In Vitro Propagation of Vanilla in Rwanda

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Abstract

Vanilla ***(Vanilla planifolia)*** *is an herbaceous, perennial, climbing orchid belonging to the family Orchidaceae and order Orchidales. Vanilla is a high value export crop and it is cultivated for its beans that contain sweet scent, aroma and pleasant flavor, mainly due to the presence of vanillin. It is used as flavoring substance in several items such as chocolates, ice-creams, yoghurts, soft drinks, liquors, candies, baked foods, cakes, and biscuits. It is also used in scenting tobacco, perfumery and pharmaceuticals. Vanilla is the second most expensive spice after saffron and its demand worldwide is enormous. It is often referred to as "green gold" or "princess of spices". This crop can be profitably grown in some parts of Rwanda and currently it is being grown by a small number of farmers in Eastern Province. However, lack of adequate planting materials remains the major bottleneck in exploiting its potential in Rwanda. The objective of the study was to evaluate the effect of growth regulators on* **in vitro** *regeneration of plantlets from vanilla nodal explants. Nodal explants harvested from screen house grown vanilla vines were sterilized and cultured on Murashige and Skoog medium supplemented with 100 mg/l inositol. 20 µM/l argine, 3% sucrose and gelled with 0.3 % Phytagel. The cultures were incubated in a growth room maintained at 26º C and 16 hours photoperiod. The Kruskal-Wallis test showed that there were highly (p=0.001) significant differences among the two cytokinin levels for microshoot elongation in vanilla. The two cytokinins tested induced bud break to different degrees. Among the different growth regulators evaluated, BAP (benzylaminopurine) at 2.5 µM/l gave the highest mean shoot number at 1.21±0.80 and the highest shoot length with 3.34±1.61cm. The regenerated plantlets were successfully transferred to soil and acclimatized in the greenhouse. The protocol developed in this study is a significant advance on those previously reported for vanilla due to its efficiency and reproducibility and is a major step towards large scale vanilla production in Rwanda.*

Key words: *In vitro propagation, Vanilla planifolia*

1. Introduction

Vanilla is a herbaceous, perennial, climbing orchid belonging to the family Orchidaceous and order Orchidales. It originated from the tropical area in Mexico and Central America and also cultivated in the forest around South America (Moorthy and Moorthy, 2002).

The world production of vanilla is estimated to be about 3500 tons per annum and nearly 300 tons are consumed in the USA alone. Madagascar grows 70 to 80 % of the worlds' vanilla crop (Geetha and Shetry, 2000).

Vanilla is commercially cultivated for pods (beans) from which the popular flavoring substance called vanillin is extracted (Goodenough, 1982). Vanillin is used as flavoring substance in several items such as chocolates, ice-creams, soft drinks, liquors, candies, baked foods, cakes, biscuits as well as for scenting tobacco, perfumery and pharmaceuticals.

Traditionally vanilla is propagated by stem cutting of mature vine. However, the method is slow, labour intensive and time consuming. It is also uneconomical as the harvest of stems for propagation arrests growth and development of the mother plant Giridhar *et al.* (2001). Furthermore, the numbers of seedlings produced by cuttings are few and this method can hardly meet the national demand for quality planting materials. Therefore, it is necessary to exploit the potentials of tissue culture to effectively multiply and supply the required amount of planting materials for large scale plantation in Rwanda. Tissue culture techniques have been shown to have definite and indispensable advantages over the former, as it ensures an extremely rapid rate of multiplication. It is not season dependent and requires only a limited quantity of plant tissue as a source of initial explants. Moreover, it can also aid in the production of disease free plants and true to type planting material (Pierik, 1990).

Different explant sources have been utilized for *in vitro* propagation of vanilla including regenerative callus production, root tip cultures, seed cultures (Mathew *et al*., 1999) and nodal explants (Konowicz and Janick, 1984; Jarret and Fernandez, 1984, Agarwal *et al.*, 1992 and Zerihun *et al.,* 2009). However, *in vitro* propagation of vanilla varieties in Rwanda has not been reported. This study was conducted to develop an efficient protocol for propagating vanilla cultivars in Rwanda.

2. Material and Methods

Nodal explants were harvested from vanilla vines growing in the screen house at Rubona Research Station and transported to the laboratory in a beaker containing a fungicide (Benlate). They were gently washed with liquid detergent and kept under running tap water for one hour. The nodes were dipped in 70% alcohol inside the lamina flow cabinet for 30 seconds and then rinsed 3-4 times in sterile distilled water. .The nodes were then transferred in a bottle containing 0.5% $HgCl_2$ solution for 20 min and the bottle gently shaken. The explants were then rinsed 3-4 times. The nodal explants were sterilized a second time by dipping them in 25 % jik for 25 min then rinsed three times in sterile distilled water to completely get rid of sterilizing agents. The sterilized nodal explants were cultured in Murashige and Skoog (MS) medium supplemented with 100 mg/l inositol, 20 µM/l argine, 3 % sucrose, 0.3 % phytagel, and 2.5, 5, 10 20 µM/l of BAP and kinetin. In a second trial, the above media were supplemented with a combination of BAP and Kinetin (2, 4µM/l.) The cultures were incubated in a growth-room maintained at 26°C and 16 hour photoperiod. The data was analyzed using one way ANOVA.

3. Results

The effects of BAP on shoot proliferation and growth from vanilla nodal explants is presented in Table 1. Among all the concentration evaluated, the highest mean number (1.21±0.80) of shoots per explants with a mean length of 3.34±1.61cm was observed from the medium supplemented with 2.5 µM/l. Increasing the concentration of BAP from 2.5 to 20 µM decreased the number of shoots per explants and the mean shoot length.

The effects of different concentrations of Kinetin on shoot proliferation and growth from vanilla nodal explants are shown in Table 2. There was no regeneration of microshoot at the lower concentration (2.5 µM/l) of Kinetin. Kinetin at 10 µM/l, gave the highest mean number of shoots (0.54±0.51) and the highest mean length (0.95±1.56cm) was achieved with kinetin at 20 µM/l. Increasing the concentration of kinetin from 2.5 µM to 10µM increased mean number of shoots per explant and mean length of shoots.

The effects of combining BAP and Kinetin on shoot proliferation and growth is shown in Table 3. The highest number of shoots per explant (0.71±.0.46) with a mean length of 2.43±1.82cm was observed from the medium supplemented with 2 μM/l of BAP and 2 μM/l of Kinetin. The protocol developed during this study involves culturing nodal explants harvested from the screen house in a MS medium supplemented with cytokinins (Plate 1a). Regeneration of microshoots (Plate 1b,c,d), elongation and rooting of microshoots (Plate 1e) and weaning of the plantlets in the green house (Plate 1f).

Table 1: Effects of different BAP concentrations on microshoot proliferation and growth in Vanilla.

BAP Concentration (μM/l)	**Mean microshoots number per explants (±SE)**	**Mean microshoots length (cm) (±SE)**
2.5	1.21±0.80	3.34±1.61
5	0.46±0.51	1.35±1.64
10	0.21±0.42	0.71±1.55
20	0.21±0.42	0.53±1.19

Table 2: Effects of different concentrations of Kinetin on microshoot proliferation and growth in Vanilla.

Kinetin Concentration (μM/l)	**Mean microshoots number per explant (±SE)**	**Mean microshoots length (cm) (±SE)**
2.5	0.00±0.00	0.00±0.00
5	0.50±0.51	0.58±0.89
10	0.54±0.51	0.48±0.58
20	0.29±0.46	0.95±1.56

Table 3: Effects of BAP and Kinetin combinations on microshoot proliferation and growth in Vanilla.

BAP and Kinetin Concentration (μM/L)	Mean microshoots number per explant (±SE)	Mean microshoots length (cm) (±SE)
BAP(2) +KIN(4)	0.62±0.50	1.42±1.59
BAP(4) +KIN(2)	0.21±0.42	0.50± 1.27
BAP(2) +KIN(2)	0.71±.0.46	2.43±1.82
BAP (4)+KIN(4)	0.57 ±0.51	2.13±2.25

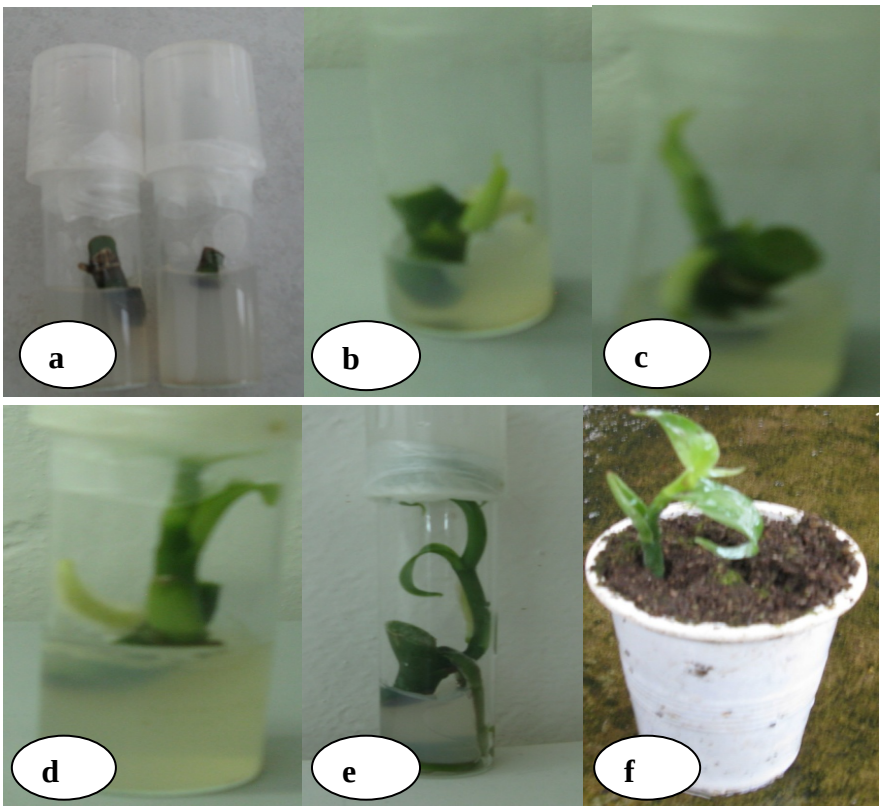

Plate 1: Regeneration of Vanilla plantlets - **a:** Freshly inoculated nodal explant, **b:** Regeneration of microshoots after 1week in culture, **c:** Regeneration of microshoots after 2 weeks, **d:** Regeneration of microshoots after 3 weeks, **e:**Elongation and rooting of microshoots after 4 weeks, and **f:** Weaned plantlet.

4. Discussion

In tissue culture, plant growth regulators are critical media components in determining the developmental pathway of the plant cells. Cytokinins such as benzylaminopurine (BAP) are known to reduce the apical meristem dominance and induce both axillary and adventitious shoots formation from meristematic explants in banana (Madhulatha *et al.*, 2004). The results of the current investigation revealed that the growth and differentiation of explants are under hormonal control.

It was observed in the study that the proliferation rate of explants cultured on the medium supplemented with Kinetin was generally lower than BAP. BAP is considered to be one of the most effective cytokinins for the induction of shoot regeneration in plant tissue culture (Janarthanam and Seshadri, 2008). Furthermore, a few studies showed that BAP was more effective than Kinetin in enhancing shoot multiplication in several plant species, such as *Crossandra infundibuliformis* (Girija *et al*., 1999), *Geoderum purpureum* (Mohapatra and Rout, 2004) and *Curculigo orchioides* (Nagesh, 2008). Combining BAP and Kinetin was found to induce multiple shoots. This result is concurrent with those reported by Zerihun *et al.* (2009) on *in vitro* propagation of *Vanilla planifolia* using nodal explants. This study also showed that increasing the concentration of BAP resulted in decrease in the shoot length and number of shoots. Similarly, Bhatt (1994) observed that high levels of cytokinins have deleterious effects on shoot growth and protocorm production in vanilla. In conclusion, information from the current study will assist mass propagation of vanilla in Rwanda.

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