stage and it increases gradually in post-anthesis stage. While the quantity of total phenolics increases considerably at all stages of development.

Phenolics compounds are water soluble and they most frequently occur combined with sugar as glycosides. Phenolics substances occur in exudates of stigmas of most and not all ester and glycosides of hydrocinnamic acids (Martin, 1969)

Fig. 01. Quantitative estimation of total phenolics in all developmental stages of anther and pistil of *M. Parvifolia.*



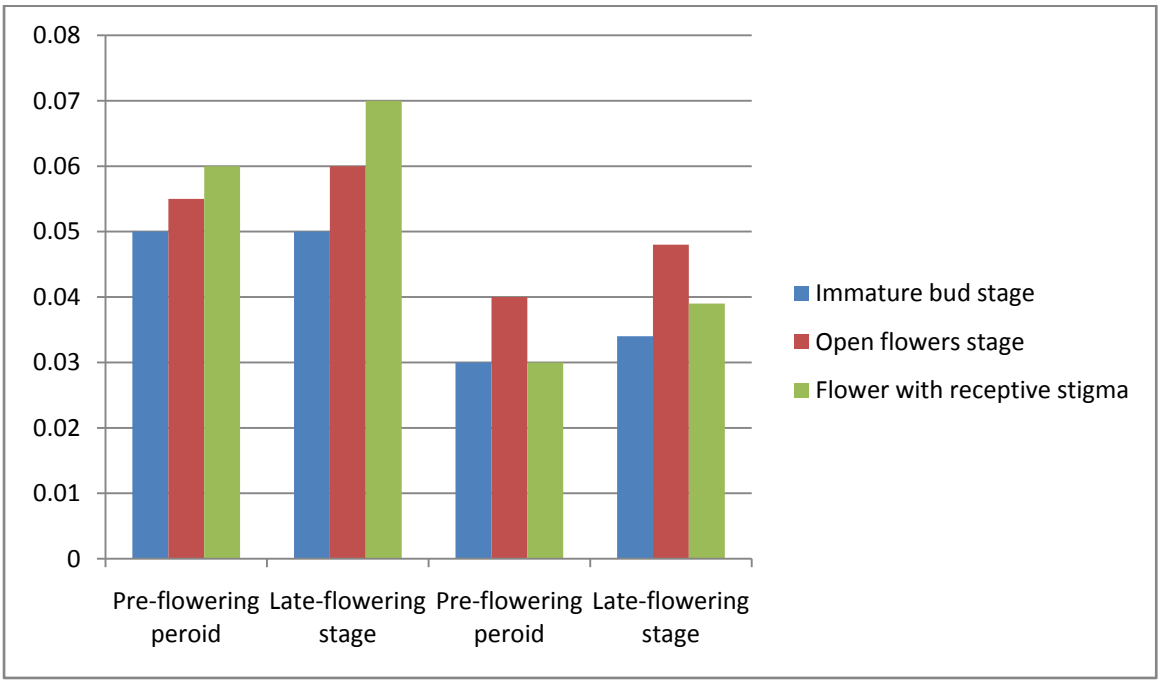
Quantitative Estimation of Free Proline in Anthers and Pistils:

In anthers the quantity of free proline in the anthers of *M. parvifolia* during the early flowering period (May-June) when the plants exhibit only 40-47% pollen fertility, when temperature during this period is high 38-42°C with low RH (20-30)%, the anthers at all stages development contain only traces of free proline. The quantity of free proline increases during various developing stages of anther development (immature bud, open flowers, fower with receptive stigma). The quantity of free proline at immature bud stage is est (0.05 mg/g), it is 0.055 and 0.06 mg/g at open flowers and flower with receptive stigma stage respectively (Fig 02).

In pistils the quantity of free proline in the pistils of *M. parvifolia*During the early- flowering period (May-June), the quantity of free proline in the immature bud stage is low (0.03 mg/g). At the open flowers and flower with receptive stigma stages, the quantity of free proline is 0.04 and 0.03 mg/g respectively.During late-flowering period (July-August), the quantity of free proline in the immature bud stage is 0.034 mg/g. However, it increases and reaches to the maximum at the open flowers and flower with receptive stigma stages are (0.048 and 0,039 mg/g) respectively (Fig. 02)

Foregoing observations have clearly indicated that the quantity of fee proline in *M. parvifolia* during early-flowering and late flowering peroid at all stages of pistil and anther development showed significant differences. During early flowering period the amount of free proline in authers and pistil is low at every stage as compared to that late flowering period

Fig. 02. Quantitative estimation of total proline in all developmental stages of anther and pistil of *M. Parvifolia.*



Proline, a major amino acid connected with pollen fertility is believed to play important role in sexual reproduction in plants (Tupy, 1964, Britikoy *et al*., 1964) have analyzed the quantity of free and partially bound proline in pollen and pistil at times, in vegetative organs of about 200 plant species belonging to 63 families of 42 orders of Angiospermae and Gynospermae. According to them, proline of pollen is involved in more fundamental reaction

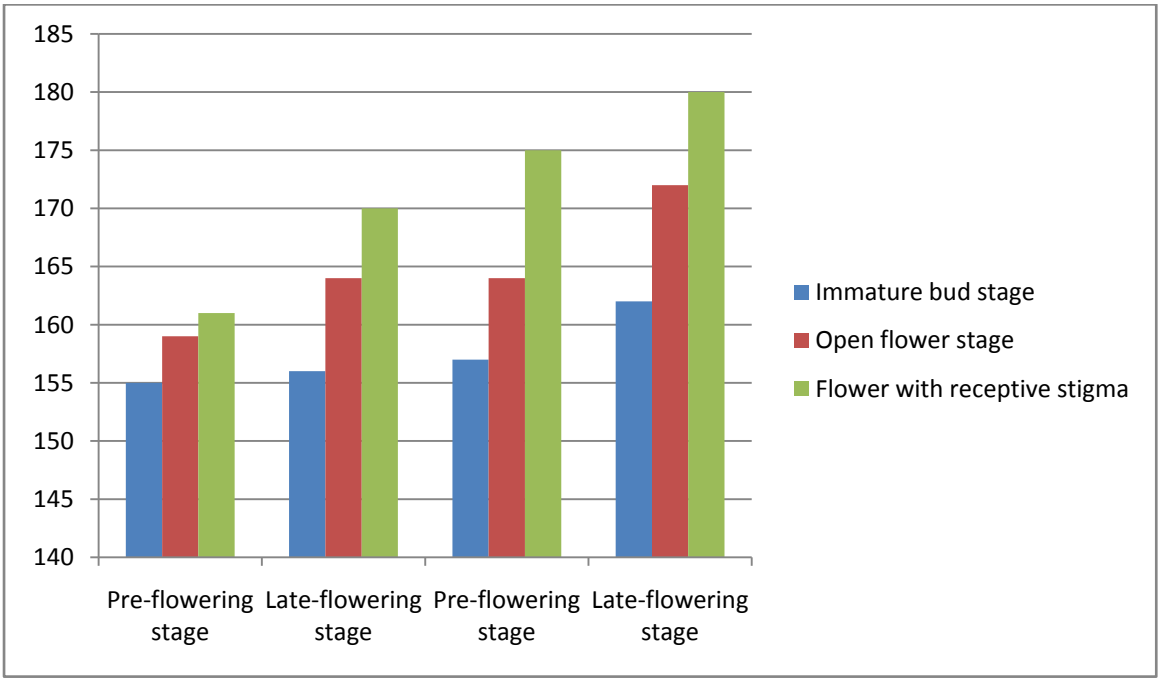
of different sexual types and is utilized in the direct intersection of pollen and pistil tissues following pollination. They have also experimentally proved that proline of pollen readily diffuses into pistil tissue and as models experiments with L-proline-C 14 ho shown that it is metabolically rapidly in cooperated after pollination. It is believed that proline in the pollen grains and finally in pollen tubes enhances proline content of the pistil. The present observations also support this fact because during early flowering period, when the anthers showed lower percentage of pollen fertility with reduced amount of free proline in months of May and June. The amount free proline in the pistils in pre-pollination and post-pollination stages was also low as compared to that in late flowering period. It increased nearly 50-80 percent during late flowering period. High rates of pollen germination after pollination showed considerable increase in free proline content in the pistil at post-pollination stage.

Quantitative Estimation of Reducing Sugar in Anther and Pistil:

In anther the quantity of reducing sugars in anthers of *M. parvifolia*at immature bud stage is lowest (155 mg/ml) However, the quantity of reducing sugar increases gradually, at the open flowers and flower with receptive stigma stages are (159 and 161 mg/ml) respectively. During late-flowering period (July-August), the quantity of reducing sugar in the immature bud stage is 156 mg/ml. However, it increases and reaches to the maximum at the open flowers and flower with receptive stigma stages are (164 and 170 mg/ml) respectively (Fig. 03).

Pistil : The quantity of reducing sugar in the pistils of *M. Parvifolia*during the early flowering period (May-June), the quantity of reducing sugar in the immature bus stage is low (157 mg/ml). At the open flower and flower with receptive stigma stage, the quantity of reducing sugar are 164 and 175 mg/ml respectively. During late-flowering period (July August), the quantity of reducing sugar in the immature bud stage is 162 mg/ml. However, it increase and reaches to the maximum at the open flower and flower with receptive stigma stages are 172 and 180 mg/ml respectively (Fig 03).

Fig. 03. Quantitative estimation of total reducing sugar in all developmental stages of anther and pistil of *M. Parvifolia.*



The present observations have clearly indicated significant difference in the quantity of reducing sugar in the anthers and stigma of *Mitragyna parvifolia* during early and late-flowering periods at all the developing stages. During early flowering period, the quantity of reducing sugar in the nectar of flowers was low in pre-anthesis as compared to late flowering period.

Nectar is a major and primary attractant and is a consequence of different complex physiological process of special glands called' nectaries'. It is derivative of phloem lamp (Gunning and Steer, 1975). Nectar sugar instant energy to the flower visitors. Common sugar present in nectar are the hexose monosaccharides, glucose, fructose and the disccharide sucrose. These sugars occurs in combination or separately. Baker and Baker (1982) called these common sugars as 'big-three sugars'. Among the sugars, arabinose and galactose are frequent (Gattosberger *et al*, 1973; Watt *et al*., 1974), while mannose is rarely found in the floral nectar.

Sugars and amino acid was present in the nectar, while alkaloids, lipids, phenols ard reducing acids were absent. The range of sugar concentration was 21-30% *M.cardifolia* has a balanced sugar ratio (Galetto, 1989).

Conclusion

The biochemical studies revealed that the Stigmatic exudates showed the presence of Carbohydrates and phenolics, while proteins and lipids were more or less absent. The quantity of free proline in the anthers and pistils during early flowering period was low. The quantity free proline increased gradually as the flowering period progresses towards late flowering period. The quantity of total phenolics in the anthers and pistils was low in early flowering period as compared to late flowering period. The quantity of total phenolics was low in pre-anthesis stage and it increased gradually in post-anthesis stage. The quantity increased considerably at all the stages of development. The quantity of reducing sugar in anthers and pistils was higher in late flowering period. The quantity of reducing sugar in the nectar of flowers is low in mature bud stage as compared to late flowering period.

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