CHAPTER V

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Though much work has been carried out on different aspects of ergot alkaloid biosynthesis in Claviceps species, Aspergillus fumigatus and other microorganisms, very little work has been done in higher plants regarding the production of ergot alkaloids. This prompted the researcher to examine the various aspects of ergot alkaloid biogenesis in higher plants.

"In vivo" studies showed maximum quantity of alkaloids in seeds in comparison to other parts of plant. The alkaloids were present in the young leaves but not detected in stem and flowers.

The present investigations were carried out with the tissues derived from the leaves of Evolvulus alsinoides L. This callus demonstrated a higher percentage of ergot alkaloids compared to the intact plant parts including the seeds, hence offering a suitable material for the type of research envisaged.

The hormonal supplement in the medium had a pronounced influence on ergot alkaloid production. Of the various auxins (2,4-D, NAA, IAA and IBA) and their levels studied NAA at 5 mg/l supported the highest growth of tissue. On the other hand, alkaloid production was maximum at 2.0 mg/l 2,4-D. Kinetin alone also promoted growth of tissue, but the alkaloid production was

comparatively much less. GA_3 did not have any promotory effect on growth and alkaloid production. Studies with auxins or GA_3 along with kinetin levels indicated that 2 mg/l 2,4-D + 0.4 mg/l kinetin was the most effective combination for growth of tissue as well as alkaloid production.

Comparative studies in callus and suspension cultures showed that growth of tissue and alkaloid production was higher in suspension cultures. The time course study with the callus and suspension cultures indicated that maximum alkaloid synthesis occurred during the rapid growth phase, the peak values of tissue growth and alkaloid production were attained on day 25 of culture. The tryptophan synthetase activity reached its peak value on day 20, thus preceeding the highest accumulation of alkaloids.

Experiments on inoculum size/medium volume ratio, indicated that 200 mg tissue by fresh weight in 25 ml of medium gave higher growth compared to other treatments. Hence, further experiments were carried out in suspension cultures with an inoculum of 200 mg tissue by fresh weight in 25 ml of medium.

Experiments with different sugars (sucrose, fructose and glucose) showed that sucrose was a more suitable source of carbohydrate and energy for tissue growth and alkaloid production. Further, of the different levels of sucrose tested, 2% sucrose supported maximum growth and alkaloid production.

Studies on the effect of total nitrogen on growth and

production of alkaloids clearly indicated that normal supply of nitrogen (840 mg/l) as present in MS standard medium supported maximum growth of tissue and alkaloid production.

Further studies with different levels of microelement and macroelement salts revealed maximum growth of tissue at the highest levels of microelements and macroelements (2 + 2); whereas the maximum alkaloid production was registered at 1 micro + 2 macro and also at 2 micro + 2 macro element salts levels.

The effect of Tween-40, Tween-60 and Tween-80 as surface reactants on growth and alkaloid production was examined. Of these, Tween-80 was found to be more effective for alkaloid production; whereas growth of tissue was higher in Tween-40. Further experiments with Tween-80 showed that addition of Tween-80 on day 0 was more effective for alkaloid production; while the growth of tissue was higher in treatments where Tween-80 was added on day 20.

The effects of Tryptophan, Mevalonic acid and Methionine, the main precursors of ergot alkaloids, on alkaloid production were studied. In addition to these precursors, the effect of Indole, Serine, Anthranilic acid and 5-methyl tryptophan was also examined. Tryptophan, 5-methyl tryptophan and Mevalonic acid were found to have stimulatory effect on alkaloid production. Addition of Tryptophan at 10 mM concentration promoted the alkaloid production more than 2 times over that

of control. But the tissue growth was suppressed as the concentration of Tryptophan was increased in the medium.

Mevalonic acid promoted growth of tissue comparatively more than the control at all levels tested; while 5-methyl tryptophan completely inhibited the growth of tissue. Methionine did not have any stimulatory effect on alkaloid production. On the other hand, growth of tissue was inhibited.

Anthranilic acid and Indole were found to have adverse effect on growth of tissue and alkaloid production; while DL-serine promoted growth and alkaloid production. In the experiments with Indole + DL-serine, it was observed that the growth of tissue as well as alkaloid production was inhibited.

In all the above studies with precursors, the tryptophan synthetase activity was less than the control. In treatments with Indole and Methionine the enzyme activity was at the lowest value, whereas Anthranilic acid completely inhibited the enzyme activity.

Studies on amino acids during the course of culture showed that the tissue contained in all 7 amino acids. Quantitative studies revealed that Tryptophan was present in maximum quantity on day 20 of culture; whereas Methionine and Serine were present in high amounts on day 15.

Addition of different levels of inorganic phosphate in the medium showed that the increasing levels of inorganic

phosphate had an inhibitory effect on alkaloid production. At the highest level of inorganic phosphate tested, the tryptophan synthetase activity was completely inhibited; thereby limiting the availability of tryptophan as precursor.

Tryptophan, Mevalonic acid and 5-methyl tryptophan were incorporated in the medium containing high levels of phosphate to find out if these precursors can restore the phosphate inhibition of alkaloid production. Tryptophan could enhance the alkaloid production in phosphate inhibited tissues. Mevalonic acid or 5-methyl tryptophan, however, could not counteract the phosphate inhibition of alkaloid production. In all the above cases, tryptophan synthetase activity was not restored. This clearly indicated that 5-methyl tryptophan played a role in ergot alkaloid production by inducing alkaloid synthesising enzymes.

Experiments with cofactors of dimethylallyl pyrophosphate : L-tryptophan dimethylallyl transferase (DMAT synthetase) on alkaloid production showed that higher levels of Ca^{2+} stimulated the highest production of alkaloids compared to other cofactors. On the contrary, the growth of tissue was suppressed. Mg^{2+} at higher levels than control, increased the production of alkaloids, but inhibited the tissue growth. Increasing the levels of Fe^{2+} beyond 1 mM had an inhibitory effect on growth and alkaloid production. Though indirect, these studies with cofactors indicated the regulatory role of DMAT synthetase in higher plants also.

Examination of the changing patterns of growth and alkaloid production with passages in culture, revealed that growth and alkaloid production were enhanced at 8th and 12th subcultures; while they declined thereafter.

The present investigation thus illustrated the basic similarity in ergot alkaloid biosynthetic processes of ergot fungus and a higher plant. It further represents an in-depth study in ergot alkaloid biogenesis by a higher plant, which had not been carried out so far.

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

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