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Biochemical methods for identification of male and female plants in *Garcinia gummigutta*

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ABSTRACT

Garcinia gummigutta, belonging to Guttiferae family is a medium sized, evergreen tree, mostly found in hilly tracks of Western Ghats of South India and also distributed over South East Asia. *Garcinia gummigutta* is a dioecious plant, whose male and female plants are separate. Gender identification methods have been developed for this species based on peroxidase isoenzyme studies. Apart from laboratory methods, a field method has also been developed to identify male and female plants.

KEYWORDS

Garcinia gummigutta;
Gender identification;
Peroxidase enzyme;
PAGE electrophoresis;
Field method.

INTRODUCTION

Garcinia gummigutta is one of the 400 species in Garcinia groups belonging to Guttiferae family. It is a medium sized, evergreen tree, mostly found in hilly tracks of Western Ghats. It is also distributed over South East Asia. The fruit rind of tree is very important and is used as a substitute for tamarind to impart flavour. It has got medicinal properties too. The anti obesity property of L–hydroxy citric acid (HCA) present in fruits has gained much importance in present days and generated great demand for the fruits..

Garcinia gummigutta is a dioecious plant, who's male and female plants are separate. The plant is propagated from seeds. In its cultivation or in natural condition, the presence of one male plant is essential for every seven to eight plants. But gender of a plant will be known only after six to eight years, when female plants start bearing fruits. In case of *Bursera penicillita*, a dioecious plant, we have reported earlier that peroxidase isoenzyme pattern could be used as index for gender identification, Parthasarathi *et al*[3] and Parthasarathi and Angadi[4]. Recently, we have reported simple field method for gender identification of *Myristica fragrans Houtt*, Angadi *et al*[2]. But no such reports are available for *Garcinia gummigutta* at laboratory or field level. Hence the present study.

MATERIALS AND METHODS

In the present study, the difference in peroxidase enzyme activity in leaf and tender twig tissue was used for identification of male and female plants. Following three methods were developed based on peroxidase enzyme (POD) activity.

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1. Qualitative method using isozyme studies – Laboratory method.
2. Quantitative method for gender identification based on peroxidase enzyme activity – Laboratory method.
3. Simple, less expensive and user friendly colour reaction - a field method to be used by forester / farmer / common man.

Qualitative method using isozyme studies - laboratory method

For the experiment, leaf and tender twig tissue were used as a source of enzyme. In case of leaf tissue, healthy, green and matured tissue was used for the study. Healthy tender twig tissue of 0.3 cm internal diameter was used as source of POD.

Extracting medium**:** Ascorbic acid at 0.1 % was used as extracting medium and 10 ml of solution was used for 2 gm of both leaf and tender twig tissue.

Loading concentration for electrophoresis**:** For loading, 0.1 ml of the enzyme extract was used both for leaf and tender twig tissue. All other methodologies followed in the experiment have been described earlier, Parthasarathi *et al*[3].

Quantitative method for gender identification based on peroxidase enzyme activity - laboratory method

In this method, difference in activity of peroxidase enzyme in male and female leaf tissue was used for gender identification. The POD in leaf tissue can be estimated by using the method described here. Enzyme was extracted from the tissue as described above. Activity of POD was assayed at room temp in 50 ml conical flasks containing 5.0 ml Tris -Glycine buffer (pH 9), 1.0 ml of 1 % Guaiacol in 50 % ethyl alcohol, 1.0 ml 0.33 % hydrogen peroxide, 2.9 ml of distilled water and 0.1 ml of enzyme solution. Solution containing 5.0 ml Tris -Glycine buffer (pH 9), 1.0 ml of 1 % Guaiacol in 50 % ethyl alcohol, 1.0 ml 0.33 % hydrogen peroxidase and 3.0 ml of distilled water acts as blank. The reaction was initiated by the addition of enzyme and the flask was thereafter swirled continuously. Absorbance at 470 nm was measured at 1 minute interval and POD activity was expressed as increase in absorbance (A) or O.D (Optical density) at 470 nm per minute (TABLE 1).

TABLE 1 : Peroxidase enzyme activities in leaf tissue of male and female plants of *Garcinia gummigutta*

Enzyme source: Healthy, green and matured leaf tissue
Quantity of enzyme used in reaction mixture: 20 mg
Optical density measured at 470 nm

Time in minute	Increase in absorbance i.e. A at 470 nm	
	Male	Female
1 Min	0.124	0.007
2 Min	0.098	0.009
3 Min	0.111	0.008
4 Min	0.122	0.009
5 Min	0.118	0.007
6 Min	0.124	0.008
	Avg : 0.116	**Avg : 0.008**

Simple, less expensive and user friendly colour reaction for gender identification - a field method to be used by forester / farmer / common man

The above two methods developed for gender identification are rather time consuming or non field oriented. Keeping this fact in view, an attempt was made to develop a simple field method to distinguish male and female plants of *Garcinia gummigutta* on lines of the colour reaction developed for identification of high yielders of sandal in the field, Angadi *et al*[1]. For this purpose, tender twig tissues and leaf mid rib tissues were used as source of POD for colour reaction. In case of tender twig tissue, the tissue size of (whole tender twig tissue) 30 x 0.3 cm (l x b) was selected; cut into small bits and taken into 50 ml conical flask containing peroxidase reagent to study the colour reaction. In case of leaf mid rib tissue, tissue size of 30 cm was selected; cut into small bits and taken into 50 ml conical flask containing peroxidase reagent to study the colour reaction.

Following two peroxidase reagents were used to develop colour reaction.

Guaiacol peroxidase reagent (GPR)

The substrate used in GPR reaction was guaiacol. A reaction mixture consisting of 2 ml of Tris- Glycine buffer pH 9, 2 ml of 1 % hydrogen peroxide and 2 ml of 1% Guaiacol in 50% ethanol was used in GPR reaction. Tris-Glycine buffer and hydrogen peroxide were first mixed and reaction was initiated by adding guaiacol solution. The contents in the conical flask were shaked well occasionally for 30 minutes. At the end of 30 minutes, reaction was stopped by adding 0.2 ml of

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1 N hydrochloric acid. The colour of the solution was then noted.

Bezidine peroxidase reagent (BPR)

The substrate used in BPR reaction was benzidine. A reaction mixture consisting of 2 ml of Tris- Glycine buffer pH 9, 2 ml of 1% hydrogen peroxide and 2 ml of 1% benzidine in 25 % acetic acid was used in BPR reaction. Tris-Glycine buffer and hydrogen peroxide were first mixed and reaction was initiated by adding benzidine solution. The contents in the conical flask were shaked well occasionally for 15 minutes. At the end of 15 minutes, reaction was stopped by adding 0.2 ml of 1 N hydrochloric acid. The colour of the solution was then noted.

RESULTS AND DISCUSSION

Qualitative method

Pattern of multi-molecular forms of POD in case of leaf tissue is illustrated in the given figure 1. The male plant was represented by 3 bands (Rf values: 0.4, 0.76 and 0.86). In case of female plant, no bands were formed and gel was clear and transparent.

Pattern of multi-molecular forms of POD in case of tender twig tissue is illustrated in the figure 2. The male plant was represented by 3 bands (Rf values: 0.88, 0.92 and 0.96) and the female plant is represented by only one band (Rf value: 0.92). Thus bands with Rf values 0.88 and 0.96 are representing male genes.

The difference in banding pattern of multi-molecular forms of POD both in leaf and tender twig tissue could be of helpful in separating male and female plants of *Garcinia gummigutta*

Quantitative method

Peroxidase enzyme activities in leaf tissue of male and female plants of Garcinia gummigutta are given in TABLE 1.

From the results, it is found that POD activity in male tissue is more (average A is 0.116) than female tissue (0.008) and is comparable. The method also could be helpful in identifying male and female plants.

Field method

In case of GPR reaction, green or yellowish green colour with turbidity is formed for male tissue and clear solution with no colour is formed for female tissue (Figure 3). In case of BPR reaction, dark brownish black colour with turbidity is formed for male tissue and light brownish black colour is formed for female tissue (Figure 4). The difference in colour for male and female tissue is comparable and could be used for identification of male and female plants. It is very important to note that, the above colour reactions should be carried out within one hour of sample collection. Otherwise samples should be kept at 0° C in refrigerator before use (up to 4 to 6 hours).

Laboratory methods are certainly more accurate and reliable. The field method is also reliable provided

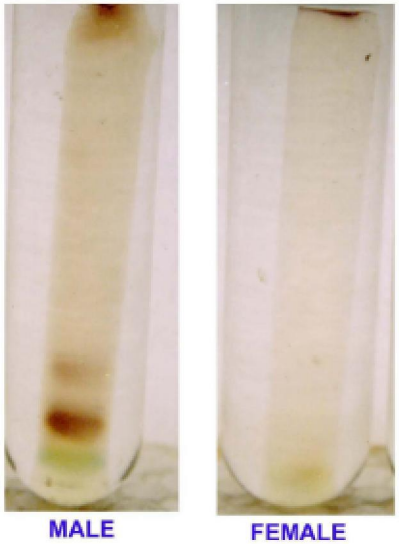

Figure 1 : *Garcinia gummigutta* - Peroxidase isoenzyme pattern

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all precautions are taken and procedures done properly. It can be performed by any one with a little training and gender of the sapling could be known before planting to maintain male female ratio.

ABBREVIATIONS

BPR: Bezidine Peroxidase Reagent
GPR: Guaiacol Peroxidase Reagent
HCA: L–hydroxy citric acid
OD: Optical density
POD: Peroxidase enzyme

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