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Plant regeneration and induction of coloured callus from henna (*Lawsonia inermis* syn. *Lawsonia alba)*

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Abstract

Tissue culture studies of *Lawsonia inermis*, syn. *Lawsonia alba* (henna) were carried out to induce multiple shoots, plant regeneration and coloured callus on MS media supplemented with various hormones. Various explants, such as sections of leaves, stems and roots, from 4-month-old aseptic seedlings were used. Different concentrations of 2,4-dichlorophenoxyacetic acid (2,4-D), napthalene acetic acid (NAA), benzyl aminopurine (BAP), kinetin, and indole acetic acid (IAA) hormones were used. The results showed that efficient regeneration system for henna plant *in vitro* could be achieved. Different coloured callus were obtained from henna tissue culture on MS supplemented with different combinations and concentrations of hormones 0.5 mg/L 2,4-D and 0.5 mg/L IAA, 2.0 mg/L NAA and 2.0 mg/L BAP, 0.5 mg/L NAA and 0.5 mg/L BAP, 1.5 mg/L NAA and 1.5 mg/L BAP, and also 0.5 mg/L IAA and 0.5 mg/L BAP, and thus production of important secondary metabolites *in vitro* is possible for this important species. Formation of embryogenic callus was observed during the plant morphogenesis in MS medium supplemented with combination of 0.5 mg/L IAA and 0.5 mg/L 2,4-D hormones which will enhance more production of *in vitro* plantlets and ultimately promote new propagules of this species.

Key words: Tissue culture, Murashige and Skoog (MS), multiple shoot, aseptic seedlings, 2,4-dichlorophenoxyacetic acid (2,4-D), napthalene acetic acid (NAA), benzyl aminopurine (BAP), indole acetic acid (IAA), *in vitro,* embryogenic callus.

Introduction

Henna (*Lawsonia inermis*) is under the family Lythraceae. It is best known as a source of natural dyes. It is a native of North Africa and South-West Asia, widely cultivated as an ornamental hedge and dye plant. *L. inermis* contains lawsone, a red–orange pigment. Chemically, the molecule of lawsone is 2-hydroxy-1,4-naphthoquinone [1]. The molecule known as hennotannic acid is the chief constituent of henna leaves. The flowers are yellowish white to brick red, and the fruit is a dry berry. The seeds are blue-black, angular and small [2]. This plant has been used for centuries, in many cultures mainly as a dye for hair and nails [3]. The secondary metabolites found in henna can be used for medicinal purposes. Dry powdered henna leaves contain lawsone, mannite, tannic acid, mucillage, gallic acid and naphtoquinone. In ancient times, henna was recommended as a remedy for jaundice, leprosy, smallpox, and skin complaints [2]. Previous studies showed that henna possess antimicrobial activity, antitubercular activity and anti-inflammatory activity [4]. Therefore, the present research was done on this species due to the high medicinal properties of the plant.

However, tissue culture work on this species is lacking. Very few work reported on the regeneration of this plant *in vitro.* Based on previous work, it shows that, in order to improve the regenerative capacity of *L. inermis*, several nutritional factors such as Fe-EDTA must be added to enhance adventitious shoot formation [5]. Previous studies [6] reported that plant tissue culture produce offspring identical to the parent plant, and callus has the potential in micropropagation and to extract secondary metabolites. Callus is non-organized cell which can be stimulated to embryogenic organization.

Production of embryogenic callus is desirable for plant regeneration because embryogenic callus culture offers the greatest opportunity for high regeneration capacity in relation to those derived from other explants [7]. Hartmann *et al.* [8] reported that somatic embryogenesis would create a complete reversion from a mature state to a juvenile state. Previous studies reported that somatic embryogenesis shows several advantages compared to other *in vitro* propagation systems. It has high multiplication rates, the potential to scale up in liquid suspension cultures and is suitable target tissues for gene transfer [9]. High amount of callus formation from desired explants is important for plant regeneration because callus culture offers the greatest opportunity for *in vitro* selection and production of genetic variability. Desired secondary metabolite can be manipulated according to conditions required from the callus [10]. Studies by Behrouz and Rosna [11] show that there are differences between embryogenic and non-embryogenic calluses. The embryogenic cells were surrounded by straight and thick walls, while in non-embryogenic callus the cell walls were wavy and thin.

Plant tissue culture generally requires supplement of plant hormones such as auxin and cytokinin to the nutrient medium. Various explant sources of henna plant cultured on MS media supplemented with different combinations and concentrations of hormones induced coloured callus, embryogenic callus, and multiple shoots formation. Sanavy and Moeini [12] reported that different factors such as type of medium, pH, sucrose concentration, type of hormone, temperature and light conditions can affect propagation and growth of plantlets from meristem culture under *in vitro* conditions. Germination of seed is important and useful when there is a need to obtain a uniform set of seedlings, and seed sterilization methods help to reduce the contamination problem that arise from the seed coats [13]. The

present studies were carried out to propagate henna species and to induce coloured callus from this species.

Materials and Methods

Seeds of *Lawsonia inermis* were purchased from Sand Mountain Herbs, Larry Chandler, U. S. A. Seeds were sterilized by soaking in chlorox solution of 70%, 50%, 30% and 10% for 5 min each. After that the seeds were rinsed three times with sterile distilled water for 5 min. They were surface sterilized with 70% alcohol in the laminar flow. Finally the seeds were rinsed again with sterile distilled water three times. Each sterilized seed was cultured on MS medium without hormone for explants sources (Fig. 1).

Henna explants from aseptic seedlings (2 months old) were cultured on MS medium with different combinations and concentrations of hormones. The initial pH of the medium was adjusted to 5.8 with 1 N sodium hydroxide (NaOH) or 1 N hydrochloric acid (HCl) before autoclaving (121°C, 20 psi, 20 min). The cultures were maintained under control environment at 25±1°C with 16 hours photoperiod and 8 hours dark. The combinations and concentrations of hormones used were: 1) MS + 0.5 NAA + 0.5 BAP; 2) MS + 1.0 NAA + 1.0 BAP; 3). MS + 1.5 NAA + 1.5 BAP; 4) MS + 0.5 IAA + 0.5 2,4-D and 5) MS + 0.5 IAA + 0.5 BAP. After 5 weeks of culture those explant segments forming multiple shoots (Fig. 2), coloured callus (Fig. 3) and embryogenic callus (Fig. 4) were recorded.

Results and Discussion

The most responsive explant was stem obtained from 5-week-old aseptic seedlings. There was no formation of multiple shoots from leaf or root explants. This results can be improved by adding ascorbic acid in culture medium. This is because previous *in vitro* studies showed that there is highest percentage of cultures with multiple shoots in henna when the media is supplemented with ascorbic acid [14]. Young stems of *Lawsonia inermis* were most responsive in MS media supplemented with 2.0 mg/L NAA and 2.0 mg/L BAP (Table 1). Also Odutayo *et al.* [15] reported the development of multiple shoots in most cultures containing higher concentration of BAP.

For embryogenic callus formation, henna plant must be cultured on MS medium supplemented with 0.5 mg/L IAA and 0.5 mg /L 2,4-D. Table 2 shows that all explants parts (leaf, stem and root) could form embryogenic callus by using the above combinations and concentrations of hormones after 2 months of induction under 25±1°C with 16 hours photoperiod and 8 hours dark.

Previous study shows that 2,4-D hormone is useful for callus induction, growth and establishment of regenerating callus cultures [16]. In this work explants cultured on MS medium with all different combinations and concentrations of hormones also could form coloured callus. Coloured callus (green) was obtained on MS medium supplemented with 0.5 mg/L IAA and 0.5 mg/L 2,4-D after 2 months of induction (Fig. 3a). Other coloured callus obtained in this experiment includes whitish green (Fig. 3b), dark brown (Fig. 3c-d) and brown (Fig. 3e). The combinations of hormones that could produce coloured callus were 2.0 mg/L NAA and 2.0 mg/L BAP, 0.5 mg/L NAA and 0.5 mg/L BAP, 1.5 mg/L NAA and 1.5 mg/L BAP, and 0.5 mg/L IAA and 0.5 mg/L BAP. Table 3 shows that the combination of NAA and BAP hormones with all different concentrations can induce the callus. This is similar with Ilahi *et al.* [17] that the most suitable hormonal combination for callus induction was found to be BAP along with NAA. Green coloured callus was formed in *Plantago asiatica* L. in the presence of BAP and IAA [18].

Table 1. Effect of different combinations and concentrations of hormones in MS medium on multiple shoot formation.

	Number of developing shoots per explant (mean ± SD)		
	Leaf	Stem	Root
MS + 0.5 NAA + 0.5 BAP	0	18±5.2	0
MS + 1.0 NAA + 1.0 BAP	0	0	0
MS + 1.5 NAA + 1.5 BAP	0	5±2.83	0
MS + 2.0 NAA + 2.0 BAP	0	35±5	0
MS + 0.5 IAA + 0.5 2,4-D	0	0	0
MS + 0.5 IAA + 0.5 BAP	0	24±8.49	0

Table 2. Effect of different combinations and concentrations of hormones in MS medium on embryogenic callus formation.

	Percentage (%) of embryogenic callus formation per explants (mean ± SD)		
	Leaf	Stem	Root
MS + 0.5 NAA + 0.5 BAP	0	0	0
MS + 1.0 NAA + 1.0 BAP	0	0	0
MS + 1.5 NAA + 1.5 BAP	0	0	0
MS + 2.0 NAA + 2.0 BAP	0	0	0
MS + 0.5 IAA + 0.5 2,4-D	65±5	9±1.41	31.7±2.36
MS + 0.5 IAA + 0.5 BAP	0	0	0

Table 3. Effect of different combinations and concentrations of hormones in MS medium on non-embryogenic callus formation.

	Percentage (%) of non embryogenic callus formation per explant (mean ± SD)		
	Leaf	Stem	Root
MS + 0.5 NAA + 0.5 BAP	9.2±1.8	38±1.73	10±0
MS + 1.0 NAA + 1.0 BAP	0	5±0	10±0
MS + 1.5 NAA + 1.5 BAP	17.5±3.54	3.5±0.71	0
MS + 2.0 NAA + 2.0 BAP	5±0	3.3±1.53	0
MS + 0.5 IAA + 0.5 2,4-D	77.5±3.54	0	0
MS + 0.5 IAA + 0.5 BAP	3.3±1.53	0	0

L. inermis is not only used as an ornamental plant, but also has a high potential for producing secondary metabolites such as lawsone, mannite, tannic acid, mucillage, gallic acid, and naphthoquinone that are useful as medicine. Depending on the plant species, traditional agricultural methods often require months to years to obtain a crop [19]. The levels of secondary metabolites produced in intact plants are affected by many factors, including pathogens and climate changes [20]. The problems in obtaining these high-value added substances from henna plants, such as decrease in plant resources and increase in labour cost, have pointed towards the use of plant cell culture for production of the compounds. Plant cell or tissue cultures offer an opportunity for continuous production of plant metabolite and pigments, and represent an attractive production source since it is scalable according to specific need [21]. Andrew and Gerald [22] also reported that tissue culture technique enables the propagation of a large number of identical plants from a single genotype. Furthermore, plant tissue culture have long been regarded as a source of commercially important secondary metabolites for pharmaceutical industry. Thus, this study shows that efficient plant regeneration for multiple shoots and embryogenic callus could be obtained. It is possible also to get coloured callus which contain secondary metabolites *in vitro*. However, further study is needed to examine the production of coloured pigments *in vitro* and to produce them on large scale basis.

Figure 1. Aseptic seedlings of henna (4 months old).

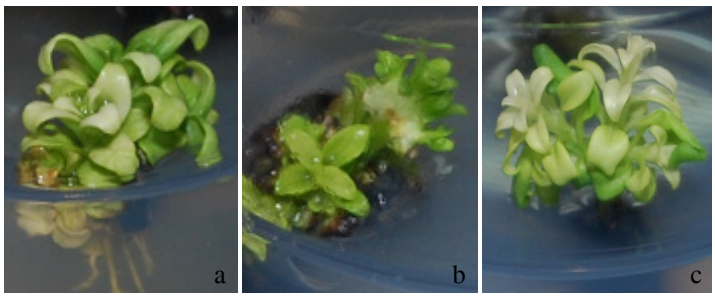

Figure 2. Multiple shoots formation.

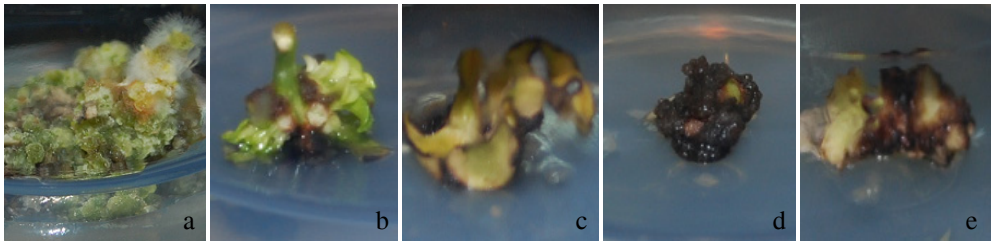

Figure 3. Non-embryogenic callus.

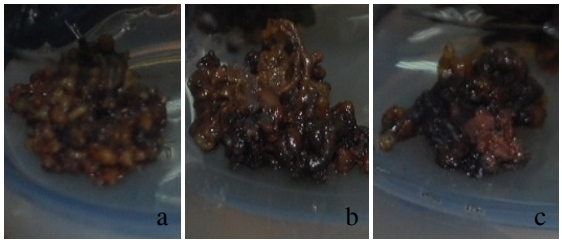

Figure 4. Embryogenic callus.

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