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In vitro Direct Regenerated Shoots from *Hypericumper foratum* L. Leaf Explants

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Abstract: A tissue culture experiment was conducted to attain the adventitious bud's initiation and growth from *Hypericumper foratum* L. *in vitro* leaves. The leaves were excited from shoot propagated *in vitro* and cultured on Murashige and Skoog (MS) medium supplemented with different concentrations of benzyl adenine (BA) and indole acetic acid (IAA) or naphthaline acetic acid (NAA) for 4 weeks. Then explants were transferred to MS medium (salts and vitamins) free of plant growth regulators for 4 weeks. The highest value most of the growth parameters (fresh and dry weights of the explant (g), number of shoots per explant, number of leaves per explant and shoot length per explant (cm))were recorded with 1.00 mg/l BA + 0.10 mg/l IAA and 0.75 mg/l BA + 0.10 mg/l NAA, respectively.

Key words: *Hypericumper foratum* · Leaf explant · BA · Shoot formation · Growth regulators

INTRODUCTION

Hypericumper foratum L. (St. John's Wort) of the family *Hypericaceae* is an herbaceous perennial plant used as a medicinal plant for over 2000 years [1]. Extracts from aerial parts have been shown to have active ingredients effects, including anti-depressant activity, antioxidant, anti-viral and wound healing [2-5]. The pseudohypericin, hypericin and naphtodianthrones belong to the antidepressant action of this species and most *Hypericum* phytomedicines are presently standardized according to their hypericin content [6]. It has been acceptedthat these biologically active substances are synthesized oraccumulated in different types of secretory structuresincluding the dark glands, translucent glands and secretorycanals located on the margins ofleaves and petals [7, 8].

In vitro regeneration has been successfully achieved for many *Hypericum* species with different growth regulators. In this study, *H. perforatum* leaves were used as explants which were very rarely used in previous studies, as well as this study pertains to morerapid *in vitro* propagation of the plant. The biotechnological tools are important to multiply, select and conserve the genotypes of medicinal plants. *In vitro* regeneration holds tremendous potential to produce high-quality plant-based medicine [9]. Micropropagation can serve in the production of a great number of genetically uniform plants and pathogen-free transplants in a limited time and space [10]. Murch *et al*. [11] consider that *in vitro* propagation of medicinal plants is a successful strategy that addresses the problems associated with supply and variability in product quality. Furthermore, plant tissues and cell cultures are also important tools that allow extensive manipulation of the biosynthesis of secondary compounds and yield higher productivity compared to that of intact plants [12]. Plant regeneration of the *Hypericum*species has been achieved by using as explants of the whole seedling or their excised parts, hypocotyl sections, leaves and leaf discs and stem segments, adventitious rootsusing various types and concentrations of cytokinins and auxins [13-19].

Therefore, this research aimed to establish a medium allowing for effective regeneration of shoots from the standardized leaf explants *in vitro* conditions to achieve a high frequency of the plant multiplication system of this plant.

MATERIAL AND METHODS

Plant Material: Seeds of *Hypericumper foratum* L. were obtained from Sekem Company for biological products, El-Salam City, Cairo, Egypt. The experiments were conducted in the plant tissue culture laboratory, Agricultural Botany Department, Faculty of Agriculture, Ain shams University, Shoubra El-Khaima, Cairo, Egypt, during the years 2018-2019.Seeds were washed using mild detergent (Twin 20) followed by tap water for 15 min.

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Seeds were sterilized by immersion in 70% ethanol solution for 10 s, followed by immersion in 15% H_2O_2 solution for 20 min. Sterile seeds were germinated on MS medium [20], 30 g/l sucrose and solidified with 7 g/l agar. The pH was adjusted at 5.7 before autoclaving at 121°C for 20 min. Cultures were incubated in a growth chamber at 16-8 h (light/dark) photoperiod, temperature 23°C ± 2 and light intensity 3000 lux for 4 weeks.

Induction of Organogenesis (Shoot): Leaf explants which obtained from 4-week-old plants were cultured on MS medium supplemented with 0.50, 0.75, 1.00, 1.25 and 1.50 mg/l BA + 0.10 mg/l IAA or 0.10 mg/l NAA for 4 weeks. The explants were subcultured on an MS-free hormone medium for another 4 weeks.The cultures were preserved in 5 replicates in each replicate 3 explantsin a growth chamber at 16-8 h (light/dark) photoperiod, temperature 25°C ±2 and light intensity 3000 lux for 4 weeks then the explants were transferred to MS free medium for 4 weeks.

Growth Parameters: The following parameters were measured: fresh and dry weights of the explant (g), number of shoots per explant, number of leaves per explant and shoot length per explant (cm). The parameters were measured after 4 weeks from subcultured on organogenesis medium. Six plants were taken from each treatment for different measurements.

The experiment was conducted in a complete randomized design with five replicates (jars) each replicate has three leaf explants. The obtained results were subjected to factual investigation of difference as indicated by the strategy portrayed by Sndecor and Cochran [21] by using SAS[22] computer program and means were compared by Duncen method.

RESULTS AND DISCUSSION

Results illustrated in Figures (1 & 2) represent the effect of different concentrations of BA and IAA or NAA on developing both of shoot fresh and dry

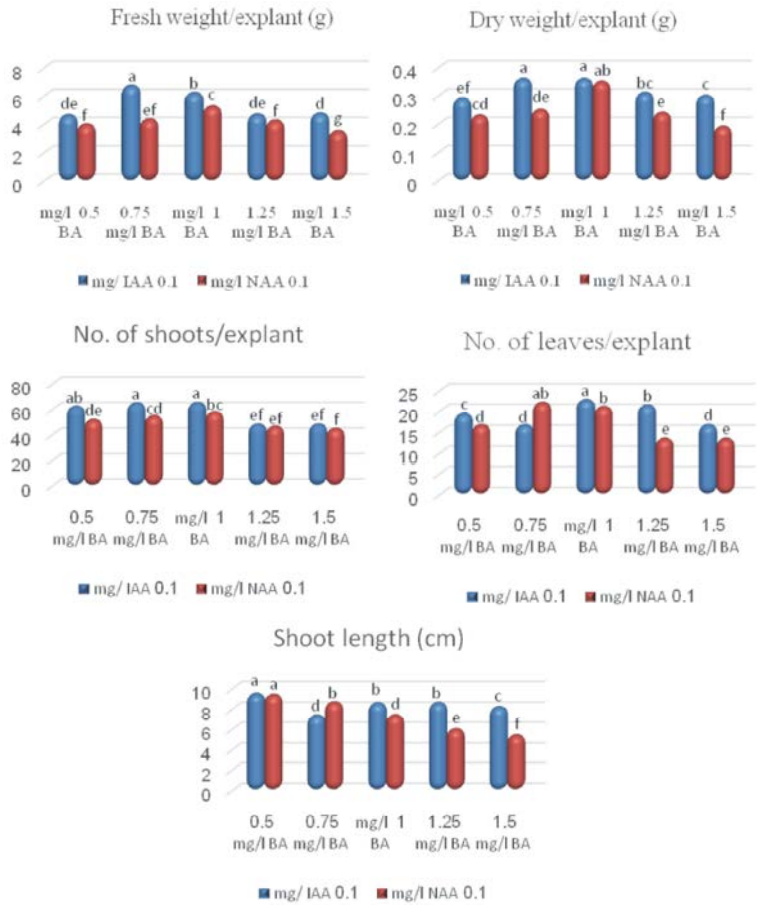

Fig. 1: Effect of different concentrations of BA in combination with IAA or NAA on growth parameters of *Hypericumper foratum* shoots *in vitro*.

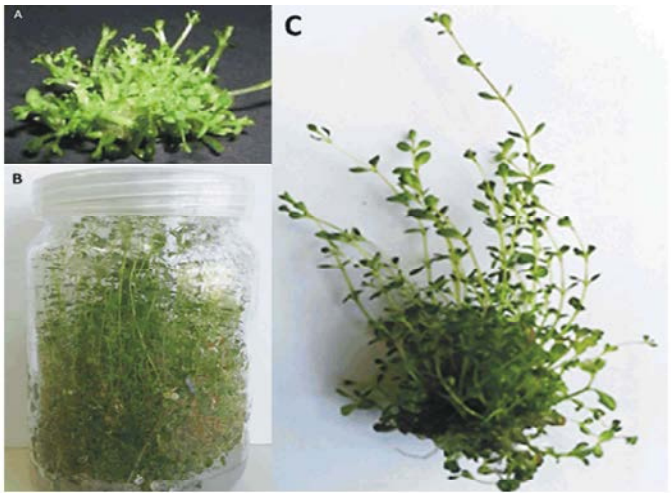

Fig. 2: Propagation stages and growth of *Hypericumper foratum* shoots from leaf explant *in vitro*. (A) Initiation stage of the leaf in the MS medium supplemented with BA + IAA or NAA. (B)and(C) Proliferation stage (after subculture) on free hormone MS medium

weight/explant. It is evident that 0.75 mg/l BA + 0.10 mg/l IAA achieved maximum shoot fresh weight (6.75 g) followed by1.00 mg/l BA + 0.10 mg/l IAA (6.23 g). On the other hand, there is no significant difference in shoot dry weight between 0.75 mg/l BA + 0.10 mg/l IAA and 1.00 mg/l BA + 0.10 mg/l IAA (0.36 & 0.35 g) respectively. By comparing the effect of auxin types (IAA and NAA), IAA showed an evident increasing in both fresh and dry weights in all treatments compared with NAA. In this respect, Abdollahpoor *et al*. [23] expressed that using MS medium supplemented with 0.10 mg/l BA + 0.05 mg/l 0.05 IBA led to increasethe shoot weight of *hypericumper foratum*.

It was observed that, the number of shoots/explant was increased significantly with the first three concentrations of BA (0.50, 0.75 and 1.00 mg/l) + 0.10 mg/l IAA. While the higher concentration of BA led toa decrease in all studied growth parameters. The highest number of shoots/explant was recorded with 1.00, 0.75 and 0.50 mg/l BA (65, 64.66 & 62), respectively.

In fact, it affirmed that by using 1.00 mg/l BA + 0.10 mg/l IAA and 0.75 mg/l BA + 0.10 mg/l NAA, number of leaves/explant increased and recorded the highest significant number (22.6 & 22 leaf/explant). On the other hand, the lowest concentration of BA (0.50 mg/l) exhited the highest significant shoot length values with both IAA and NAA concentrations (9.46 & 9.35 cm), respectively.

In this respect, Santarem and Astarita [24] mentioned that the presence of 4.5 µmol/l BA and 0.05 µmol/l NAA showed the highest average number of shoot induction of *Hypericumper foratum* plants. In the same manner, Akbaş, *et al*. [25] found that the most effective concentration of BAP for producing maximumadventitious shoot number and shoot length of *H. spectabile* was 1.0 mg/l. Also, they mentioned that increasing BA concentration led to reducing the adventitious bud formation.Moreover, McCoy and Camper [26] evaluated the effect of the combination of different concentrations from BA and IAA on *H. perforatum*. They found that the highest shoot formation was obtained with 2.85 µM IAA + 4.44 µM BA.

CONCLUSION

It could be concluded that supplying the MS medium with both concentration of 0.75 mg/l and 1 mg/l combined with the concentration of 0.1mg/l of either IAA or NAA enhanced all recorded growth parameters.

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