

# The usage of exogenously supplied plant growth regulator on the formation of adventitious roots on Cassava shoots developed *in vitro*.

**Bright Chima Megbo**

Department of Plant Physiology, Faculty of Science, Charles University Prague, Czech Republic.

## ABSTRACT

Auxillary shoots developed from buds explants from the 14<sup>th</sup> day after culture. Indole-3-butyric acid (IBA) achieved 100 percent *in vitro* rooting of shoots after 30 days of culture. Kinetin promoted vigorous shoot development and elongation. IBA promoted rooting of auxillary shoots *in vitro* as well as root regeneration when transplanting rooted plants. Further, it promoted new root initiation, increased root elongation rate, number and uniformity of rooting. IBA accelerated the translocation of nutrients from the upper part of the auxillary shoots to their basal ends by increasing the activity of the enzymes which increased hydrolysis of carbohydrates that became additional energy source in the rooting response of cells. IBA also promoted the qualitative features of the root.

**KEYWORDS:** Indole-3-butyric acid; Rooting; Plant hormones; Cassava

## 1.0 INTRODUCTION

Cassava, *Manihot esculenta* Crantz, belongs to the family of *Euphorbiaceae*. It is a perennial shrub, growing to a height of between 1 to 3 metres, grown for its starchy roots. The leaves are simple, consisting of the lamina and petiole; the leaf is lobed. The upper surface of the leaf is covered with a shiny, waxy epidermis. Cassava plant is a monoecious species producing both female (i.e. staminate) and male (i.e. pistillate) flowers. The mature stem is woody, cylindrical and formed by alternating internodes and nodes. Cassava plant primary storage organ is the root. The plant may be propagated either by true seeds or stem cuttings. If the plant is grown from stem cuttings, it can produce a number of primary stems from viable buds on the cutting. Where the plant is raised from true seed, the radicle of the germinating seed grows vertically downwards to form a taproot from which adventitious roots spring up. Cassava is a true root and cannot be used for the purpose of vegetative propagation. Cassava is a tropical root crop which takes 6 to 8 months to produce. Cassava can be classified as either sweet or bitter depending on the absence or presence of the toxic levels of cyanogenic glucosides. Sweet cultivars can produce as little as 15 mg of HCN per kg of fresh roots, while the bitter ones may produce more than 35 times as much.

## 2.0 MATERIALS AND METHOD

The selected stock plants were sprayed with antibiotic sprays to reduce the probability of disease. Terminal buds were excised from healthy shoot tips of Cassava plants. After screening for the incidence of internal microbial contaminants, the explants were used for the aseptic initiation and establishment of healthy shoots. The buds were cultured in a modified Murashige and Skoog medium (Murashige and Skoog, 1962) solidified by agar and supplemented with a cytokinin,

10mM kinetin, and an auxin, 20mM indole-3-butyric acid (IBA). The shoots were subcultured in kinetin with an interval of 21days. IBA was employed for the rooting of the elongated shoots. Plantlets were then acclimatized by lowering the relative humidity over 28days in the greenhouse under controlled temperatures between 20 and 27°C. The root length, number of roots, and percentage rooting were recorded.

### 3.0 RESULTS AND DISCUSSION

Auxillary shoots developed from buds explants from the 14<sup>th</sup> day after culture. Indole-3-butyric acid (IBA) achieved 100 percent *in vitro* rooting of shoots after 30 days of culture. IBA-treated offshoots rooted earlier and with a much higher frequency while untreated shoots rooted poorly, later, and had the propensity to produce fewer roots. Also, the number of roots per offshoot was higher in the treated offshoots, while the untreated produced fewer roots. Likewise, Naija et al. (2008) found that a period of 5 days in rooting medium with IBA was sufficient to induce 97% rooting in microcuttings of *Malus × domestica* Borkh. rootstock MM 106, and the exposure to IBA for periods longer than five days produced undesirable side effects on shoots, such as callus formation and leaf necrosis. Metivier et al. (2007) indicated that the percentage of rooted shoots of *Cotinus coggygria* Mill. increased with increasing time of exposure to the IBA-containing medium, from 40% after one day of exposure, to 100% after 5 days in the presence of IBA. Kinetin promoted vigorous shoot development and elongation. IBA promoted rooting of auxillary shoots *in vitro* as well as root regeneration when transplanting rooted plants. Further, it promoted new root initiation, increased root elongation rate, number and uniformity of rooting. IBA accelerated the translocation of nutrients from the upper part of the auxillary shoots to their basal ends by increasing the activity of the enzymes which increased hydrolysis of carbohydrates that became additional energy source in the rooting response of cells. IBA also promoted the qualitative features of the root. The results of this study is in accord with those obtained by Qaddoury and Amssa (2004) as they reported that exogenously supplied indole-3-butyric acid had a significant positive effect on the rooting response of date Palm (*Phoenix dactylifera* L.) shoots. According to George et al. (2008) and Hasan et al., (2010), IBA yielded good results of root number because it is very effective at increasing endogenous auxin contents and showed higher stability against catabolism and inactivation by conjugation with growth inhibitors. Adventitious root formation is an energy-demanding process, and starch stored in the rooting zone of cuttings is utilized to provide the energy required (Husen and Pal 2007). The presence of IBA in the medium increased the rooting percentage, number of roots, percentage of lateral roots, and length of the shoots. The rooting capacity varies with the genotype, and is generally lower in woody species than in herbaceous species (Hackett 1988). Efficient rooting treatment yields a high percentage of rooted shoots and a high-quality root system. The latter involves number of roots per shoot, length of roots and absence of callus formation, and determines the performance after planting in soil (De Klerk et al. 1997). Evidence for the involvement of IBA, but not IAA, in lateral root development was reported for lateral root induction in rice (Wang et al., 2003). While IBA was able to induce lateral roots, the same response was found only at 20-fold higher concentrations of IAA (Chhun et al., 2003, 2004). Similarly, Ludwig-Muller,

Vertocnik and Town (2005) reported that IBA, but not IAA, efficiently induced adventitious rooting in *Arabidopsis* stem segments at a concentration of 10  $\mu$ M.

#### 4.0 CONCLUSION

The use of auxins, especially indole-3-acetic acid (IBA), in rooting of cuttings has proved to be successful in a number of plant genera. IBA is naturally occurring and it is also produced commercially. The solvent, such as alcohol, used for dissolving it may affect some physiological processes in the cuttings. The potassium salt form of IBA is soluble in water, therefore does not need a solvent. The concentration of ABA used as a soaking medium and the length of time depends on the kind of plant and part of the plant used as explant.

#### REFERENCES

1. Chhun, T., Taketa, S., Tsurumi, S., Ichii, M. (2003). The effects of auxin on lateral root initiation and root gravitropism in a lateral rootless mutant Lrt1 of rice (*Oryza sativa* L.). *Plant Growth Regulation* 39, 161–170.
2. Chhun T, Taketa S, Tsurumi S, Ichii M. (2004). Different behaviour of indole-3-acetic acid and indole-3-butyric acid in stimulating lateral root development in rice (*Oryza sativa* L.). *Plant Growth Regulation* 43, 135–143.
3. De Klerk, G. J., Arnholdt-Schmitt, B., Lieberei, R., and Neumann, K.H. (1997). Regeneration of roots, shoots and embryos: physiological, biochemical and molecular aspects. *Biologia Plantarum* 39(1): 53–66.
4. George EF, Hall MA, Deklerk GJ (2008). *Plant propagation by Tissue culture*. Springer, 1: 206-217.
5. Hackett, W.P. (1988). Donor plant maturation and adventitious root formation. In: Davis, T.D., Haissig, B.E. & Sankhla, N. (eds.). *Adventitious root formation in cuttings*. Dioscorides, Portland, Ore. (USA). p. 11–28.
6. Husen, A. & Pal, M. (2007). Metabolic changes during adventitious root primordium development in *Tectona grandis* Linn. F. (teak) cuttings as affected by age of donor plants and auxin (IBA and NAA) treatment. *New Forests* 33(3): 309–323.
7. Ludwig-Muller, J., Vertocnik, A., and Town, C. D. (2005). Analysis of indole-3-butyric acid-induced adventitious root formation on *Arabidopsis* stem segments. *Journal of Experimental Botany*, Vol. 56, No. 418, pp. 2095–2105.
8. Metivier, P.S.R., Yeung, E.C., Patel, K.R., Thorpe, T.A. (2007). In vitro rooting of microshoots of *Cotinus coggygria* Mill., a woody ornamental plant. *In Vitro Cellular & Developmental Biology – Plant* 43(2): 119–123.
9. Naija, S., Elloumi, N., Jbir, N., Ammar, S. & Kevers, C. (2008). Anatomical and biochemical changes during adventitious rooting of apple rootstocks MM 106 cultured in vitro. *Comptes Rendus Biologies* 331(7): 518–525.
10. Murashige T, Skoog F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiologia Plantarum* 15, 473–497.

11. Qaddoury, A. and Amssa, M. (2004). Effect of exogenous indole butyric acid on root formation and peroxidase and indole-3-acetic acid oxidase activities and phenolic contents in date Palm offshoots. *Bot. Bull. Acad. Sin.* **45**: 127-131.
12. Wang S, Taketa S, Ichii M, Xu L, Xia K, Zhou X. 2003. Lateral root formation in rice (*Oryza sativa* L.): differential effects of indole-3-acetic acid and indole-3-butyric acid. *Plant Growth Regulation* 41, 41–47.

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