(Fig. 1 B and 1 C). While the donor B_3 formed one somatic (*a*) and two internal antigen (*b*, *c*) bands with its antiserum, the transformants did not show any cross reaction when the antigens of these were prepared on YEMA (Fig. 1 B). Only B_1 and B_2 formed cross reacting heterologous '*b*' band with B_3 antisera when cultured on Ashby's medium (Fig. 1 C). Such non-specific bands formed due to antigens or haptens of intracelluar origin would be expected in a drastically changed medium. But the absence of somatic bands with the B_3 antiserum excludes the possibility of these strains being derived from *Azotobacter*.

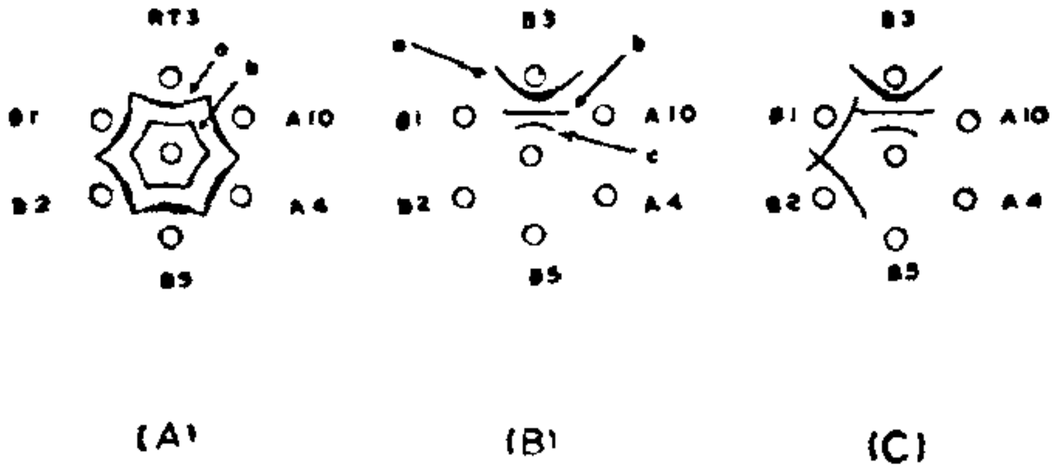

FIG. 1. Precipitin bands formed in agar diffusion against antisera of *R. trifolii* RT/3 recipient (A), and *A. chroococcum* B_3 donor (B and C). Transformantsl B_1, B_2, B_5, A_4 and A_{10} were grown on yeast extract mannitol agar slopes. When transformants were shifted to Ashby's medium, changes in antigenic properties of transformants B_1 and B_2 were observed (*c*) in all the three hexogones.

Nodules were not observed in uninoculated control plants and in those inoculated with *A. chroococcum* B_3, while the recipient strain RT/3 and other transformants formed nodules on *T. alexandrinum* (Table I). The mean number of nodules varied from 9 to 14 and the variation was within statistical limits.

TABLE I

Nodulation of Trifolium alexandrinum (*variety tetra ploid C*) *by* A. chroococcu n, R. trifolii *and transformants under pot culture conditions*
(*values average of* 28 *plants*)

Strains	No. of nodules/plant
Control	0
A. chroococcum B_3 (Donor)	0
R. trifolii RT/3 (Recipient)	11±0·33
Transformants	
B_1	14±0·99
B_2	11±0·69
B_5	9±0·62
A_4	11±0·97
A_{10}	13±0·6

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REGENERATION OF HYBRID TOMATO PLANTS FROM LEAF CALLUS

THE use of tissue culture method for rapid propagation[1] and protoplast fusion[2] is already described in literature. Tomato plants (*Lycopersicon esculentum*) have been regenerated from leafcallus by Kartha *et al.*[3]. A hybrid tomato TH (Pol × Pusa) has been developed in this laboratory[4] by artificial pollination between Pusa variety (red colored fruits bearing) and Pol variety (high β carotene containing fruits). This hybrid TH has lycopene and high β carotene containing fruits which are nutritious and easily marketable. The present work describes the differentiation of leaf callus of Pol and TH varieties of tomato into full plants. The work was taken up with the objectives of (1) rapid propagation of hybrid and (2) for the study of protoplasts fusions.

Standard methods of White[5] were used for the cultivation of leaves gathered from 4–5 weeks old plants, and plants which were flowering. The sterile leaves were cut into small pieces and put into different media for callus development and differentiation.

The basal medium contained Murashige and Skoof's[6] major and minor salts, vitamins of B 5 medium[7] with 3% sucrose as the carbohydrate source. Agar (1%) was used to solidify the medium. PH was adjusted to 6 before autoclaving and standard techniques were used for autoclaving and culturing. Cultures were incubated at 26° ± 1°C with a light intensity of 1000 Lux and relative humidity of 50–60%. Controls were maintained in all the experiments. Each experiment had a minimum of 10 replicates and all experiments were conducted twice atleast.

For the Pol leaf explants 12 different media were tried using basal medium with different hormones at various concentrations. It was observed that of the various cytokinins used, benzyladenine and zeatin

were better than kinetin and, of the various auxins used, indole acetic acid (IAA) was better than naphthyl acetic acid (NAA) for callus and shoot development. The best five media are listed in Table I. On subculturing to media containing, only zeatin (0·1, 1, 2·5 and 5 μM), only IAA (1, 10, 100 and 500 μM) and zeatin and IAA together at various concentrations, plantlets were formed on zeatin at 5 μM and roots were developed on IAA at 1 μM. At 10 μM IAA, with various concentration of zeatin only callus was formed. When these were transferred to media listed in Table II, it was observed that even calli subcultured from previous subculture, could easily differentiate into full plantlets in media having isopentenyl adenosine (6 iPA) at 2·5 μM and IAA at 1 μM. These plantlets could be transplanted into pots and grown to maturity.

TABLE I

Leaf explant cultivation of Pol variety

No.	Media	Results
1.	A* + BA + 50	Root and shoot with good callusing.
1.	A + BA 100 + IAA 100	Profuse shoot formation
3.	A + BA 10 + IAA 100	Better than 2.
4.	A + Zeatin 1 + IAA 100	Green healthy callus and shoot.
5.	A + Zeatin 5	Slight callusing.

* Basal medium—see text.
\+ Benzyl adenine
Hormone concentration in μM.

TABLE II

Growth of differentiated and non-differentiated callus of Pol variety on 2nd transfer

No.	Media	Results
1.	A + Zeatin 5 + IAA 1	Good callusing and plantlet formation
2.	A + Zeatin 5	No plantlets only shoot formation
3.	A + IAA 1	Roots and shoot formation
4.	A + 6 iPA 1	Callus and shoot formation
5.	A + 6 iPA 2·5 + IAA 0·2	Good plantlets formation Better than 1
6.	A + 6 iPA 5 + IAA 2	Mostly callusing

Hormone conc. in μM, A—Basal Media.

For hybrid tomato (TH) explants, 20 different media compositions using different hormones with basal medium were tried. Zeatin, 6 iPA and BA alone did not develop callus or shoots. But zeatin and 6 iPA with IAA gave good callus and shoot formation. The best plantlet formation was observed in media with zeatin and IAA Table III. These plantlets when transferred to media having 6 iPA 2·5 μM and IAA 10 μM developed healthy roots and were easily transplanted into pots.

TABLE III

Leaf explant cultivation of hybrid (Pol × Pusa) variety

No.	Media	Results
1.	A + Zeatin 1	No good callusing and differentiation.
2.	A + Zeatin 5	do.
3.	A + 6 iPA 1	do.
4.	A + 6 iPA 2·5	do.
5.	A + BA 10	do.
6.	A + BA 50	do.
7.	A + Zeatin 1 + IAA 1	Callus, shoot and plantlet formation.
8.	A + Zeatin 5 + IAA 1	Not as good as 7.
9.	A + Zeatin 1 + IAA 2·5	Green callus and plantlets.
10.	A + Zeatin 5 + IAA 10	No growth.
11.	A + 6 iPA 1 + IAA 10	do.
12.	A + 6 iPA 1 + IAA 100	do.
13.	A + 6 iPA 2·5 + IAA 2·5	Callus and very good shoot formation.
14.	A + 6 iPA 2·5 + IAA 100	Callus and shoot primordia
15.	A + 6 iPA 5 + IAA 1	do.
16.	A + BA 10 + IAA 10	No growth.
17.	A + BA 10 + IAA 100	do.
18.	A + BA 50 + IAA 2·5	Slight callus and shoot formation.
19.	A + BA 50 + IAA 100	No growth.
20.	A + BA 100 + IAA 10	Slight callus formation.

Abbreviations and concentrations as in Tables I and II.

The above method for regeneration of tomato plant is faster and also the segregation of hybrid characters is minimized. As the media for callus development of one of the parents (Pol) and the meida for regeneration of TH are known, the fusion of Pol callus protoplast with Pusa leaves and development of hybrid can easily be studied.

Plate I describes the various stages of *in vitro* development of Pol and TH from leaf.

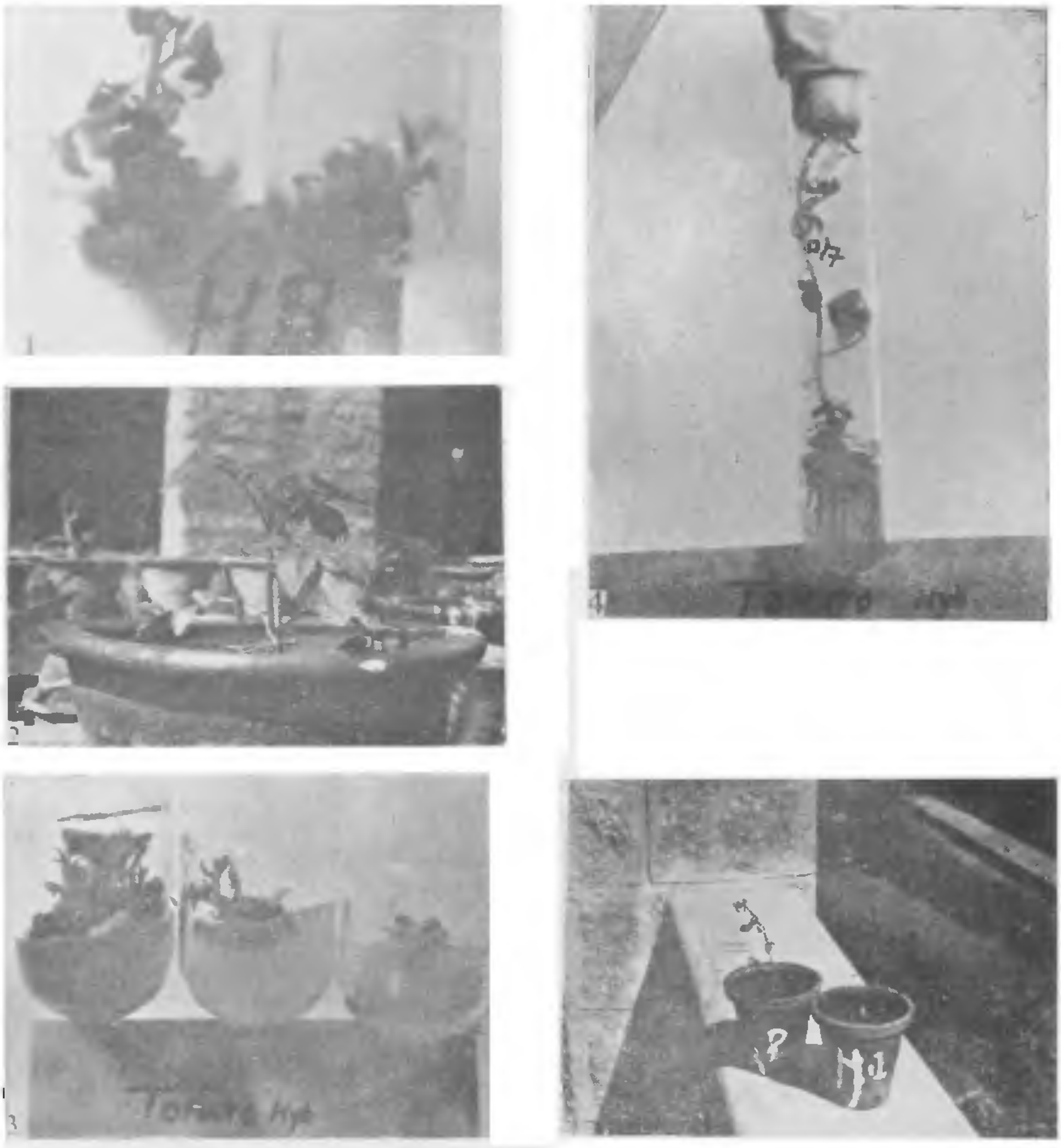

PLATE 1 (FIGS. 1–5)

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