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Effect of cytokinins on growth and maturation of direct somatic embryos in arecanut (*Areca catechu* L.)

Keywords : 2-iP, BA, direct somatic embryogenesis, kinetin, thidiazuron, zeatin

In vitro multiplication of arecanut has been successfully achieved at Biotechnology laboratory of CPCRI. The protocol developed has been applied to mass multiplication of YLD resistant arecanut palm (Karun *et al* 2004, Radha *et al.*, 2006). Direct and indirect somatic embryogenesis from inflorescence explants of healthy palms identified from YLD disease hot spot of Karnataka has been reported (Radha *et al.*, 2006). Previous studies on effect of different cytokinins at fixed concentration on the growth of indirect somatic embryos conducted in our laboratory led to observation that somatic embryos could be effectively grown on a medium supplemented with fixed concentration of 5mg/l BA. However the above trials were not effective on direct somatic embryos of Yellow Leaf Diseases (YLD) resistant arecanut palms. So the present study was undertaken to select the best cytokinin for conversion/maturation of direct somatic embryos into normal plantlets.

Direct somatic embryos derived through inflorescence culture were utilized for the experiment (Fig.1). The size of the embryos ranged from 0.5-0.8 cm. Different types of somatic embryos were observed in culture, *viz.* elongated, round and globular with roots. In order to achieve uniform plantlet development, different cytokinins were tested with direct somatic embryos.

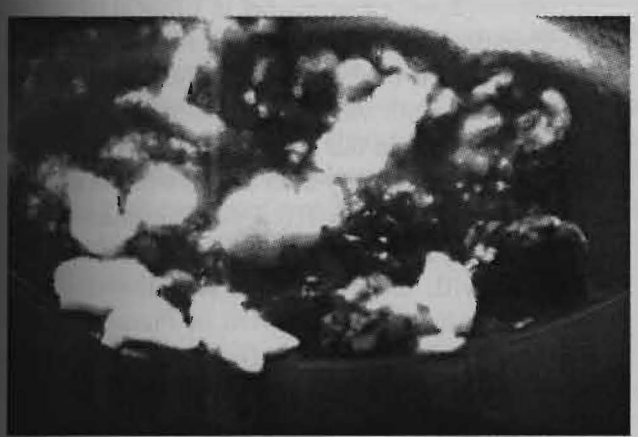

Fig. 1. Direct somatic embroyos

Equal concentration (2mg/l) of the following cytokinins: Thidiazuron, BA, Zeatin, 2-iP and Kinetin were explored for their influence on shoot development. For each treatment 15 somatic embryos were used. Before the treatment, the size of the embryos was recorded and embryos were subjected to various treatments in culture condition. The media (MS) also contained sucrose (3%), charcoal (0.1%) and agar (0.6%). The pH of the media was adjusted to 5.7 ± 02 using 1N NaOH/1N HCl and the media were autoclaved at 121°C for 20 minutes. TDZ and Zeatin were filter sterilized whereas BA, KN and 2-iP were autoclaved along with medium. Sub-culturing was carried out at 4-5 weekly intervals with same concentration of growth hormone. The cultures, which were initially maintained in the dark, were transferred to light (40 μ Em^{-2} S^{-1}) provided by white, cool fluorescent tubes (Philips) with a photoperiod of 16 h light and 8 h dark. Data recorded every month included growth parameters like shoot length, number of leaves, number of roots, root length etc.

The effect of five fixed concentrations of cytokinins on conversion of direct somatic embryos into plantlets was studied. No significant effect was observed among the treatments upto 4 weeks of culture. Variation in shoot elongation was observed after fourth week of culture on MS medium supplemented with the following cytokinins *viz.* TDZ, BA, kinetin, 2iP and zeatin. After twelfth week of subculture, significant effect on shoot growth was noticed (Table 1) in TDZ and BA treatments (Fig. 2 and 3). After 20th week, the maximum shoot length, root length and number of roots were observed in TDZ, followed by BA, kinetin, zeatin and 2-iP respectively. Statistical analysis showed that there were significant differences in shoot length and root length between TDZ and other cytokinin treatments (Fig.4). TDZ is highly significant for shoot length and root length. Where as, in the case of leaf production, three cytokinins such as TDZ, BA and Kinetin were on par. Similarly in root production TDZ and BA were on par. Slower growth rates in all the

parameters were noticed in control. Germination of somatic embryos was observed by simultaneous development of shoot and roots. To achieve rapid growth and development of plantlets, MS medium supplemented with 2mg/l TDZ was again used.

Table 1. Mean shoot growth observed after 12th week of *in vitro* culture of arecanut

Cytokinin	Shoot growth Mean
2iP	1.163
BA	1.523
Kinetin	1.240
Control	0.807
TDZ	1.820
Zeatin	1.207
General Mean	1.2933
Std.Error	0.3701

CD (P=0.05) = 0.3136

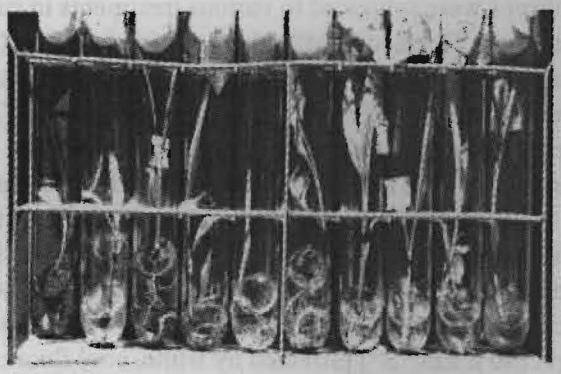

Fig. 2. Direct somatic embryors treated with TDZ

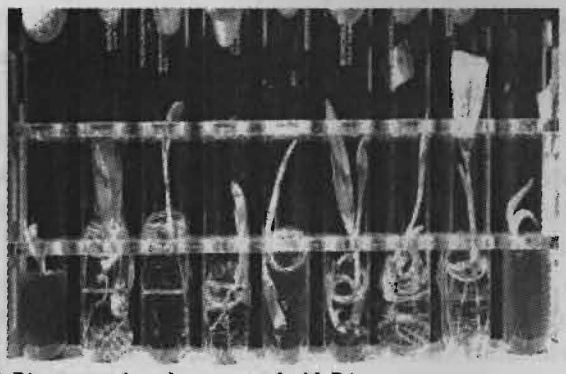

Fig. 3. Direct somatic embryos treated with BA

The type of cytokinin supplemented in the medium greatly influenced the shoot as well as root growth. Maximum growth rate of somatic embryos were noticed in TDZ-supplemented medium. Cultures in kinetin-supplemented medium displayed simultaneous shoot and root development where as cultures in zeatin and 2-i,P supplemented medium had lesser root development. More than 80 % of the cultures in TDZ-supplemented medium, showed vigorous shoot growth and plantlet development. The results also revealed that TDZ had a high degree of activation of shoot growth than the other

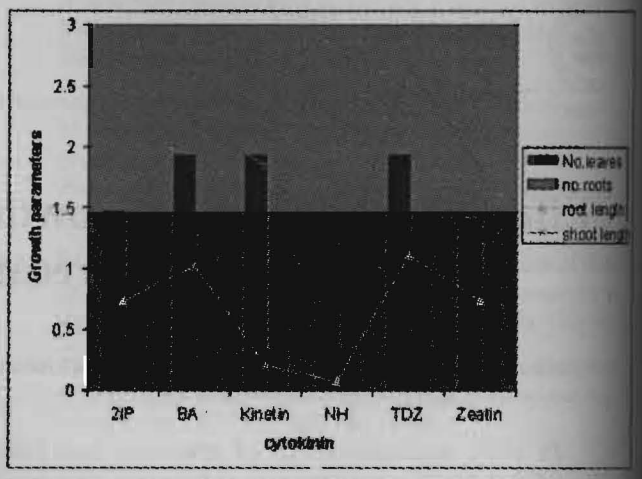

Fig. 4 Mean of morphological parameters observed after 20 week.

cytokinins tested.

In order to achieve further shoot elongation and full root development, the germinated somatic embryos were transferred into MS liquid medium supplemented with the same concentrations of cytokinins. Fully developed plantlets (after 30 weeks of culture) were transferred to pots containing sterilized sand and soil 5:1 ratio for primary hardening. Fully hardened plantlets were moved to a shaded net-house and maintained there for further hardening (Fig. 5). Plantlets were watered with MS macro-solution once in a week till it was transferred to polybags.

Fig. 4. Plantlets transferred to pots

Rout (2004) reported that in *Clitoria ternatea* L., the elongation of the shoot from somatic embryos was observed within one week of culture on MS media supplemented with varying concentration of BA and Kinetin, where as in our study, the slight elongation of shoot was observed only after fourth week of culture in MS supplemented with fixed concentration of either TDZ, BA, Kn, 2-i,P or Zeatin. The growth rate of shoot increased with the number of subcultures. Similar observations have been reported in *Picrorhiza kurroa* (Upadhyay *et al.*, 1989) and in *Gentiana kurroo* (Sharma *et al.*, 1993). Rout *et al.* (1999) demonstrated a significant improvement in shoot multiplication rate by subculturing *Plumbago zeylanica* at 4-weekly intervals.

Previous studies conducted in our laboratory to find out the effect of cytokinins on indirect somatic embryogenesis from leaf explants of arecanut that supplementation of 20 µM BA gave 74.02 % germination. However, in the case of direct somatic embryos, BA was not found to be effective for conversion of somatic embryos to plantlets. Karun *et al.* (2004) earlier reported that BA was the best cytokinin for germination of arecanut somatic embryos. But our study proved that TDZ was the best and effective cytokinin for conversion of somatic embryos into plantlets.Thidiazuron has received considerable attention as a potent regulator of *in vitro* propagation systems and as an effective means of stimulating the development of adventitious shoots and somatic embryos in a wide variety of plants (Huetteman and Preece 1993, Lu 1993). El-Zeiny (2007) reported that in *cucumber* 0.1 to 1,2,4 mg/l concentration of TDZ and BA suppressed the development of roots. They also reported that the maximum shoots were obtained with the supplementation in the media of any one cytokinin (TDZ or BA) and among these, TDZ was better than BA. The same results were obtained in our study where 2mg/l TDZ gave maximum shoot length, number of leaves and root growth compared to other cytkinins tested. Van Niewkerk *et al.* (1986) reports also supported that TDZ was better than BA for increasing the shoot numbers as well as shoot growth. This is in contrast with studies on oil palm (Karun and Sajini, 1996), where plantlet regeneration was achieved mainly through meristemoids in a medium supplemented with zeatin riboside. Giridhar *et al.* (2004) reported that *Coffea arabica* L. they could achieve rapid repetitive somatic embryogenesis from regenerated plantlets by using TDZ (0.91µM). Singh *et al.* (2000) reported that in *Dendrobium strictus supplementation* of 0.01 mg/l concentration TDZ in the medium increased the conversion of shoot buds into shoots giving 94 % cultures, while in control about 11 % cultures showed regeneration by producing single shoot bud. Maxwell *et al.* (2007) reported that TDZ induced morphogenesis of somatic embryos and shoots. Further more an increase in the concentration of TDZ in the culture medium caused a significant increase in the number of somatic embryos but reduced the number of shoots. In contrast, in *Picea glauca* adventitious shoots elongated better when 0.01 or 0.1 µM TDZ combined with BA or Zeatin up to 100 µM, compared to use of the latter two cytokinin individually (Ellis *et al.*, 1991).

To conclude, among the five cytokinins tested, TDZ was found to be the most efficient cytokinin for maturation and conversion of direct somatic embryos of arecanut into complete plantlets.

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