be present as a complex in plant cells[9]. OPRTase and ODCase were found in dry seeds and in cotyledons. PRPP synthetase was also detected in dry seeds, but its level decreased over 48 h. Activities of these enzymes were slightly higher in cycloheximide-treated cotyledons than in controls (figure 1c, d). Ross *et al*[1] reported that biosynthesis of UMP from [6-^{14}C]orotate in pea cotyledons was limited during early stages of germination, although high levels of OPRTase, ODCase, and PRPP were present in the cotyledons. Similar results have been found in germinating black gram seeds[3]. The present results indicate that, at least in the case of black gram, the limitation in biosynthesis of UMP from externally supplied [6-^{14}C]orotic acid seems to be due to the poorer permeability of cells to orotate during early stages of germination. Significant amounts of [6-^{14}C]orotate, taken up by cotyledons, failed to reach the site of active synthesis of nucleotides.

From the present results, it appears that enzymes for *de novo* and salvage pathways of pyrimidine biosynthesis are present in dry seeds and are functional after imbibition, while degradation of pyrimidines is mostly catalysed by enzymes synthesized in cotyledons during germination.

1 October 1988

1. Ross, C., Coddington, R. L., Murray, M. G. and Bledsoe, C. S., *Plant Physiol.*, 1971, **47**, 71.
2. Ross, C. and Murray, M. G., *Plant Physiol.*, 1971, **48**, 626.
3. Ashihara, H., *Z. Pflanzenphysiol.*, 1977, **81**, 199.
4. Koshiba, T., Suzuki, Y. and Minamikawa, T., *Plant Cell Physiol.*, 1983, **24**, 1291.
5. Kanamori-Fukuda, I., Ashihara, H. and Komamine, A., *J. Exp. Bot.*, 1981, **32**, 69.
6. Wasternack, C., *Biochem. Physiol. Pflanzen*, 1978, **173**, 467.
7. Arora, S. K., *Chemistry and biochemistry of legumes*, Edward Arnold, London, 1983, p. 359.
8. Ashihara, H., *Acta Physiol. Plant.*, 1981, **3**, 77.
9. Ashihara, H., *Z. Pflanzenphysiol.*, 1978, **87**, 225.

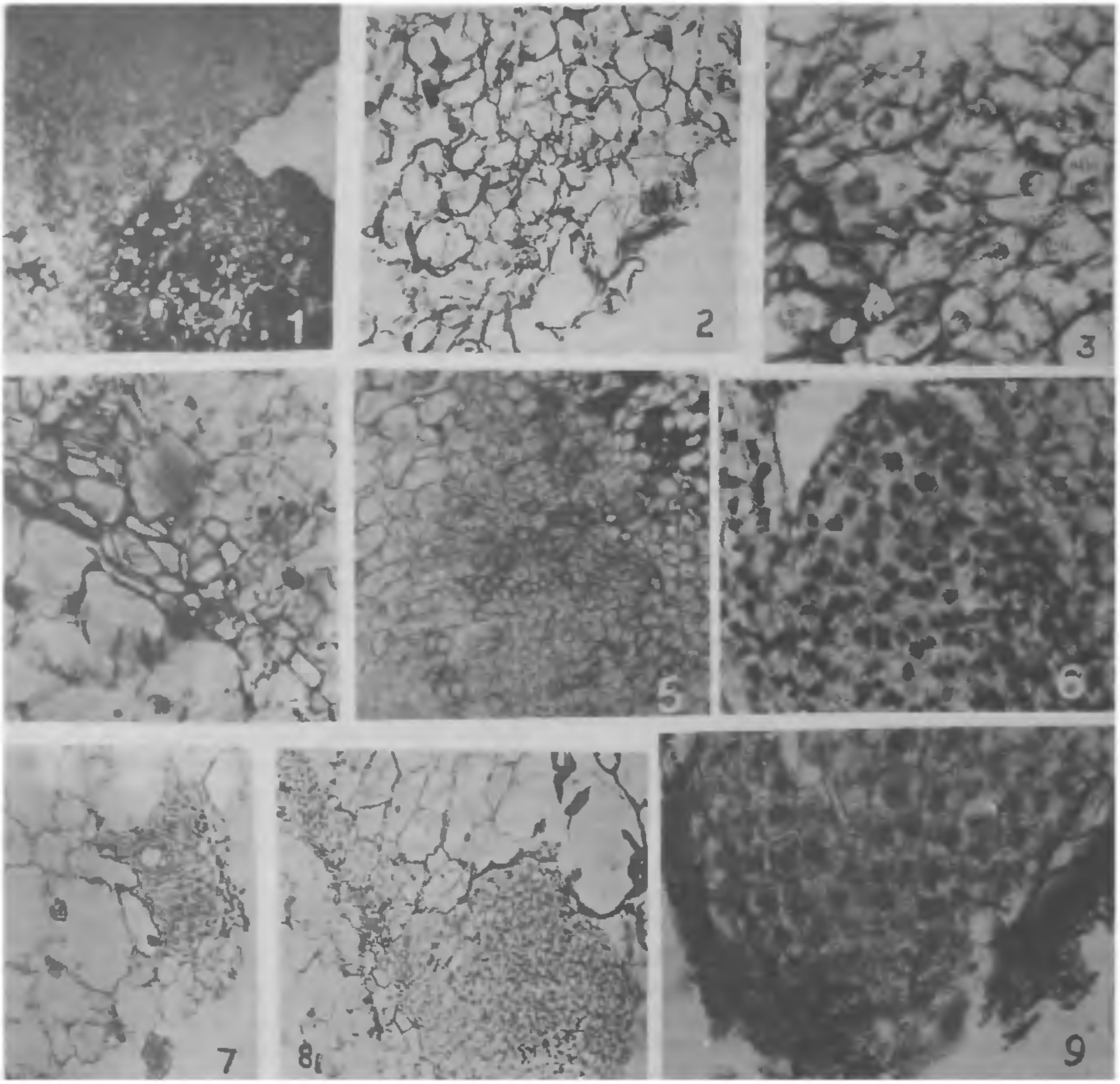
MORPHOGENETIC STUDIES IN ANDROGENIC CALLUS OF *SOLANUM MELONGENA* L.

NEELAM R. YADAV*, T. M. VARGHESE and D. R. SHARMA*
*Departments of Botany and *Genetics, Haryana Agricultural University, Hisar 125 004, India*

STUDIES on morphogenesis in callus cultures have not received due attention. However, some scattered reports are available[1-3]. It is important to study plant regeneration at morphological, physiological and biochemical levels for successful application of plant tissue culture techniques to crop improvement. In this communication, we describe the morphogenetic events leading to shoot and root formation in androgenic callus cultures of *Solanum melongena* L. cv. H4.

Androgenic callus was obtained by culturing anthers at the uninucleate stage of development on GD medium[4] supplemented with 2 mg/l IAA and 1 mg/l kinetin in the dark at 25±2°C. Calli were subcultured on differentiating medium containing GD basal medium, 0.1 mg/l NAA and 2 mg/l kinetin. Shoots and roots developed on the same medium. These cultures were incubated in a 16 h light, 8 h dark cycle. Calli at different stages of development were fixed and sectioned by conventional procedures and stained with safranin and fast green[5]. Details of callus development and differentiation have been discussed earlier[6].

The callus that grew out of anthers started growth by the activity of peripheral meristematic cells. The cell walls of intermittent group of cells became suberized, resulting in irregular growth of the callus (figures 1 and 2). The callus cells were large and loosely arranged. The cells destined to form meristemoids were rich in starch (figure 3). The first indication of vascularization was the appearance of tracheidal cells and vessel elements. These were randomly distributed at different positions over the callus but formed a continuous ring marking the initiation of procambium (figures 4 and 5). The procambium gave rise to xylem towards the outer side and phloem towards the inner side, and a multi-layered cambium in between. The protoxylem showed exarch condition at many places, which is a feature of roots. In shoot-forming callus, meristematic activity was located in peripheral regions. These cells were small, with prominent nuclei and dense cytoplasm. The meristematic tissue later



Figures 1–9. **1.** Callus with irregular margins, showing suberized cells and meristematic tissue (× 192). **2.** Inactive cells on the margin of callus (× 288). **3.** Meristematic cells with starch grains (× 288). **4.** Cambial tissue development in callus (× 288). **5.** Part of vascular ring showing xylem (× 192). **6.** Shoot apex with leaf primordia (× 1125). **7.** Meristematic tissue developed at the margin of callus (× 288). **8.** Initial development of shoot apex (× 288). **9.** Root apex (× 192).

developed into dome-like structures, which ultimately gave rise to shoot meristem (figures 6–8). In early stages shoot-forming and root-forming meristems could not be distinguished. The shoot apex had a well-defined tunica of 4–5 layers overarching the corpus. Fast divisions in the tunica cells of the apical meristem produced the first leaf primordia, which often overgrew the shoot apex. After the production of a few leaf primordia, the tunica region lost its identity in the shoot apex. Most of the cells of the leaf primordia were larger, with small nuclei, compared to shoot apex cells (figure 6). Root development was not clearly understood. The initials for different histogens were not demarcated. A

characteristic feature noted in the root primordium was the absence of root cap (figure 9).

Random vascularization in callus cultures has been reported by several workers[7,8]. Starch accumulation is reported to be essential in mesistematic tissues, leading to organogenesis[9]. However others have found no correlation between bud formation and starch accumulation[3,10]. In our studies the meristems were indistinguishable at early stages of development. Similar reports are also available in the literature[8]. Meristematic activity is found in peripheral regions or in deeper layers depending upon the crop species. In cauliflower, meristematic activity was found in deeper layers while in *Punica granatum* meristems were superficial[8,11]. Although all seed plants have root cap, it was missing in the *in vitro* formed roots. But these may degenerate under culture conditions, as reported in vanilla[12].

4 July 1988

1. Halperin, W., *Annu. Rev. Plant Physiol.*, 1969, **20**, 395.
2. Hicks, G. S., *Can. J. Bot.*, 1987, **65**, 1913.
3. Maherchandani, N., Yadav, N. R., Jain, R. K., Chowdhury, V. K. and Singh, S., *Proc. symp. plant cell and tissue culture of economically important plants*, (ed.) G. M. Reddy, Hyderabad, 1987, p. 337.
4. Gresshoff, P. M. and Doy, C. H., *Planta*, 1972, **107**, 161.
5. Johansen, D. A., *Plant microtechnique*, McGraw Hill, New York, 1940.
6. Misra, N. R., Varghese, T. M., Maherchandani, N. and Jain, R. K., *Plant cell culture in crop improvement*, Plenum Press, New York, 1983, p. 465.
7. Gayatri, M. C., *Phytomorphology*, 1978, **28**, 395.
8. Jaidka, K. and Mehra, P. N., *Can. J. Bot.*, 1986, **64**, 1644.
9. Thorpe, T. A. and Murashige, T., *Can. J. Bot.*, 1970, **48**, 277.
10. Von Arnold, S., *Ann. Bot.*, 1987, **59**, 15.
11. Singh, S. and Mathur, M., *Acta Bot. Indica*, 1987, **15**, 98.
12. Philip, V. J. and Nainar, S. A. Z., *Ann. Bot.*, 1988, **61**, 193.

VIRUS PARTICLES IN CHLOROPLAST PREPARATIONS FROM INFECTED LEAVES OF *NICOTIANA TABACUM*

G. P. RAO and K. SHUKLA
Department of Botany, University of Gorakhpur, Gorakhpur 273 009, India.

THE presence of virus particles inside the chloroplast has been proved by electron microscopy[1,2]. In the present investigation, the presence of virus particles inside the chloroplast has been proved by the following experiment.

Chloroplast was isolated as described earlier[3,4]. The cultures of tobacco mosaic virus (TMV) and potato virus X (PVX) were maintained on systemic host (*Nicotiana tabacum* cv. White Burley) by successive inoculations at intervals of 3 days, in an insect-free glass house. *Chenopodium amaranticolor* Coste et Reyn was used as local lesion host (test plant). The three types of virus inoculums were prepared as follows:

(i) Diseased leaf (with TMV and PVX) showing severe symptoms was macerated and crushed in pestle with distilled water in 1:1 (w/v) ratio (inoculum I).
(ii) Chloroplast was isolated from chlorotic areas of virus-infected leaf. This isolated chloroplast in 0.2 M NaCl solution served as inoculum II.
(iii) Isolated chloroplast (in 0.2 M NaCl solution) from green areas of the same virus-infected leaf served as inoculum III.

For chloroplast isolation, two types of tissues were taken from each of the same virus-infected (TMV and PVX) leaf of *N. tabacum*, i.e. the green and chlorotic tissues. The chlorotic and green patches were cut separately from virus-infected leaves with the cork borer. Fifty g of leaf tissues was taken for chloroplast isolation. After isolation, the chloroplast was kept in 0.2 M NaCl solution at 4°C for 6 h and this was treated as inoculums II and III.

The inoculation was done by rubbing the inoculum gently (I, II and III) on the upper surface of leaves of test plant with 600 mesh carborundum powder. All the inoculums were inoculated separately on the leaves of test plants of the same age and size. Five plants were taken for each experiment. Local lesions were counted after seven days of treatment to compare the potency in infection of each inoculum. All the experiments were repeated thrice and the average value recorded.