In vitro assessment of tea (Camellia sinensis) explant types to vegetative propagation potentials in Nigeria.

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ABSTRACT

Seven types of seed explants were cultured *In Vitro* in an effort to evolve a rapid clonal propagation protocol for tea industry in Nigeria. The Murashige-skoog (M-S) medium was modified for the series of studies in which two thousand cultures were involved. The modified M-S medium was devoid of Zinc and Copper Sulphate salts but was fortified with complex Organic addenda such as coconut milk (CM), Casein hydrolysate (CH) and plant growth substances auxin (NAA) and cytokinin (BA). The explants of mature embryos were superior in seedling and regenerative growth forms than the immature embryo explants. Somatic embryos, embryogenic nodules, regenerated cotyledons, seedling establishment, adventitious roots and friable callus were observed in cultures of most explants either singly or in combinations of these morphogenic structures.

Some seedlings produced multiple shoots while some that had single shoots produced leaves and nodes which are considered necessary for further micro-propagation studies.

INTRODUCTION

Tea (Camellia sinensis) (L) O. Kuntze, a non-alcoholic beverage drink is native to South East Asia, (Njuguna, 1984),

In Africa, Kenya is the leading producer with about 11% of total world production. Nigeria officially joined the list of African tea producing nations in 1972 with the introduction of hybrid and clonal teas and experienced tea techniques from Kenya, and by about 1985 Nigeria commenced Black tea export trade on a rather low scale. Prior to this period, Nigeria was predominantly an importing, blending and packaging nation. All Nigerian tea plantations, estates and farms are established from clonally (vegetatively) propagated (VP) plants in areas determined to be characterized by the most ideal ecophysiological climate.

With the rapidly expanding areas of tea cultivation in plantations, estates and farms dotted both in highland and few lowland locations in Nigeria, the conventional production of VP-tea plants has begun to be inadequate in meeting the demands of interested farmers.

Consequently, there is the need to explore new and non-conventional methods and technologies such as the plant micro-propagation first reported for tea in 1969 (Forest) for the rapid clonal multiplication of VP-tea plants obtained from both the reproductive and vegetative parts in order to meet with increasing rate of demand by the farming tea communities in Nigeria.

Therefore, the objectives of this study was to evaluate the potentials of seven different types of embryo sourced explants to either produce somatic embryos or regenerate plantlets as potential methods for rapid multiplication of those elite commercial tea clones in Nigeria.
MATERIALS AND METHODS

Plant Materials: All Brooke Bond Clones available were used: 35, 31/8, 6/8, 14/3 and 236.
Explant Types: Whole mature embryos, whole immature embryos, mature embryo axis, vertical segments with embryo axis, horizontal segments with embryo axis, vertical segments without embryo axis, horizontal segments without embryos axis.

CULTURE MEDIA: Murashige and Skoog medium (MS) salts and vitamins devoid of zinc and copper sulphate salts was used. However, the MS was supplemented with inositol 100mg/l sucrose 30%/l, coconut milk 20%/l, casein hydrolysate 100mg/l, Naphthanlene acetic acid (NAA) 0.1mg/l, Benzyl amino purine (BAP) 0.1mg/l, Ascorbic acid 10mg and PH 5.6 prior to autoclave sterilization.

RESULTS AND DISCUSSION

In vitro morphogenic responses observed within 45 days on nutrient media ranged from somatic embryos, adventitious roots and shoots to plantlets.

Somatic embryos developed from the cotyledon sections proximal to the embryonic axis than from the plumule regions of the excised embryo axis and the induction of these somatic embryos were highly dependent on the nature of the explant types and the absence of 2, 4D and NAA. They were maintained for a long period on BAP (0.1mg) for conversion to plantlets. In Vitro morphogenic response were observed in all the explant types used similar to those reported by Kato, 1986, Nadamitus et al 1986, Agarwal et al 1992).

The study showed that somatic embryos can be developed readily from cotyledon explants than from other explant types and the embryos maintained on MS medium with BAP 0.1mg. Plantlets can also be regenerated in vitro from cotyledon explants with embryo axis intact.

CONCLUSION

Thus, in conclusion since the plantlets regenerated are either with multiple shoots/nodes, they can then be used for vegetative propagation. Nevertheless, this achievement is still far from being commercialisable.

Germination and seedling establishment were suppressed in explants types of whole immature embryos while the cotyledon sections with embryo axis regenerated new cotyledons or developed into plantlets which had high potentials for further in vitro micro-propagation.

The immediate future trials on the tea tissue culture will endeavour to emphasize and dwell on the use of stamens of floral buds or the filaments only, since a single flower produces 90-260 stamens which are easy to collect, preserve and transport and are naturally potential explants in an uncontaminated form. This idea is being proposed as a result of the successes recorded with the cocoa plant globally.
Fig. 1A: Plantlet regenerated from a cross Section of cotyledon with embryo Axis intact

Fig. 1B: Plantlet with several leaves and nod nodes from cotyledon section

Fig. 2A: Somatic embryo developing From excised embryo on Secondary regenerated cotyledon

Fig. 2B: Somatic embryo and callus from mature whole embryo
**TABLE 1:**  *In vitro* morphogenic response of tea explants after 45 days in culture

<table>
<thead>
<tr>
<th>Explant types</th>
<th>Survival**</th>
<th>NM*</th>
<th>Morphogenic Responses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole embryos (Mature)</td>
<td>50</td>
<td>20</td>
<td>Plantlets (Fig. 1A)</td>
</tr>
<tr>
<td>Whole immature embryo</td>
<td>65</td>
<td>5</td>
<td>Somatic embryos/Adventitious shoot root</td>
</tr>
<tr>
<td>Embryo axis (Mature)</td>
<td>80</td>
<td>15</td>
<td>Shoots/somatic embryos (Fig. 2A)</td>
</tr>
<tr>
<td>Vertical cotyledon segment with</td>
<td>85</td>
<td>2</td>
<td>Callus, plantlet (Fig. 1B)</td>
</tr>
<tr>
<td>Embryos axis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Horizontal cotyledon segment with</td>
<td>90</td>
<td>6</td>
<td>Callus, Adventitious root somatic embryo</td>
</tr>
<tr>
<td>Embryo axis</td>
<td></td>
<td></td>
<td>(Fig. 2C)</td>
</tr>
<tr>
<td>Vertical cotyledon segment</td>
<td>45</td>
<td>3</td>
<td>Friable callus</td>
</tr>
<tr>
<td>Without embryo axis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Horizontal cotyledon segment</td>
<td>47</td>
<td>3</td>
<td>Friable callus</td>
</tr>
<tr>
<td>Without embryo axis</td>
<td></td>
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</tbody>
</table>

** Explant enlarged, turned green and became dormant.

*NM – Number showing morphogenesis.

REFERENCES


