This paper forms part of the Ph.D. thesis submitted to IARI, New Delhi by the senior author.

9 January 1987

1. Tcacenco, F. A., Fordlloyd, B. V. and Astley, D., *Z. Pflanzenücht.*, 1985, **94**, 192.
2. Raut, R. N. and Prakash, S., In: *genetic manipulation in crop plants*, (ed.) V. L. Chopra, Oxford and IBH Publishers, Delhi, 1985, p. 205.
3. Sarla, N., Ph.D. thesis, 1986, IARI, New Delhi.
4. Frandsen, K. J., *Dan. Bot. Ark.*, 1947, **12**, 1.
5. Olsson, G., (ed.) A. Hagberg, *Recent research in plant breeding, Svalof 1944–1961*, 1963, 179–192, New York and London.
6. Pearson, O. H., *J. Am. Soc. Hort. Sci.*, 1972, **97**, 397.
7. Erickson, L. R., Straus, N. A. and Beversdorf, W. D., *Theor. Appl. Genet.*, 1983, **65**, 201.

ERGOT ALKALOID PRODUCTION IN SUSPENSION CULTURES OF *IPOMOEA BATATAS* POIR.

P. B. KAVI KISHOR* and A. R. MEHTA

Department of Botany, M. S. University of Baroda, Baroda 390 002, India.

**Department of Botany, Kakatiya University, Warangal 506 009, India.*

DIFFERENT aspects of ergot alkaloid biosynthesis in *Claviceps* species, *Aspergillus fumigatus* and other microorganisms have been carried out by earlier workers[1–3]. Some plants belonging to the family Convolvulaceae also contain ergot alkaloids[4,5]. But very little work has been done in higher plants regarding the production of ergot alkaloids *in vitro*. The present communication deals with the influence of various growth regulators on the growth and alkaloid production in suspension cultures of *Ipomoea batatas* Poir.

Tuber callus of *I. batatas* Poir. was initiated on Murashige and Skoog's (MS) medium[6] containing 2 mg/l 2,4-dichlorophenoxyacetic acid (2,4-D) and 0.4 mg/l kinetin (KN). The culture vessels were incubated at a constant temperature (26 ± 2°C) and light (500 lux) conditions. Callus cultures were later maintained on the same medium subculturing every 30 days. Cell suspensions were initiated by inoculating 300 ± 30 mg of fresh tissue into 40 ml of MS basal medium with additives as above (but without agar-agar). Culture vessels were continuously agitated on a horizontal rotary shaker at 26 ± 2°C under constant illumination (500 lux). Growth of cell suspensions was measured as an increase in fresh and dry weights. Five replicate cultures were harvested at the end of 30 days for growth measurements and alkaloid production.

Dry tissue (100 mg) was macerated with 0.4 mg of ammonium hydroxide, 0.5 ml of ethanol and 0.5 ml of diethyl ether overnight, mixed in 25 ml of chloroform and boiled for about 15 min. The extract was then passed through a specially prepared glass column. The filtrate was evaporated to dryness and spotted on thin layer chromatographic plates (Kieselgel G Type 60). The plates were run in chloroform:ethanol (9:1) solvent system and sprayed with modified Van Urk's[7] reagent. The spots were eluted, dissolved in ethanol and total ergot alkaloids were measured in a colorimeter at 580 nm using Ergocrystine as standard.

Different concentrations (0.2, 1.0, 2.0 and 5.0 mg/l) of indole-3-acetic acid (IAA), indole-3-butyric acid (IBA), naphthaleneacetic acid (NAA) and 2,4-D were tested on suspension cultures of sweet potato for their influence on growth and ergot alkaloid production. Increasing concentrations of IAA increased the growth slightly but the alkaloid production was not much influenced (table 1). While maximum growth was obtained with the incorporation of IBA and NAA in the medium, highest production of total alkaloid was noticed on 2,4-D containing medium (on μg/100 mg dry weight basis). NAA is known to enhance RNA content[8] by as much as 50% which would result in the increased synthesis of structural and functional proteins. NAA is also found to accumulate rapidly in plant tissues and is then metabolized slowly to a series of unidentified derivatives[9]. The optimum level of NAA in the system can thus be maintained resulting in the rapid growth of tissues. Though higher concentrations of 2,4-D suppressed the growth, alkaloid production was maximum at 1 mg/l level (table 1) as was also reported by Nambiar[10] in callus cultures of *Evolvulus alsinoides* L. The total alkaloid content on 1 mg/l 2,4-D medium at the end of 30 days was almost equal to that of intact tubers. *In vivo* studies showed the maximum quantity of alkaloids in tubers (17 μg/100 mg of dry tissue) compared to leaves and stems. Hence, suspension cultures derived from tuber callus of sweet potato can be used further for commercial exploitation of these ergot alkaloids.

Low concentrations of KN and 6-benzylaminopurine (BAP) promoted the growth of the tissues considerably but higher concentrations decreased both fresh and dry weight of the tissues (table 2).

Table 1 *Growth and alkaloid production in suspension cultures of sweet potato as influenced by auxins*

Conc. of auxin mg/1	Fresh weight mg/culture	Dry weight mg/culture	Alkaloid μg/culture	Alkaloid μg/100 mg of dry weight
0.2 IAA	833 (±41)	73 (±12)	5	7
1.0 IAA	908 (±46)	82 (±14)	8	10
2 0 IAA	1116 (±52)	99 (±19)	11	11
5.0 IAA	809 (±37)	69 (±11)	8	11
0.2 2,4-D	2410 (±63)	113 (±20)	11	10
1.0 2,4-D	1870 (±57)	94 (±16)	18	19
2.0 2,4-D	1697 (±55)	93 (±16)	16	17
5.0 2,4-D	1423 (±55)	80 (±15)	10	12
0 2 NAA	1429 (±53)	101 (±18)	9	9
1.0 NAA	3105 (±77)	172 (±28)	19	11
2.0 NAA	2956 (±69)	161 (±26)	21	13
5.0 NAA	1903 (±60)	129 (±22)	12	9
0.2 IBA	1321 (±54)	90 (±17)	7	8
1.0 IBA	1810 (±57)	103 (±18)	10	10
2.0 IBA	2236 (±62)	144 (±24)	10	7
5.0 IBA	3900 (±83)	164 (±27)	10	6

Figures in parentheses represent standard error.

Table 2 *Effect of KN, BAP and GA_3 on growth and alkaloid production in suspension cultures of sweet potato*

Conc. of organics mg/l	Fresh weight mg/culture	Dry weight mg/culture	Alkaloid μg/culture	Alkaloid μg/100 mg of dry weight
0.04 KN	3960 (±89)	183 (±29)	15	8
0.4 KN	3125 (±75)	174 (±25)	14	8
1.0 KN	2348 (±64)	116 (±20)	8	7
2.0 KN	2168 (±58)	114 (±20)	11	10
0.04 BAP	3989 (±90)	180 (±27)	16	9
0.4 BAP	3207 (±76)	173 (±23)	12	7
1.0 BAP	1902 (±55)	96 (±21)	9	9
2.0 BAP	1490 (±52)	87 (±16)	7	8
5.0 GA_3	2106 (±57)	84 (±15)	7	8
25.0 GA_3	2243 (±60)	93 (±16)	7	7
50.0 GA_3	1917 (±54)	82 (±15)	6	7
100.0 GA_3	1434 (±50)	78 (±14)	5	6

Figures in parentheses represent standard error.

Growth of the tissues was better in media containing KN and BAP than auxin and GA_3 but total alkaloid production was much less. The indispensability of KN for the growth of callus tissues has been demonstrated by Murashige and Skoog[6] and Steward *et al*[11]. KN-induced growth is accredited to the enhanced RNA and protein synthesis[12]. KN has further been reported to have additive effect to that of auxin for growth promotion of spruce and sycamore callus cultures[13,14]. Gibberellic acid

(GA_3) has neither any promotory effect on the growth nor on alkaloid production (table 2) in sweet potato suspension cultures.

Further work is needed for maintaining and/or enhancing the biosynthetic potentiality of the tissue, by isolation and selection of stable cell lines of sweet potato by plating the cells and subjecting them to selection pressures.

One of us (PBK) is grateful to UGC, New Delhi for a fellowship.

9 October 1986

1. Tabler, W. A. and Vining, L. C., *Can. J. Microbiol.*, 1960, **6**, 355.
2. Splisbury, J. F. and Wilkinson, S., *J. Chem. Soc.*, 1961, 2085.
3. Rao, K. K., Patel, V. P. and Patel, B., *Indian J. Exp. Biol.*, 1974, **12**, 76.
4. Hofmann, A. and Tscherter, A., *Experientia*, 1960, **16**, 414.
5. Hylin, J. W. and Watson, D. P., *Science*, 1965, **148**, 499.
6. Murashige, T. and Skoog, F., *Physiol. Plant.*, 1962, **15**, 473.
7. Van Urk, H. W., *Pharm. Weekbl.*, 1929, **66**, 473.
8. Leaver, C. J., Ph.D. thesis, University of London, England, 1966.
9. Glasziou, K. T., Gayler, K. R. and Waldron, J. C., In: *Biochemistry and physiology of plant growth substances*, (eds) F. Wightman and G. Setterfield, Runge Press, Ottawa, Canada, 1968, p. 433.
10. Nambiar, G. R., Ph.D. thesis, M.S. University of Baroda, Baroda, India, 1980.
11. Steward, F. C., Mapes, M. O. and Ammirato, P. V., In: *Plant physiology: A treatise*, (ed.) F. C. Steward, Academic Press, New York, 1969, **B5**, 329.
12. Kende, H. and Gardner, G., *Annu. Rev. Plant Physiol.*, 1976, **27**, 267.
13. Steinhart, C. E., Standifer, L. G. and Skoog, F., *Am. J. Bot.*, 1961, **48**, 465.
14. Digby, J. and Wareing, P. F., *J. Exp. Bot.*, 1966, **17**, 718.

UNCOMMON CUTICULAR ORNAMENTATION ON THE STIGMA SURFACE OF MANGO (*MANGIFERA INDICA* L.)

PHILIP JOHN, Y. RAGHURAM and G. M. NAIR

Department of Botany, Faculty of Science, The Maharaja Sayajirao University of Baroda, Baroda 390 002 India.

Mangifera indica L. produces staminate and bisexual flowers on the same panicle. Though, more than 3,000 flowers are borne on a single panicle[1], only a few of them give rise to fruits. To understand the reasons for the poor fruit-set in mango, the present investigation was undertaken to study its reproductive cycle. An unevenly thick and ornamented cuticle covering the papillae cells and its uncommon nature has been observed.

The style in mango is formed laterally on the ovary with the pointed stigma surface having compactly arranged finger-shaped papillae cells. The stigmatic surface including the protruding convex surfaces of papillae cells is covered by a wavy and unevenly thick cuticle (figure 1). The cuticular deposition is massive at the corners of the compactly arranged cells and comparatively thin at their convex surfaces (figure 4). The cuticle is also deposited deep into the spaces between some of the loosely arranged papillae cells (figure 3). There are narrow channel-like configurations in the cuticle, which presumably facilitate the components of the pellicle to ooze out and spread out over the cuticle. The outer layer of the pellicle is electron opaque and is thin (figure 4, thick arrows). The papillae cells also contain abundant phenolics in their vacuoles (figure 4).

The ornamented region comprising the curticle showed positive fluorescence when mounted in auromine O (figure 2). The presence of such a thick and wavy cuticle on the stigmatic surface is uncommon. Comparatively thick and continuous cuticle enclosing the stigmatic papillae cells and the secretion product has been reported in some wet stigmas[2-4]. In *Trifolium pratense*, the highly impermeable cuticle covering the exudate makes a mechanical blocking of pollen at the stigma, which could be a significant determinant of breeding behaviour in self-fertile species[2]. In *Vicia faba*, the cuticle is thickened over the prominences left by epidermal papillae, thinning out between[4]. In all these plants with wet stigmas, mechanical 'tripping' of the cuticle is essential to allow the pollen to come in contact with the secretion for its germination.