

EFFECT OF SOME GROWTH REGULATORS ON PRODUCTION OF OPIUM ALKALOIDS *IN VITRO* TISSUE CULTURES OF *PAPAYER SOMNIFERUM* L.¹

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ABSTRACT

Twelve months old unorganised callus tissues of *P. somniferum* L., grown on revised Murashige and Skoog's medium (RT) and RT medium supplemented with various concentrations (1, 1.5 and 2 ppm each) of indole acetic acid (IAA), naphthalene acetic acid (NAA), 2, 4-dichloro-phenoxyacetic acid (2, 4-D) and kinetin (K) were analysed separately for their alkaloidal content. Six major alkaloids—morphine, codeine, thebaine, papaverine, narcotine and narceine were observed in each case. The percentage of alkaloids increased considerably in the tissues fed with growth regulators and the maximum (5.95%) amount of alkaloids was observed in the tissues fed with kinetin (1.5 ppm).

INTRODUCTION

Production of six major opium alkaloids in callus cultures of *Papaver somniferum* Linn. has been reported (Khanna and Khanna, 1976). Effect of tyrosine and ascorbic acid on the production of alkaloids has also been reported in tissue cultures. The present investigation is in continuation with our previous studies (Khanna and Khanna, 1976; Khanna *et al.*, 1978). In the present communication effect of growth regulators on the growth and production of major opium alkaloids has been reported.

MATERIAL AND METHODS

Unorganised callus tissue of *P. somniferum* maintained on revised (Khanna and Staba, 1968) Murashige and Skoog's (1962) medium (RT) for twelve months was transferred to fresh RT medium and RT medium supplemented with various

concentrations. (1, 1.5 and 2 mg/L each) of indole acetic acid (IAA), naphthalene acetic acid (NAA), 2, 4-dichlorophenoxyacetic acid (2, 4-D) and kinetin (K). The tissues grown on different media were harvested periodically (after 3, 4, 6 and 8 weeks), dried and extracted separately in aqueous solution at definite pH (Khanna and Khanna, 1976; Longmann and De-Bussy, 1972) for their alkaloidal content. Growth indices of the tissues grown on various media were also calculated from time to time (GI—Final dry weight of the tissue/Initial dry weight of the tissue). The various media on which the tissues were grown were also measured and extracted separately for their alkaloidal content.

RESULTS AND DISCUSSION

Six major opium alkaloids (Morphine, codeine, thebaine, narceine, narcotine and papaverine) were isolated and identi-

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fied by co-chromatography, mp, mmp and IR spectral studies from each tissue sample.

The maximum (6.96) growth index of tissue was observed in the tissue grown on RT medium supplemented with 1.5 ppm of 2, 4-D (Table I IV (b) and the maximum total alkaloidal content (5.95%) was observed in the tissue grown on RT medium supplemented with 1.5 ppm of kinetin (Table I v(b)).

The percentage of alkaloids increased

considerably in the tissue fed with 1.5 ppm of kinetin (Codeine, 0.82%; papaverine oxalate, 1.03%; and total alkaloidal content, 5.95%; Table I v (b) when compared with that of the tissue grown on RT medium alone (Codeine, 0.26%; papaverine oxalate, 0.27%; and total alkaloidal content, 4.71%; Table I i). The amount of narceine was found maximum (2.82%) in the tissue fed with 1 ppm of kinetin (Table I v(a) and those of morphine (1.12%) and narcotine oxalate

TABLE I

TISSUE GROWTH AND ALKALOIDAL CONTENT OF *PAPAYER SOMNIFERUM* L. INN. STATIC CULTURES GROWN ON RT MEDIUM AND RT MEDIUM SUPPLEMENTED WITH VARIOUS CONCENTRATIONS (1, 1.5 AND 2 PPM) OF IAA, NAA, 2, 4-D AND KINETIN.

Age of tissue (weeks)	GI _A	Alkaloidal content (%)						
		Morphine	Codeine	Thebaine	Narceine	Narcotine oxalate	Papaverine oxalate	Total alkaloidal content
(i) <i>RT Medium</i>								
2	4.00	0.54	0.05	0.20	1.01	0.13	0.08	2.01
4	5.25	0.75	0.18	1.15	1.38	0.21	0.27	3.94
6	3.20	0.83	0.11	1.02	1.72	0.90	0.13	4.71
8	2.71	0.31	0.09	0.01	1.03	0.39	0.04	1.87
(ii) <i>RT+IAA Medium</i>								
(a) <i>1 ppm IAA</i>								
2	3.82	0.42	0.21	0.72	0.92	0.12	0.08	2.47
4	5.27	0.82	0.45	0.86	1.03	0.23	0.11	3.50
6	6.00	0.90	0.51	1.20	1.22	0.52	0.67	5.02
8	4.48	0.61	0.81	0.72	1.14	0.24	0.42	3.94
(b) <i>1.5 ppm IAA</i>								
2	4.24	0.39	0.27	0.67	0.62	0.17	0.09	2.21
4	6.12	0.71	0.51	0.80	0.82	0.24	0.15	3.23
6	6.43	0.88	0.56	0.96	0.97	0.42	0.07	3.86
8	5.87	0.32	0.45	0.87	0.89	0.14	0.05	2.72

(c) 2 ppm IAA

2	2.99	0.24	0.19	0.54	0.19	0.03	0.05	1.29
4	3.65	0.76	0.27	0.71	0.63	0.19	0.09	2.65
6	4.76	0.52	0.34	0.89	0.88	0.17	0.04	2.84
8	4.00	0.32	0.12	0.77	0.78	0.09	0.07	2.15

(iii) RT+NAA Medium

(a) 1 ppm NAA

2	2.42	0.64	0.13	0.24	0.82	0.09	0.05	1.97
4	3.97	0.82	0.28	0.45	1.11	0.15	0.09	2.90
6	4.43	0.69	0.34	0.72	1.40	0.29	0.13	2.57
8	2.96	0.71	0.24	0.36	0.96	0.12	0.06	2.45

(b) 1.5 ppm NAA

2	4.12	0.69	0.21	0.31	0.96	0.06	0.07	2.30
4	4.48	0.75	0.48	0.42	1.24	1.00	0.15	4.04
6	5.06	0.90	0.52	0.46	1.55	1.24	0.18	4.85
8	3.56	0.57	0.32	0.35	1.13	0.81	0.05	3.23

(c) 2 ppm NAA

2	1.53	0.82	0.43	0.47	1.35	1.00	0.13	4.20
4	2.68	1.10	0.58	0.34	1.16	0.92	0.35	4.45
6	3.41	1.12	0.61	0.52	1.45	1.06	0.29	5.05
8	1.27	0.86	0.50	0.29	0.87	0.84	0.25	3.61

(iv) RT+2, 4-D Medium

(a) 1 ppm 2, 4-D

2	3.00	0.41	0.09	0.18	1.26	0.18	0.09	2.21
4	4.00	0.67	0.16	0.24	1.33	0.30	0.07	2.77
6	4.86	0.82	0.24	0.48	1.59	0.25	0.12	3.50
8	2.80	0.57	0.19	0.39	1.60	0.21	0.06	3.02

(b) 1.5 ppm 2, 4-D

2	5.11	0.51	0.04	0.21	1.31	0.27	0.03	2.43
4	6.26	0.67	0.09	0.28	1.42	0.29	0.25	3.00
6	6.96	0.89	0.26	0.36	1.56	0.32	0.20	3.59
8	5.53	0.48	0.19	0.22	1.46	0.13	0.16	2.64

(c) 2 ppm 2, 4-D								
2	5.33	0.53	0.05	0.26	1.26	0.06	0.05	2.21
4	4.48	0.83	0.08	0.32	1.51	0.08	0.06	2.28
6	4.55	0.90	0.36	0.54	1.78	0.13	0.19	3.90
8	3.96	0.70	0.18	0.28	1.61	0.10	0.08	2.95
(v) RT+Kinetin Medium								
(a) 1 ppm kinetin								
2	2.45	0.62	0.02	0.38	1.24	0.05	0.03	2.34
4	2.80	0.87	0.06	0.27	1.56	0.08	0.05	2.89
6	3.23	0.91	0.13	0.67	2.82	0.16	0.18	4.87
8	1.19	0.36	0.05	0.52	2.02	0.18	0.08	3.21
(b) 1.5 ppm kinetin								
2	3.87	0.66	0.68	0.51	1.16	0.14	0.06	3.21
4	3.92	0.89	0.82	0.74	2.67	0.09	0.05	5.26
6	4.64	0.93	0.59	0.83	2.42	0.16	1.03	5.16
8	1.87	0.51	0.16	0.32	0.14	0.06	1.00	2.19
(c) 2 ppm kinetin								
2	1.16	0.15	0.12	0.04	0.75	0.62	0.38	2.06
4	2.00	0.39	0.40	0.54	0.91	0.78	0.07	3.09
6	3.08	0.94	0.21	0.85	1.50	0.90	0.10	4.50
8	2.50	0.73	0.02	0.37	0.87	0.25	0.02	2.26

*GI Growth Index=final dry weight of tissue—initial dry weight of tissue/initial dry weight of tissue.

(1.24%) in the tissue fed with 2 ppm and 1.5 ppm NAA (Table I iii (c) & (b) respectively. Thebaine was, however, observed maximum (1.2%) in the tissue fed with 1 ppm of IAA Table I ii (a). The media on which the tissue was grown contains only traces of alkaloids.

The present study shows that growth regulators play a role in the production of alkaloids in *P. somniferum* tissue culture and RT medium supplemented with kinetin (1.5 ppm) is the best medium for the production of major opium alkaloids.

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