exudate at the expense of the inorganic fraction. Darkness showed similar effect. Under such dark conditions, DNP also increased the percentage of organic–^{32}P in the root exudate, ATP was uneffective, while DNP + ATP appreciably increased it as compared with that in the control plants.

Discussion

The results indicate that light enhances the uptake of phosphorus by the roots of cotton plants and its translocation towards the stem thus confirming the results obtained by others (Linser, 1965 and Ashour *et al.*, 1968). Such effect was suggested by McEvoy (1967) to be due to the increased supply of the photosynthate under light conditions. In the light, the decrease in the uptake of ^{32}P after treatment with DNP, may be due to the inactivation of the phosphorylation processes in plant tissues. Under such conditions the formation of ATP was found to be partially blocked (Jachson *et al.*, 1962). However, in the dark, when only the oxidative phosphorylation was acting and not the photosynthetic phosphorylation, the DNP and the ATP were without effect on the uptake of ^{32}P. Thus, it seems that the high energy compounds formed during photosynthesis alongside with the downwards photosynthate may take part in the metabolic active uptake of phosphorus. On the other hand, when ATP is present in the root medium, the uptake of ^{32}P was not activated, but on the contrary, may be slightly retarded. A competitive effect between the molecule of ATP or its derivatives and the ion of phosphorus for a certain carrier was suggested for the explanation of such phenomenon (Vakhmistrov and Listova, 1967).

The translocation of ^{32}P from the root to the shoot was enhanced under both conditions of light due to DNP or ATP treatments, while DNP + ATP seemed to have an additive effect. It seems that high energy phosphorus compound is required for translocation. Randal and Vose (1963) found that DNP had a major possitive effect on the translocation of phosphorus to the shoots. In addition, it seems that when the translocation of phosphorus from the root was enhanced, the organic fraction of the translocated phosphorus was increased indicating a change in phosphorus metabolism. Further studies are needed to clarify the problem of translocation of phosphorus compounds in connection with the role of DNP, ATP and light.

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PROPAGATION OF *DIOSCOREA FLORIBUNDA* FROM *IN VITRO* CULTURE OF SINGLE-NODE STEM SEGMENTS

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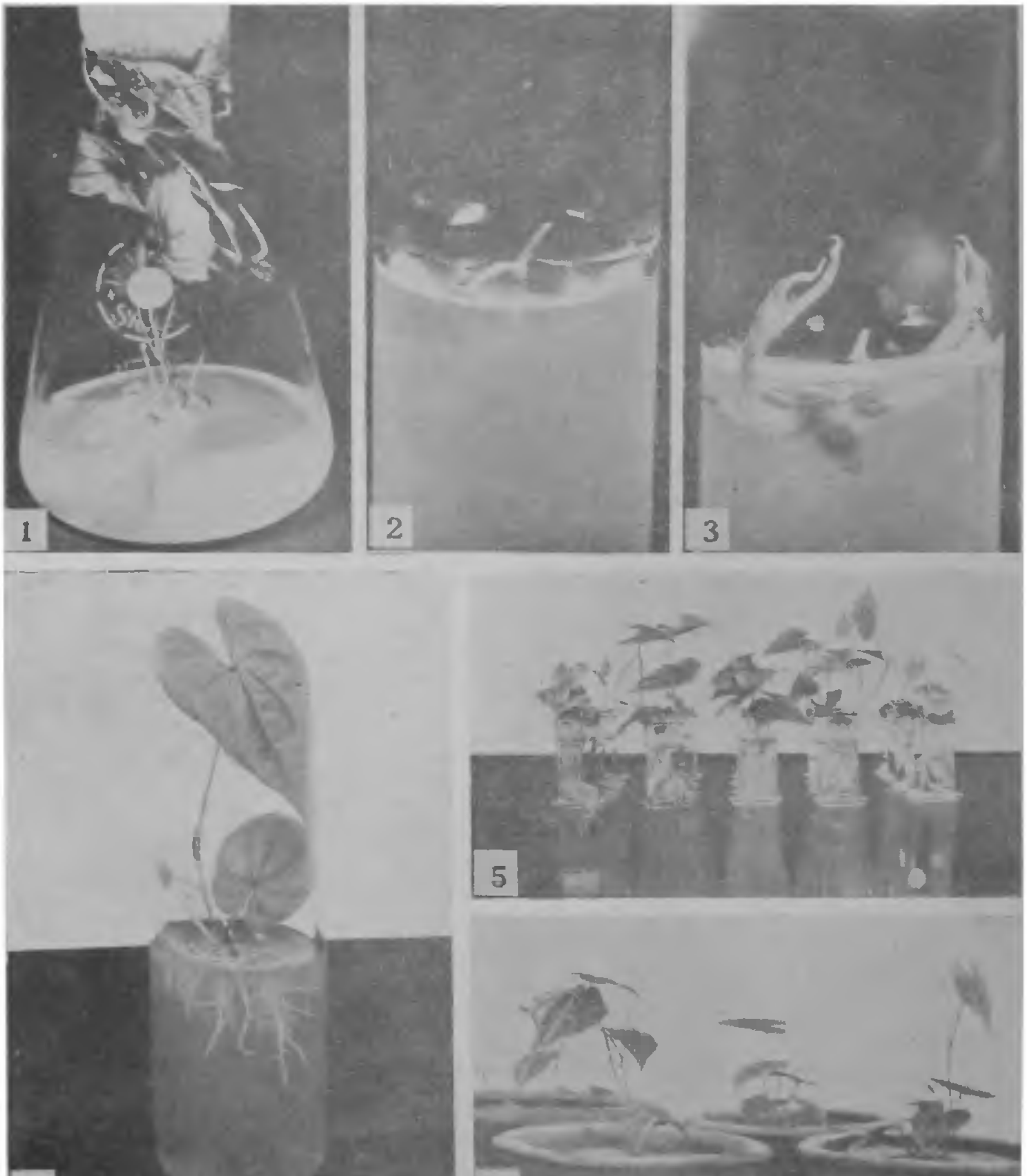
Abstract

Dioscorea floribunda plants were established in aseptic cultures from surface-sterilized single-node stem segments of field-grown vines. Axillary buds of nodal segments proliferated in presence of 6-benzylaminopurine (2 mg/l) unaccompanied by root formation. Whereas, shoot apices and single-node leaf cuttings rooted 100% in presence of NAA (0·5 mg/l), resulting into plantless, 100% of which were successfully grown in potted soil. It took about 40 days to obtain a 5–6 leaved plantlet in potted soil from single-node cutting taken from a plant grown in aseptic culture.

Introduction

DIOSCOREA FLORIBUNDA Mart. and Gal. is one of the three *Dioscorea* spp. (the other two are *D. composita* Hemsl. and *D deltoidea* Wall.) commercially yielding diosgenin, a main precursor, from plant source, for the synthesis of steroidal drugs, namely, cortisone, sex hormones, oral contraceptive pill, etc, which are so important in

modern medicine. The great medicinal value of *Dioscorea* necessitates its large-scale cultivation as a crop since wild resources can hardly meet the ever-increasing demand for diosgenin. To avoid



FIGS. 1–6. Cultures of *Dioscorea floribunda*. Fig. 1. A plant in aseptic culture obtained from single-node stem segment of field-grown vine (× 0·7). Fig. 2. A single-node stem segment taken from an aseptically growing plant as it looked at the time of inoculation (× 1·71). Fig. 3. Proliferation of axillary bud of an explant as shown in Fig. 2 (× 1·8). Fig. 4. A rooted shoot (× 0·9). Fig. 5. Liquid culture of rooted shoots—plantlets—in an inorganic nutrient solution (× 0·27). Fig. 6. About 50-day-old plantlets, regenerated from single-node cutting, growing in potted soil (× 0·3).

variation, its high yielding strains have to be multiplied vegetatively. Propagation from tuber segments is a slow process, whereas consistent success for its rapid propagation from leaf or stem cuttings has not been achieved and with certain commercial species the latter method has failed[1]. *D. floribunda* could be propagated from single-node leaf cuttings[2,3], but as reported by Bammi and Randhawa[4] the percentage of rooting of such cuttings has been quite low, *i.e.*, only 20–30%. Application of the methods of tissue and organ culture for induction and proliferation of shoot buds and their 100% rooting, as has been demonstrated for *Nicotiana*[5], *Chrysanthemum*[6] and *Citrus*[7], holds a great promise for rapid multiplication of *Dioscorea* too. Preliminary results of such a study for propagation of *D. floribunda* are reported here.

Experimental Procedure

About 2-cm-long stem segments consisting of a single node, a portion of petiole with its axillary bud and small portions of internode on either side were obtained from middle region of vigorously growing stem of *D. floribunda* vines cultivated at the National Botanic Gardens, Lucknow. The segments were pretreated with a detergent (Teepol 5%) for 5 min. and surface-sterilized by first dipping them in 95% ethanol for 5 sec. followed by immersion in $HgCl_2$ solution (0·2%) for 30 min. Such segments, after being thoroughly washed with sterile distilled water, were inoculated one per culture tube with their cut basal ends inserted in the nutrient agar.

A modification of Murashige and Skoog's medium[8] was used as the basal medium. The medium was adjusted to pH 5·8 and sterilized by autoclaving at 1·08 kg/cm^2 for 15 min. The cultures were grown under 3000 lux fluorescent light for 14 hr. daily at 27° ± 1° C. Humidity of the culture room was maintained at 70 ± 4%.

Results

Stem segments cultured in the basal medium supplemented with 15 mg/1 adenine sulphate and 0·1 mg/1 NAA remained quiescent for about 20 days, after which swelling appeared at the site of axillary bud denoting the formation of new tuberous tissue, from which roots came out first followed by the development of one or two shoots. Such plantlets, on being subcultured in the same medium contained in Erlenmeyer flasks, grew vigorously giving rise to more shoots (Fig. 1), and constituted the source material for further cuttings.

Single-node explants (Fig. 2) taken from *in vitro*-growing plants when cultured in a medium containing 2 mg/1 6–benzylaminopurine produced 5–6 or sometimes more shoot buds from its axil after an incubation of 20–25 days (Fig. 3). In this way, multiplication of shoots was effected. Shoot apices (1–2 cm long) as well as single-node leaf cuttings from *in vitro*-growing plants 100% rooted profusely in a medium containing 0·5 mg/1 NAA within a period of 10–15 days (Fig. 4). A large number of such cuttings could be obtained from a single culture of aseptically growing plant as several "crops" of new shoots continued to develop from the rooted basal portion of the plant after the excision of older shoots.

The rooted leafy shoots, after being reared for about 20 days on an auxin-free medium supplemented with 25 mg/1 adenine sulphate to promote shoot growth, were then taken out from aseptic cultures and grown in a liquid inorganic nutrient medium (Fig. 5). When the plantlets got acclimatized during their liquid culture for 10–15 days, they were transplanted into sterilized potted soil (Fig. 6). For the first 2–5 days after transfer in liquid culture and in potted soil, the plantlets were covered by glass-jars to prevent them from desiccation. All the plants transplanted in this way grew normally and vigorously in potted soil.

It took about 40 days to obtain 5–6 leaved plantlets in potted soil from the cuttings taken from an aseptically growing plant. Further experimentation is continuing to achieve still faster rate of multiplication of plantlets before this process could be developed as a practical method for clonal propagation of *Dioscorea*.

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