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Callus tissue was successfully initiated and established from the floral buds of Nicotiana tabacum L. var. Anand-2. It was maintained on MS medium supplemented with 2.0 mg/l each of IAA, NAA and KN. The callus cells (upto 60%) remained diploid during the first four transfers. Thereafter, the ploidy level went on increasing.

Using anthers of N. tabacum L. var. Anand-2, haploid plants were raised. The pathway of pollen embryogenesis was traced. The frequency of haploid formation was low - 16%. IAA (0.2 mg/l) or coconut milk (15% v/v) alone or in combination were essential for haploid induction. Cold pretreatment of flower buds did not enhance frequency of haploid initiation.

Haploid callus was initiated from the shoot explants of haploid N. tabacum plantlets. The haploid callus thus initiated maintained its ploidy level (upto 60-70%) during the first four subcultures.

Of all the auxins tested maximum growth response of both haploid and diploid callus was obtained with incorporation of NAA (2.0 mg/l) into the MS basal medium. 2,4-D antagonized growth. However, at low concentration of 2,4-D (0.5 mg/l) the growth of haploid callus was stimulated.

The cytokinin, kinetin stimulated growth of both haploid and diploid callus. MAP, the other cytokinin tested, caused growth inhibition in both callus types.

$GA_3$ could bring about growth stimulation of both diploid and haploid callus only at high concentrations, lower levels being ineffective.

Of all the auxin and kinetin combinations tested, 2.0 mg/l each of IAA, NAA and KN supported maximum growth of both haploid and diploid callus.

Maximum phenolic accumulation in diploid callus occurred in response to IAA (0.5 mg/l). However, in haploid callus 2,4-D (4.0 mg/l) brought about maximum phenolic accumulation. Increasing KN levels (0.1 - 1.0 mg/l) resulted in decreasing accumulation of phenolics in both the callus types. MAP, however, favoured phenolic accumulation in diploid callus but not so in haploid callus.

Increasing $GA_3$ concentration in the medium resulted in decline of phenolic accumulation in both haploid and diploid callus.

IAA and KN combinations when tested with varying levels of sucrose, brought about high phenolic accumulation at high sucrose level in the medium. Both diploid and haploid callus responded in identical manner.

The organogenetic behaviour of both diploid and haploid callus was essentially comparable. Both callus types produced shoots on MS basal medium. In both the tissues organogenesis was induced in presence of IAA. All other auxins (IBA, NAA, 2,4-D) and cytokinins (KN, MAP) proved ineffective. IAA at low levels (0.175 - 0.3 mg/l) invariably induced shoots in haploid and diploid callus. Higher levels of IAA (0.5 - 2.0 mg/l) favoured root differentiation. Root differentiation could also be achieved by increasing sucrose level in the low IAA (0.3 mg/l) containing medium. IAA and KN when taken together in the ratio of 10:1 proved totally ineffective in promoting organogenesis. However, with their use in 1:1 ratio shoots were either differentiated exclusively or accompanied by roots. High IAA and KN levels in the medium though effective in shoot induction, however, reflected on the shoot morphology. The shoots differentiated were short, stunted and in clumps.

Incorporation of phenolic acids - t-cinnamic acid, caffeic acid, ferulic acid and p-hydroxybenzoic acid - into the shoot inducing (MS + 0.3 mg/l IAA) and root inducing (MS + 2.0 mg/l IAA) media resulted in total suppression of organogenesis from diploid callus. Incorporation of t-cinnamic acid (500 μM) and caffeic acid (100 μM) into the IAA (0.3 mg/l) containing medium blocked shoot differentiation from haploid callus and favoured instead, the differentiation of roots. All other phenolic acids

inhibited caulogenesis. All the phenolic acids with the exception of t-cinnamic acid (100 μM) and caffeic acid (500 μM) inhibited root differentiation from haploid callus in response to IAA (2.0 mg/l).

The root, shoot and leaf explants of diploid tobacco plants differentiated numerous shoots when cultured on MS medium supplemented with 2.0 mg/l IAA and 0.4 mg/l KN. On the same medium shoots were differentiated from leaf and root explants of haploid tobacco plants. The shoot of haploid tobacco plant did not respond.

Physiological and biochemical studies carried out during callus initiation revealed interesting facts. The floral buds in culture demonstrated two growth phases, one before callus induction and the other after it. A lag period acted as an interlude between the two growth phases. High activity of peroxidase, IAA Oxidase, MDH and PAL was registered with callus induction. The activity of all enzymes decayed thereafter. The anodic isoperoxidases $A_{15}$, $A_{17}$ and $A_{21}$ were detected in callus. These isoperoxidases were synthesized either earlier (day 15) or on the day of callus induction (day 18). All three cathodic isoperoxidases $C_4$, $C_5$ and $C_9$, found in diploid callus were synthesized many days before callusing occurred.

Both diploid and haploid tobacco callus exhibited only lag and exponential growth phases. The enzyme activity of peroxidase,

IAA Oxidase, MDH and PAL was high during the lag phase and declined with the progress of exponential period. Similar pattern was followed by phenolics in both callus types. The anodic and cathodic isoperoxidase spectrum changed with the growth phases. Isoenzymes were identified and related to a particular growth period.

In haploid callus rhizogenesis on various media was accompanied with decline in peroxidase activity. Same did not hold true for diploid tobacco callus. The enzyme activity in haploid callus remained in the range of 0.35 - 0.38 units, whereas in diploid callus it remained in the range of 0.83 to 0.88 units. The IAA Oxidase activity declined during rhizogenesis from diploid callus, however, the same did not hold good for haploid callus. The MDH activity during rhizogenesis from haploid and diploid callus on various media did not follow any definite pattern of development. However, in haploid callus the activity was in the range of 3.06 - 5.63 units on the day of rhizogenesis. Rhizogenesis in haploid and diploid callus occurred concomitant with increasing PAL activity, though the activity on different rhizogenic media varied. The phenolic content of haploid callus increased with rhizogenesis whereas it declined in diploid callus.

Though peroxidase isoenzyme patterns changed with the media used, of significance was the synthesis of anodic

isoperoxidase PR ($R_m$ = 0.3) before rhizogenesis in both haploid and diploid callus. This isoperoxidase was also detected in regenerated roots.

Physiological studies made with diploid and haploid tobacco callus during shoot differentiation revealed that the differentiation of shoots in both callus types occurred concomitant with increasing peroxidase activity which was in the range of 1.01 - 1.11 units on the day of caulogenesis. Though in both the callus types the IAA Oxidase activity increased during early culture period it, nevertheless, declined during the phase of shoot induction and remained in the range of 2.48 - 2.72 units. Caulogenesis in both haploid and diploid callus occurred concomitant with increase of MDH activity. However, the MDH activity in haploid callus was 6-7 times more than in diploid callus. The PAL activity decayed in both haploid and diploid callus during the phase of shoot differentiation.

The phenomenon of shoot differentiation in both haploid and diploid callus was signalled by the synthesis of three anodic isoperoxidases $PS_1$, $PS_2$ and $PS_3$. These isoperoxidases were synthesized at least 3 days before the actual emergence of shoots.

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