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EFFECT OF VARIOUS AUXINS ON GROWTH INDEX AND PRODUCTION OF SAPOGENINS IN TISSUE CULTURES OF *M. EMERGINATA*

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ABSTRACT

Background/Aim: *Plant hormones are chemicals that regulate plant growth; these are signal molecules produced within the plants and occur in extremely low concentration. Auxin is considered as growth regulators, they positively influence cell enlargement and root initiation. Auxins also promote production of other hormones. The present study objective: is to estimate and compare the effect of auxin concentration on unorganized tissue.* ***Materials and Methods:*** *In this study unorganized tissues were transferred to fresh standardized MS medium and MS medium singly supplemented with different concentrations (1,3,5 mg/L) of IAA, NAA and 2,4-D. Tissues samples were dried at 1000 °C for 15 minutes to inactivate enzymes followed by 60°C till constant weight was achieved. The dried tissue samples were then powdered and analyzed separately for their sapogenin content. Five such replicates were taken.* ***Results and observations****: Maximum amount of sapogenins was found in the tissue grown on standardized MS medium fed with 5 mg/L IAA and minimum with 5 mg/L 2, 4-D. This shows that higher concentration of 2, 4-D shows inhibitory effect on production of sapogenins.* ***Conclusion:*** *Hormonal supplementation played an important role in the growth of tissues, diosgenin and kryptogenin production, however the type of hormone and its optimum concentration to be used varied from plant to plant.*

KEY WORDS

Plant hormones, Auxin, Sapogenins, Growth Index.

INTRODUCTION

Plant hormones are chemicals that in small amount promote and influence the growth, development, difference of cells and tissues. Influence of auxins and different hormones on sapogenin production in ***D. deltoidea*** and ***T. foenum-graecum*** tissue cultures. Hormones play only a minor role in the regulation of diosgenin production in ***D. deltoidea*** tissue cultures. Effect of plant growth regulators has not only been seen on growth and differentiation of cells but also on the production of secondary metabolites. Plant growth regulators in the medium influence the accumulation of secondary plant products in culture.

MATERIAL AND METHODS

Unorganized tissues of ***M. emerginata*** were transferred to fresh standardized MS medium and MS medium singly supplemented with different concentrations (1,3,5 mg/L) of IAA, NAA and 2,4-D. Tissues samples were dried at 100° C for 15 minutes to inactivate enzymes followed by 60° C for constant weight. The dried tissue samples were then powdered and analyzed separately for sapogenin content.

a. Extraction Procedure

Samples were hydrolyzed with 30% (v/v) hydrochloric acid (2 gm/20 ml) for 4 hours on a water bath. Hydrolyzed test samples were washed separately with distilled water and filtrate attained pH 7.0. Test samples obtained were dried at 60°C for eight hours and soxhlet extracted in benzene (200ml) for twenty four hours separately. [1] Benzene extract of each test sample was dried separately *in vacuo* and taken up in chloroform for analysis of its steroidal sapogenins.

b. Chromatography

Thin Layer Chromatography (TLC)

Crude extracts with reference to sapogenins (diosgenin, gitogenin, hecogenin, kryptogenin, smilagenin, tigogenin and yamogenin) were dissolved in chloroform and applied separately on silica get 'G' coated and activated glass plates. These plates were developed in an organic solvent mixture of hexane and acetone (8.2, v/v). Developed glass plates were dried and visualized under UV light revealing two fluorescent spots in each of the test samples which on spraying with 50% sulphuric acid and subsequent heating at 100°C for 10 minutes showed two spots (brown, Rf 0.43, Rf 0.22) coincided with that of the standard reference compound, diosgenin and kryptogenin.

Preparative Thin Layer Chromatography (PTLC)

Two spots (brown, Rf 0.43, Rf 0.22) coinciding with that of diosgenin and kryptogenin were separately eluted by preparative TLC (silica gel 'G' dry thickness 0.4-0.5 mm, solvent system-hexane and acetone 8:2), from unsprayed plates along with silica gel. Each mixture was eluted with chloroform dried *in vacuo* and crystallized with methanol-acetone [2] .The isolated compound and the standard reference compounds of diosgenin and kryptogenin were subjected to their mp and IR study.

c. Quantitative Estimation

Steroidal sapogenins were estimated following the spectrophotometric method of Sanchez *et al*. (1972). Standard stock solutions of diosgenin and kryptogenin were separately prepared in chloroform, out of which various concentrations were made ranging from 10µg to 120µg (each) and applied separately on silica gel 'G' coated and activated glass plates along with a parallel run of blank. These glass plates were run in a solvent mixture of hexane: acetone (8:2), air dried and kept in a chamber saturated with iodine vapours. Resulting coloured spots were marked and the plates were kept in an oven at 100°C for 15 minutes so as to evaporate excess of iodine. Spots of diosgenin and kryptogenin along blank zone from the parallel run were scrapped along with the absorbent, eluted with 5 ml of methanol and then centrifuged. From each of the sample 4 ml of aliquot was taken and evaporated to dryness on a water bath. To each of the resulting residue 4 ml of 80% methanolic sulphuric acid was added and kept for 2 hours. Absorbance from each of the known sample was measured on a spectronic-20 colorimeter (Bausch and Lomb) set at 405 nm against a blank (80% methanolic sulphuric acid) and a regression curve of various concentrations against optical densities was computed which followed Beer's law. Absorbance from each of the unknown sample was also taken in a similar manner and their concentration (%) was determined by comparing with those of their standard curves. Five such replicates of each of the samples were examined and mean values were taken.

RESULTS

Auxins effect on growth of tissue and production of sapogenins was seen on standardized MS medium supplemented with different concentrations of auxins. Amount of diosgenin

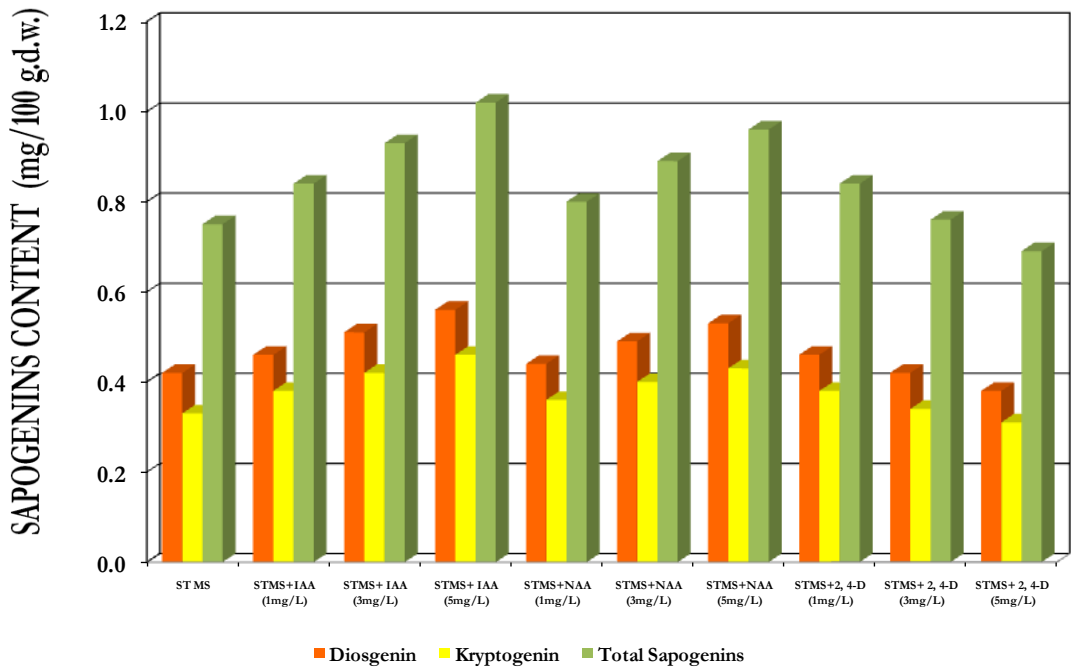
and kryptogenin increased with increase in concentration of IAA and NAA in the medium from 1 mg/L (0.46 and 0.38 mg/100 g.d.w.) to 3 mg/L (0.51 and 0.42 mg/100g.d.w.) to 5 mg/L (0.56 and 0.46 mg/100 g.d.w. respectively)for IAA and with 1mg/L (0.44 and 0.36 mg/100g.d.w.), 3 mg/L (0.49 and 0.40 mg/100g.d.w.) and 5 mg/L (0.53 and 0.43 mg/100 g.d.w respectively) for NAA. This shows that increased concentration of IAA and NAA in the medium increased the amount of sapogenins in the unorganized tissues, but 2, 4-D showed different results as amount of sapogenins was increased in tissue fed with 1 mg/L 2, 4-D (0.46 and 0.38 mg/100 g.d.w.) but it decreased from 1 mg/L to 3 mg/L (0.42 and 0.34 mg/100 g.d.w.) and ultimately upto 5 mg/L (0.38 and 0.31 mg/100 g.d.w.) **Table 1 & Figure 1**.

Table 1: Effect of auxins on production of sapogenins in eight weeks old tissue of *M.emarginata*

Sapogenins	HORMONE CONCENTRATION									
	ST MS Medium	MS Medium + IAA(mg/L)			MS Medium +NAA (mg/L)			MS Medium + 2,4-D (mg/L)		
		1	3	5	1	3	5	1	3	5
Diosgenin	0.42±.04	0.46± .02	0.51±.04	0.56±.02	0.44±.03	0.49±.04	0.53±.03	0.46± .05	0.42±.05	0.38± .04
Kryptogenin	0.33±.05	0.38±.02	0.42±.03	0.46±.02	0.36±.03	0.40±.05	0.43±.04	0.38± .04	0.34±.05	0.31± .05

Values are mean of five replicates ± SD

Figure 1: Effects of auxins on production of sapogenins (mg/100 g.d.w.) In eight weeks old tissue of *M. emarginata*



Maximum amount of sapogenins was found in the tissue grown on standardized MS medium fed with 5 mg/L IAA (0.56 and 0.46 mg/100g.d.w.) and minimum with 5 mg/L 2, 4-D (0.38 and 0.72 mg/100 g.d.w.). This shows that higher concentration of 2, 4-D shows inhibitory effect on production of sapogenins.

DISCUSSION

Hormones play only a minor role in the regulation of diosgenin production in tissue cultures of ***Dioscorea deltoidea***. [3] Maximum amount of diosgenin (2.58%) in suspension cultures of ***Trigonella foenum-graecum*** grown on RT medium supplemented with 5 ppm of kinetin as compared with that of control (2.23) indicated that hormones do play a certain regulatory role in the synthesis of steroidal sapogenins. [4] Supplementations of different hormones as well as the culture age have significant effect on steroidal contents. [5] NAA (1 ppm) was suitable for growth of callus and diosgenin production in ***Lycium barbarum*** and 2, 4-D inhibited both growth of tissue and diosgenin content. MS medium incorporated with of NAA (1 ppm, 3 ppm) and IAA (1 ppm) had more potential for growth of tissues and diosgenin production as compared to other concentrations and simple MS medium. [6]

Zygophyllaceous plants and their tissue culture have been screened for their steroidal sapogenin content and influence of different plant growth regulators on their production. IAA (3 ppm) supplemented tissues contained maximum amount of diosgenin in ***Tribulus alatus.*** [7] MS media incorporated with 2, 4-D (5 ppm) was most efficient for diosgenin production in tissue culture of ***Zygophyllum simplex, Setzenia orientalis and Fagonia cretica.*** [8,9,10] IBA (3ppm) to be most suitable for diosgenin production in ***Lycium barbarum*** [11] Effects of plant growth regulators on cell growth and ginsenoside saponin production by suspensions cultures of ***Panax quinquefolium.*** [12] In the tissue of the three cultivars of ***Gossypium*** 2, 4- D (5ppm) supplemented LS medium contained highest amount of diosgenin. [13] Diosgenin content was higher in redifferentiated ***in vitro*** cultures of ***Lycium barbarum*** as compared to the dedifferentiated cultures. [14]

CONCLUSION

As mentioned earlier, ***M. emerginata*** tissues fed with 5ppm IAA proved to be most potent for diosgenin and kryptogenin production closely followed by NAA fed tissues. Both these hormones (IAA and NAA) enhanced diosgenin and kryptogenin content in the tissues of the plant considerably, whereas 2, 4-D exhibited a somewhat inhibitory effect. These results favour the previous finding that auxins regulate growth and production of diosgenin and kryptogenin in tissue cultures. The total sapogenin content in ***M.emarginata*** tissues was significant. It can thus, be concluded that hormonal supplementation played an important role in the growth of tissues, diosgenin and kryptogenin production, however the type of hormone and its optimum concentration to be used varied from plant to plant.

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