AUXIN AUTOTROPHIC CALLUS TISSUES IN *NIGELLA SATIVA*

DIFFERENT types of auxins in combination with kinetin are generally used for the initiation and maintenance of callus tissues. Auxin-kinetin balance is responsible for the growth of callus tissues and also for regeneration into whole plant. However, crown gall, virus and genetic tumour tissues and some habituated tissues grow without any hormones[1]. Binns and Meins[2] succeeded in obtaining cytokinin–autotrophic *Nicotiana tabacum* cells at the rate of 10^{-3} per cell generation. Syono and Furuya[3] induced auxin-autotrophy in *N. tabacum* callus tissues at the rate of about 10^{-4}. Present findings deal with the study of leaf and seed callus tissues of *N. sativa* grown in hormone free medium to isolate new auxin-autotrophic lines.

Callus cultures were initiated from leaf segments as well as directly from seeds and were maintained on a modified Murashige and Skoog's[4] medium[4]. The constituents of the medium are listed in Table I. The

TABLE I

Constituents of the medium (all value expressed for 1 litre of medium)

Constituents A

$CaCl_2.2H_2O$—880 mg; NH_4NO_3—3300 mg; KNO_3—3800 mg; H_3BO_3—6·0 mg; $Na_2MoO_4.2H_2O$—0·25 mg; $FeSO_4.7H_2O$—27·85 mg; Na_2EDTA—37·25 mg; $MgSO_4.7H_2O$—615 mg; KH_2PO_4—425 mg; $MnSO_4.4H_2O$—11·15 mg; $ZnSO_4.7H_2O$—4·30 mg; KI—0·415 mg; $CuSO_4.5H_2O$—0·0125 mg; Sugar—20,000 mg.

Constituents B

Glycine—2·0 mg; *m*-Inositol—100 mg.

Constituents C

Nicotinic acid—0·5 mg; Pyridoxine HCl—0·5 mg; Thiamine HCl—0·1 mg.

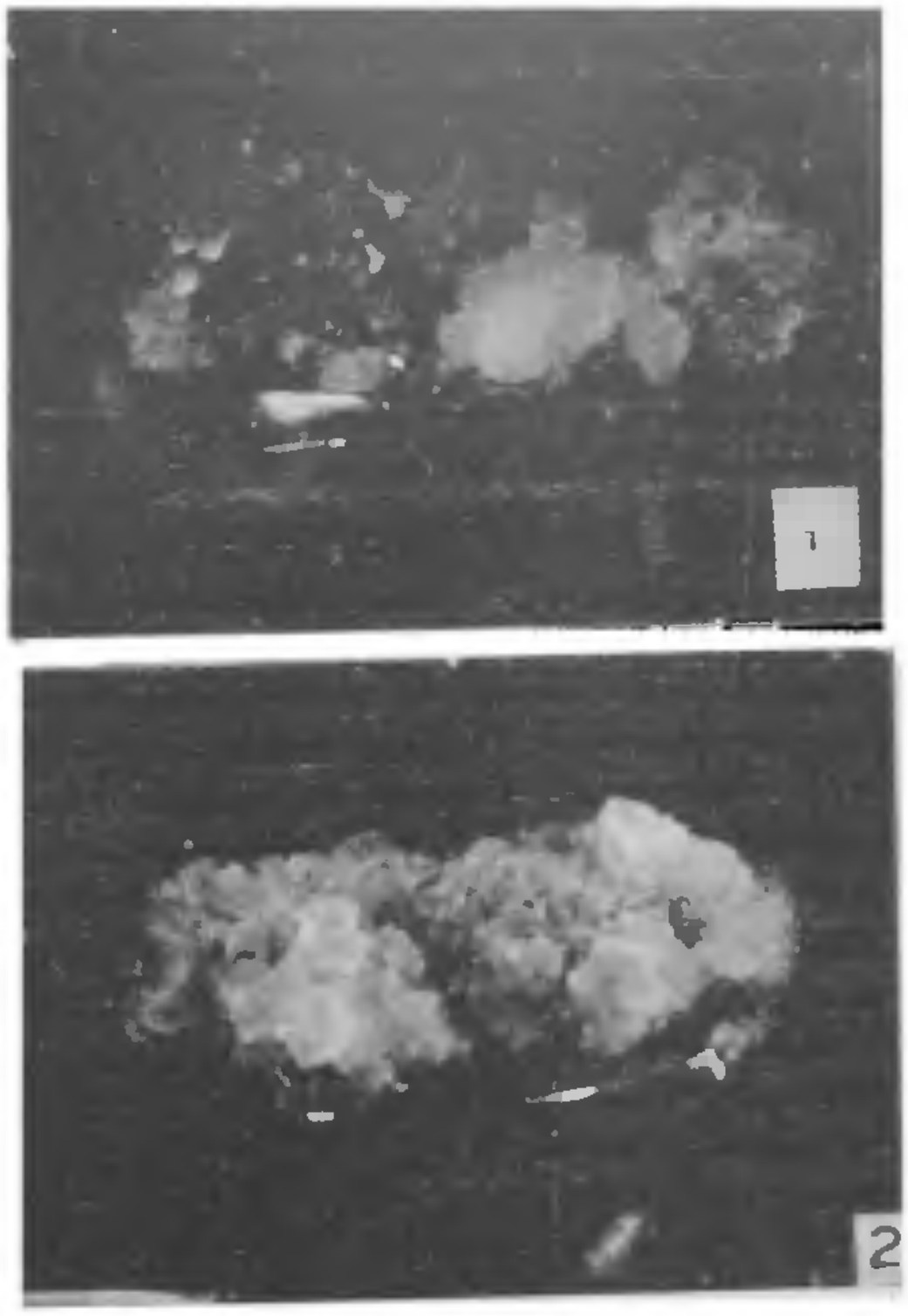
medium (N-1) was supplemented with 2·0 mg/l of 2,4–D, 0·1 mg/l of kinetin (6-furfurylaminopurine) and 100 ml/l of fresh coconut milk. These were grown for five months in the same medium (N–1) at $24 \pm 2°$ C and at 16 hour and 8 hour light-dark photoperiod (Fig. 1). Incubation time was 28 days in each case. After 5 subsequent subcultures the callus tissues were transferred to the following sets:

(*a*) Media containing constituents A, B and C of Table I (designated as N–6/0).

(*b*) Media containing constituents A and B (designated aa N–6/1).

(*c*) Media containing constituents A plus 10% fresh coconut milk of whole volume (designated as N–6/2).

(*d*) Maintained in the same (N–1) medium (Taken as control).



FIGS. 1–2. Fig. 1. Leaf callus growth of *N. sativa* in auxin containing medium (N–1 media). Fig. 2. Leaf callus growth of *N. sativa* in auxin and kinetin free medium (medium containing constituents A, B and C of Table I).

It was observed that callus tissues grow nicely in N–6/0 medium for first 4 months, and in N–6/2 medium for first 2 months and the growth was almost same as in the case of control (Table II). From 3 months onwards callus growth begins to retard in N–6/2 medium and the callus was completely dead from 6 months of subculture. Callus growth was moderate from the beginning in N–6/1 medium and the tissues completely failed to grow in this medium after 4 months of subculture. The callus tissues maintained statisfactory growth in N–6/0 medium even upto 11 months. If the 2 months old and 3 months old callus tissues from N–6/1 and N–6/2 medium were transferred to a medium containing little amount of hormones such as 0·5 mg/l 2,4-D and 0·1 mg/l kinetin, vigorous growth was again restored.

TABLE II

Growth of the leaf callus tissues grown in N-6/0, N-6/1 and N-6/2 medium

Medium	Growth pattern of different age of callus tissues					
	1 month old	2 months old	3 months old	4 months old	5 months old	6 months old
N-1 (Control)	++++	++++	++++	++++	++++	++++
N-6 0	++++	++++	++++	++++	+++	+++
N-6 1	++	++	+	Died	–	–
N-6 2	++++	++++	+++	+	+	Died

++++ = best callus growth; +++ = satisfactory growth; ++ = slow callus growth; + = very slow callus growth.

Thus, an auxin-autotrophic callus tissue of *N. sativa* was obtained from N-6/0 medium containing only basic salts and vitamins of the medium. These tissues are still maintaining their profuse growth after keeping for 11 months in hormone free medium (Fig. 2). *Further trials are needed to establish these autotrophic lines as mutants which will lead to additional areas of investigation both in basic as well as applied science.*

Chromosome Research Centre, SURESH CHAND,
SATYESH CHANDRA ROY.
Department of Botany,
University of Calcutta,
35, Ballygunge Circular Road,
Calcutta 700 019.
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CHEMICALLY-INDUCED VARIANTS IN BLACK GRAM—*PHASEOLUS MUNGO* L.

ALTHOUGH black gram (*Phaseolus mungo* L.) is an important pulse crop it has not received the attention it deserves in the matter of mutation breeding. T–9, a short duration, day-neutral variety of black gram obtained from the Pulse and Oil Seed Research Station, Berhampore, West Bengal, was used in the present study. Dry seeds were treated with 0·1% and 0·2% aqueous solutions of ethyleneimine (EI) and hydroxylamine (HA) for 4 hours at room temperature in each case and a control was maintained in distilled water (DW). After washing, the seeds were sown in the experimental plots of the Department of Botany, University of Calcutta.

Cytological studies revealed that the percentage of chromosomal aberrations induced was the highest in 0·2% HA. The aberrations consisted of breakage in metaphase and lagging and unequal separation in anaphase. Meiotic studies showed fragments and univalents at metaphase I and laggards, bridge with or without fragments at anaphase I. Control plants did not show any meiotic irregularity. Percentages of pollen sterility were 12·92 in 0·1% EI, 14·01 in 0·2% EI, 12·39 in 0·1% EI, 13·77 in 0·2% HA and 2·24 in control (DW).

Reduction in germination could be due to the fact that the treated seeds were sown directly in the field. In the M_1-generation, variations in plant height, leaf shape, pod shape and pod size were observed. Most of them did not persist in the subsequent generation, presumably because of mutation shock or other physiological causes. Seeds of normal looking M_1 plants were used to raise the M_2-generation in which plant mutants were detected.

In the M_2-generation, percentage of germination which was the lowest in 0·2% EI was 60% as compared to 90% in DW (control). Chlorophyll mutants were mostly albinos which did not survive for more than 3–4 days. Some other seedling mutants were also observed in which growth was severely affected. Most of them survived for less than a month and were grouped as lethals. Percentage of seedling mutants was maximum in 0·2% EI.

Plant mutants were those which could be detected in the adult stage as well as those seedling mutants which grew into adult plants retaining their morphological abnormalities. Percentage of these variant