International Journal of Advanced Research in Engineering Technology & Science
ISSN: 2349-2819
Email: editor@ijarets.org Volume-7, Issue-1 January- 2020 www.ijarets.org

EFFECT OF PHENYLALANINE ON PRODUCTION OF FLAVONOIDS IN TISSUE CULTURE

LALITA YADAV

Plant Tissue Culture and Biotechnology Lab, Department Of Botany,

Govt. Dungar College, Bikaner(Rajasthan) INDIA

ABSTRACT

Unorganized cultures of medicinally useful plants *C. pendulus* and *T. cordifolia* were established on MS medium supplemented with suitable combination and concentrations of growth regulators, using nodal explant in *C. pendulus* and inflorescence explant in *T. cordifolia.* Small amount of these tissues were transferred to fresh MS medium supplemented with four concentrations of dl-β -Phenylalanine separately. Tissues were harvested at different age intervals and their growth indices were calculated. PTLC, Spectrophotometry and infra-red spectral studies were used for qualitative and quantitative estimation of flavonoids. Presence of Kaempferol and Quercetin was confirmed in both the plant species and significantly higher in calli than normal plant parts. All flavonoids individually as well as total flavonoids content showed gradual increase in amount present in tissue grown on control (MS) to tissue fed with certain amount of mg PA/100ml medium. It was sufficiently higher than amount estimated in tissues grown on standardized MS medium.

KEYWORDS*: Medicinal plants, Flavonoids, MS medium, dl-β -Phenylalanine*

INTRODUCTION

Nature has provided a rich store house of herbal remedies to cure all mankind's ailments. A number of plants yielding drugs from ancient time to till now are being employed for large scale commercial production of the respective metabolites especially secondary metabolites used as medicines in remedy of several severe ailments.

Flavonoids, one of the secondary metabolites, are water soluble phenolic glycosides. They have multiple biological effect including antioxidant, anticarcinogenic, anti-inflammatory and free radical scavenging abilities etc. Their contribution to physiological function such as seed maturation and dormancy has already established (Brenda,1998) . Presence of flavonoids has been reported from many plant species like *Corchorus depressus*, *Fagonia cretica*, *Citrullus colocynthis*, *Lycium barbarum* (Harsh et al. 1983), *Passiflora palmeri* (Ulu Belen et al.,1984), *Sophora griffithi* and *Goebelia pachycarpa* (Muminova et al., 2006) .

Amino acid phenylalanine act as a precursor and play important role in flavonoid biosynthesis. Effect of dl-β-phenylalanine on flavonoids production has been studied in tissue culture of *Tribulus alatus and Lycium barbarum* (Jit and Shekhawat ,1985), *Seetzenia orientalis* (Sethia ,1988) ,*Calligonum polygonoides* and *Lasiurus sindicus* (Bhojak , 1991), *Paganum harmala* (Badia,1999), *Vigna aconitifolia* (Tyagi,2002) *Withania somnifera* (Bains,2002) *Ailanthus excelsa* (Rao,2007), *Adhatoda vasica* (Deepa , 2009).

The present paper shows that the effect of incorporation of dl-β-phenylalanine in cultures of *C. pendulus* and *T. cordifolia* has been studied with respect to flavonoids production in the cultures.

MATERIAL AND METHOD

Plants namely *Cocculus pendulus* (Falor)and *Tinospora cordifolia* (Heartleafmoonseed) were collected from the local areas.Plant parts (stem, leaves and flowers) were separated, dried and powdered for analysis of flavonoids by **Subramanian and Nagarajan** (1969) method.

Regarding *in vitro* studies various explants were used to initiate callusing. Explants presoaked in 0.1% liquid detergent for 30 minutes, were washed with running tap water and then surface sterilized with 0.1 % (w/v) mercuric chloride for 3 minutes followed by 2 or 3 rinses of sterile distilled water.

Murashige and Skoog's medium (1962) supplemented with various concentrations and combinations of growth hormones were used. Calli were maintained for six months by frequent subculturing at intervals of 6 to 8 weeks at 26 ± 1º C, 55% relative humidity and diffused light conditions (3000lux).Growth Indices (GI) of tissues were calculated at 2,4,6,8 and 10 weeks time intervals.

Tissues grown on MS medium supplemented with various concentrations of phenylalanine were harvested at maximum GI, dried, powdered, wighed and extracted for quantitative estimation of flavonoids. The experiments was carried out in five replicates.

EXTRACTION PROCEDURE

Each of the dried and powdered sample was soxhlet extracted (**Subramanina** and **Nagarajan, 1969**) in 80% ethanol. Each of the extract was concentrated in vacuo and re- extracted with petroleum ether (40-60° C, Fraction I), ethyl ether (Fraction II) and ethylacetater (Fraction III) in succession. Ethyl ether (FractionII) was used for determining free flavonoid contents whereas the ethyl acetate (Fraction III) was used for bound flavonoid determination.

Fraction third of each of the test sample was hydrolyzed by refluxing with 7% sulphuric acid (10 ml/gm residue) for two hours. The mixture was filtered and the filtrate was extracted with ethyl acetate in separating funnel. The ethyl acetate layer (upper layer) was washed with distilled water to neutrality dried in vacuo and analyzed for bound flavonoids.

QUANTITATIVE ESTIMATION

Quantitative estimation of the identified flavonoids was carried out calorimetrically following the method of **Kariyon *et al***. (1953) and **Nagasaki *et al***. (1975) in case of quercetin and Mabry et al. (1970) in case of kaempferol.

RESULTS AND DISCUSSION

Secondary metabolites are very specific in plants and even in the plant parts. There are many chemicals or compounds which are treated as the precursors of these compounds.

Growth indices (GI) of unorganized tissues of both *C. pendulus* and *T.cordifolia* were calculated on standardized MS medium along with different concentrations of growth hormones and standardized MS medium supplemented with different concentrations of dl-β- phenylalanine separately. GI of callus was found to be gradually increased from standardized MS medium and maximum (6.55) with 75 mg/100 ml phenylalanine fed medium in *C.pendulus* and standardized MS medium to 25 and maximum (8.14) with 50mg/100ml phenylalanine fed medium in *T.cordifolia*. GI of callus decreased from 75 to 100 mg/100 ml phenylalanine

fed in *C.pendulus* and continuously 50 to 75 and then 100mg/100ml phenylalanine fed in *T. cordifolia*. Maximum GI was observed at the age of eight weeks old tissues fed with different concentrations of phenylalanine as shown in table 1.1. Hence eight weeks old tissues supplemented with four concentrations of phenylalanine (PA) were selected for qualitative and quantitative analysis and comparison of flavonoids with that of tissues grown on normal MS medium.

All flavonoids individually (kaempferol and quercetin) as well as total flavonoids content showed gradual increase in amount present in tissues grown on control medium (MS) to tissues fed with 25mgPA/100ml medium, up to maximum in tissues fed with 50mgPA/100 ml medium in *T. cordifolia* and 75mgPA/100ml medium in *C.pendulus*. After that it started declining in tissues fed with 75 to 100 mgPA/100 ml medium *T. cordifolia* and 100mgPA/100ml medium in *C. pendulus* as shown in table 1.2 . In all observations of both plant species amount of kaempferol was slightly more than quercetin as in tissues grown on standardized MS medium.

TABLE NO.-7.1

EFFECT OF β- PHENYLALANINE ON GROWTH INDERX (GI) OF *C. PENDULUS* AND *T. CORDIFOLIA* TISSUE CULTURES

PLANT NAME	MEDIUM	GROWTH INDICS AT THE AGE OF					
		2 Weeks	4 Weeks	6 Weeks	8 Weeks	10Weeks	12 Weeks
C.pendulus	ST MS MEDIUM	0.67±0.01	1.24±0.02	4.01±0.01	6.38±0.03	6.02±0.02	5.34±0.01
	MS+25mgPA/100ml	0.71±0.02	1.30±0.01	4.06±0.03	6.42±0.01	6.08±0.02	5.36±0.03
	MS+50mg PA/100ml	0.74±0.04	1.35±0.03	4.12±0.01	6.49±0.02	6.13±0.01	5.41±0.04
	MS+75 mg PA/100 ml	0.79±0.05	1.42±0.02	4.21±0.02	6.55±0.02	6.20±0.02	5.44±0.01
	MS+100 mg PA/100ml	0.68±0.02	1.27±0.04	4.09±0.03	6.44±0.02	6.07±0.02	5.35±0.01
T.Cordifolia	ST MS MEDIUM	0.88±0.01	1.62±0.02	4.87±0.04	8.03±0.03	7.81±0.01	6.49±0.02
	MS+25mgPA/100ml	0.92±0.02	1.68±0.03	4.90±0.01	8.07±0.02	7.77±0.04	6.51±0.03
	MS+50mg PA/100ml	0.99±0.01	1.75±0.04	4.98±0.02	8.14±0.04	7.83±0.03	6.59±0.01
	MS+75 mg PA/100 ml	0.95±0.02	1.71±0.04	4.96±0.02	8.11±0.04	7.80±0.02	6.55±0.01
	MS+100 mg PA/100ml	0.90±0.01	1.67±0.04	4.91±0.03	8.08±0.01	7.76±0.03	6.52±0.02

Values are mean of five replicates ± SD

TABLE No. 1.2

EFFCT OF β- PHENYLALANINE ON FLAVONOID CONTNT OF

***C. PENDULUS* AND *T. CORDIFOLIA* TISSUE CULTURES**

MEDIA	C.PENDULUS			T.CORDIFLIA		
	Kaempferol	Quercetin	Total Flavnoid	Kaempferol	Quercetin	Total Flavonold
Standarized Ms	0.41±0.02	0.37±0.01	0.78±0.03	0.44±0.02	0.41±0.02	0.85±0.04
MS±25 mgPA/100Ml	0.44±0.03	0.39±0.01	0.83±0.04	0.50±0.02	0.46±0.01	0.96±0.03
MS±50 mgPA/100Ml	0.49±0.01	0.46±0.02	0.95±0.03	0.58±0.01	0.53±0.02	1.11±0.03
MS±75 mgPA/100Ml	0.58±0.03	0.52±0.01	1.10±0.04	0.54±0.01	0.50±0.01	1.04±0.02
MS±100 mgPA/100Ml	0.52±0.02	0.49±0.01	1.01±0.03	0.49±0.02	0.47±0.01	0.96±0.03

Values are mean of five replicates ±SD

REFERENCES

Badia, N. 1999. Influence of plant growth regulators and precursors on the production of primary and secondary metabolites in tissue cultures of *Peganum harmala* Linn. Ph.D. Thesis MDS University, Ajmer,Rajasthan,India.

Bains, N. S. 2002. Effect of different concentrations of phenylalanine and auxin (IAA) on production of flavonoids content in six weeks old tissue culture (with maximum GI) of *withaniasomnifera.* Ph.D. Thesis MDS University, Ajmer, Rajasthan, India.

Bhojak, S. 1991. Effect of dl-β-phenylalanine on production of flavonoids in tissue cultures of *Calligonumpolygonoides* and *Lasiurus sindicus.* Ph. D. Thesis MDS University, Ajmer, Rajasthan, India.

Chauhan, N. 2003. Effect of phenylalanine on production of flavonoid content in tissue cultures of variety of Zizyphusmauritiana. Ph. D. Thesis MDS University, Ajmer, Rajasthan, India.

Deepa 2009. Increased amount of flavonoids in *Adhatodavasica*and *Barleriaprionitis* on MS medium with phenylalanine.Ph. D. Thesis M.G.S. University, Bikaner,Rajasthan.

Jit,S. ; Shekhawat, S. S.1985. Presence of flavonoids and effect of various concentrations of phenylalanine on production in tissue culture of *Tribulus alatus* and *Lyciumbarbarum* respectively. 72thIndian Science Congress. Lucknow. India.

Kapoor, B. B. S. 1991. Phenylalanine influencethe production of flavonoids in tissues of *Fagoniacretica.*Ph. D. Thesis Rajasthan University, Jaipur, Rajasthan, India.

Kariyon,T.,Hashimoto, Y. and Kimura, M. 1953. Microbial studies of Plant components. IX. Distribution of flavonoids in plants by paper chromatography. J.Pharma. Soc. (*Japan*).73:253-256

Kaur, A. 1997. Influence of plant growth regulators and precursors on production of primary and secondary products in Cotton varieties growing *in vitro.*Ph. D. Thesis MDS University, Ajmer, Rajasthan, India.

Reddy, A. 2005. Studied effect of phenylalanine on flavonoid production in vitro in Cassia angustifoliaPh. D. Thesis MDS University, Ajmer, Rajasthan, India.

Rao, H. R. 2007. Increased amount of individual and total flavonoid in tissue of Ailanthus excelsa. Ph. D. Thesis M.G.S. University, Bikaner,Rajasthan.

Sethia, M. 1988. Marked increase in total quercetin and kaempferol content in tissue of Seetzeniaorientalis with phenylalanine.Ph. D. Thesis Rajasthan University, Jaipur, Rajasthan, India.

Shekhawat, S. S. 1985. Phytochemical investigation of some arid zone plants of Rajasthan in vivo and in vitro. Ph. D. Thesis Rajasthan University, Jaipur, Rajasthan, India.

Subramaninan, S.S. and Nagarajan, S. 1969. Flavonoids of the seeds of Crotalaria retusa and C. striata. Curr. Sci. 38: 65-68.

Ulubelen , A., mabry, T.J., Della Monica, G. and Chopin , J. 1984.Flavonoids from passiflora. Palmeri. J. Nat. Prod. 47: 384-385