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# *IN VITRO* SHOOT MULTIPLICATION IN CASTOR

**D. LAKSHMI and BIR BAHADUR**
Department of Botany, Kakatiya University, Warangal - 506 009, India.

Multiple shoots were induced from shoot apex of *Ricinus communis* L., on MS Medium supplemented with various combinations and concentrations of auxins, cytokinins, polyamines and other additives. Of the various combinations tested, medium supplemented with Adenine sulphate (2mg/1) gave maximum number of multiple shoots. Isolated shoots developed into small plantlets.

**Keywords:** Shoot multiplication; Adenine sulphate; Shoot apex.

## Introduction

Castor (*Ricinus communis* L.) is an important oil crop of industrial and medicinal value. Tissue culture technology has been recognised as a useful tool for crop improvement, rapid propagation of desirable genotypes and induction of genetic variability. Available literature on castor tissue culture mainly deals with the micro propagation from endosperm or seedling explants[1-5]. *In vitro* multiplication in castor from the seedling shoot apices was initiated in our laboratory and we reported successful multiple plantlet formation[4]. In continuation of our work, we report *in vitro* multiple shoot formation.

## Materials and Methods

Castor seeds (Var. Aruna) were germinated in pots containing vermiculite. The shoot apices (2-3 mm size meristem with adjacent leaf primordia) excised from seven days old healthy seedlings were disinfected with detergent for 5 minutes. Later, the explants were surface sterilized with 0.1% $HgCl_2$ solution for 10 minutes and washed thoroughly with sterilized distilled water. The sterilized explants were cultured on Murashige & Skoog's[6] medium supplemented with various combinations and concentrations of auxins, cytokinins, polyamines and other additives together with 3% sucrose. pH was adjusted to 5.5 gelled with 0.8% agar.

The cultures were maintained at 25 ± 2° C with a photo period of 10 hours per day of flourescent light (2000-3000 lux). 30 shoot apices were uniformly cultured in each treatment. For all treatments, sub-culture was made on the same medium after 20th day. Survival, growth and number of shoot buds per explant were noted. These shoot buds after isolation were transfered for root induction in medium containing half strength MS salts together with 1mg/1 NAA or 1mg/1 IBA.

## Results and Discussion

Multiple shoot initiation was noted directly from the seedling shoot apices within 10-15 days of cultures in all treatments tested. The number of shoot buds increased when sub-cultured in the same medium. The induction of cluster of shoot buds were observed from the terminal portion of the shoot apex similar to that earlier reported by Reddy and Bahadur[4].

Maximum number of multiple shoots were obtained when the shoot apices cultured on MS medium supplemented with Adenine sulphate (2mg/1) (Figs. 1,2). Among the shoot

**Table 1.** *In vitro* response of Castor seedling shoot apex to various combinations and concentrations of growth regulators.

| S.No. | MS medium + supplements | Number of explants showing response | Percentage response | Number of shoot buds per explant | Remarks |
|---|---|---|---|---|---|
| 1 | AdS (1mg/1) | 13 | 43.3 | 4 | Multiple shoots |
| 2 | AdS (2mg/1) | 25 | **83.3 | 12 | Multiple shoots |
| 3 | AdS (10mg/1) | 20 | 66.6 | 2 | Long shoot with two axillary buds |
| 4 | AdS (20mg/1) | 15 | 50 | - | Hypertrophy |
| 5 | AdS (1mg/1) + TDZ (1mg/1) | 12 | 40 | 4 | Multiple shoots |
| 6 | Put (1mg/1) + Spd (1mg/1) | 18 | 60 | 4 | Multiple shoots |
| 7 | TDZ (1mg/1) | 17 | 56.6 | - | Development of shoot |
| 8 | TDZ (2mg/1) | 25 | **83.3 | 5 | Multiple shoots |
| 9 | TDZ (10mg/1) | 21 | * 70 | - | Hypertrophy |
| 10 | TDZ (20mg/1) | 15 | 50 | - | Bunch of small shoots |
| 11 | KN (2mg/1) + IBA (1mg/1) | 21 | * 70 | 5 | Multiple shoots |
| 12 | KN (2mg/1) + NAA (0.5mg/1) | 14 | 46.6 | 4 | Multiple shoots |
| 13 | IBA (1mg/1) + BAP (0.5mg/1) | 20 | 66.6 | 3 | Multiple shoots |
| 14 | KN (2mg/1) + NAA (1mg/1) + BA (1mg/1) | 22 | * 73.3 | 7 | Multiple shoots |

**N.B.** 30 explants were used uniformly in each treatment.

ABBREVIATIONS:

| | | | | | |
|---|---|---|---|---|---|
| AdS | - | Adenine sulphate | KN | - | kinetin |
| TDZ | - | Thidiazuron | BAP | - | 6 - Benzyl aminopurine |
| Put | - | Putrascene | BA | - | Benzyl adenine |
| Spd | - | Spermedine | NAA | - | α naphthalene acetic acid |
| | | | IBA | - | Indole 3- Butyric acid |

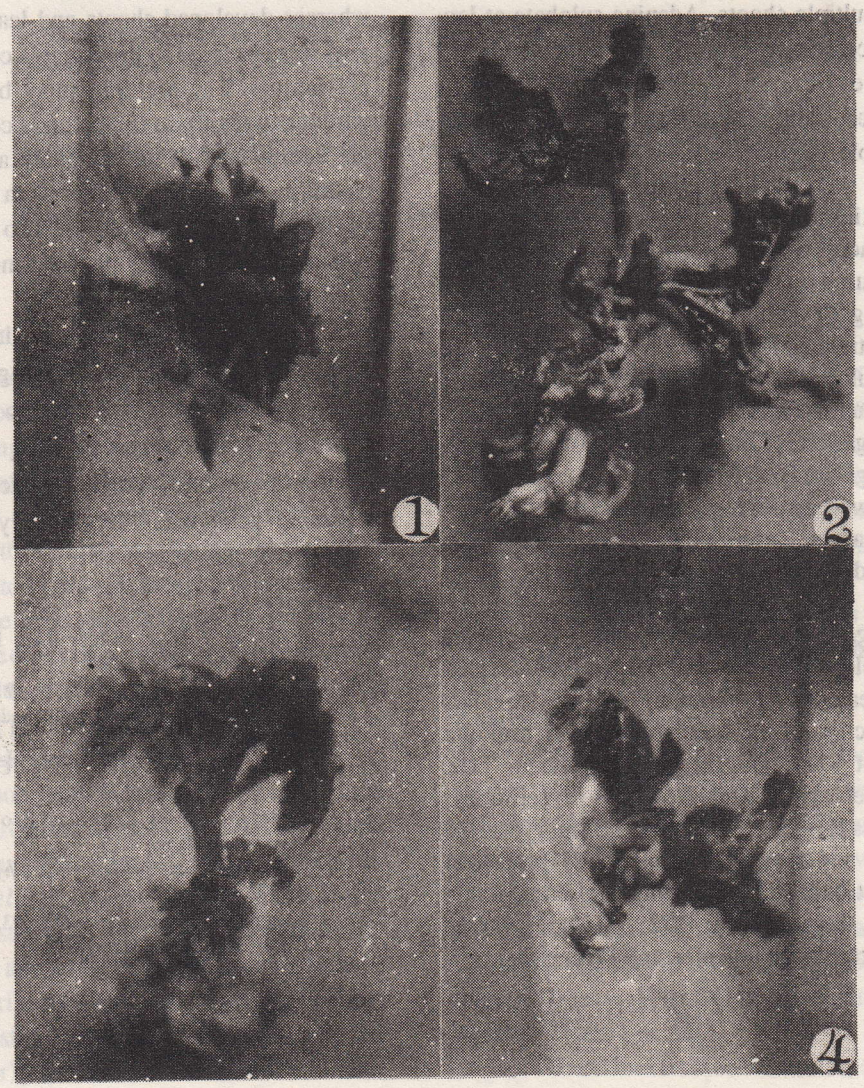


Fig. 1 : Half portion shoot apex with 6-7 multiple shoots grown on MS + Adenine Sulphate (2mg/1).

Fig. 2 : Induction of multiple shoots (10-12) on MS + Adenine Sulphate (2mg/1).

Fig. 3 : Induction of multiple shoots (4) on MS + Putrascine (1mg/1) + Spermedine (1mg/1).

Fig. 4 : Induction of multiple shoots (5) on MS + Thidiazuron (2mg/1).

apices tried, 16.6% showed good response (resulting in 10-12 multiple shoots, 50% resulted in multiple shoots (5-7), 16.6% gave 3 multiple shoots. Adenine sulphate at low concentration (1mg/1) few shoot buds (4) were obtained and with increase in concentration (20mg/1) hypertrophy resulted (Table 1).

Of the various phytohormones (auxins and cytokinins) tested, more number of shoots were obtained when the shoot apices cultured on medium supplemented with KN (2mg/1) +NAA (1mg/1) +BA (1mg/1). Multiple shoot induction was also observed in cultures containing combinations of other auxins and cytokinins viz. KN (2mg/1) +IBA (1mg/1); KN (2mg/1) +NAA (0.5mg/1); IBA (1mg/1) + BAP (0.5mg/1). Shoot apices also showed multiple shoot formation with polyamines, putrascine and spermedine (Fig. 3 and Table 1).

At low concentration of TDZ (1mg/1) small amount of white, friable callus developed at the base of the shoot apex. Increase in concentration (2mg/1) led to callus induction at the base followed by multiple shoot formation (Fig.4). Increase in TDZ concentration (10-20mg/1) led to hypertrophy, callus formation with somatic embryo at the base and 10-15 very small (0.5-1cm size) shoot buds. These findings are comparable with the effect of TDZ on woody plants reported by Huetteman and Preece[7].

The multiple shoot buds were small without the well developed shoot and leaves in the cultures containing phytohormones, whereas developed shoots and leaves as *in vivo* was observed in the cultures containing polyamines. The multiple shoot buds were vitreous in nature in the cultures containing the additives, Adenine sulphate and TDZ. Callus mediated morphogenesis in the form of shoot buds and shoots were also observed in cultures containing phytohormones and TDZ.

However, shoots formed in cultures developed into plantlets (ca 2cm long). Further work is in progress to root them so that fully developed plantlets can be used for mass micropropagation of stable pistillate lines and desirable genotypes for higher yield and disease free plants.

## References

1. Satsangi A and Mohan Ram HY 1965, *Phytomorphology 15* 26
2. Srivastava P S 1971, *Curr. Sci. 40* 337
3. Reddy K R K , Rao G.P. and Bahadur B 1986, *J. Swamy Bot. cl. 3* 119
4. Reddy K R K and Bahadur B 1989, *Curr. Sci. 58* 152
5. Bahadur B, Reddy K R K and Rao G P 1992, *Asian Jour. Pl.Sci. 4* 13
6. Murashige T and Skoog F 1962, *Physiol.Plant. 15* 473
7. Huetteman, C A and Preece J E 1993, *Plant Cell Tissue and Organ Culture 33* 105