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Plant Responses to Alternative Matrices for *in Vitro* Shoot Multiplication and Root Induction of Pineapple (*Ananas comosus* L.)

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Abstract: The quest for alternative matrices for plant tissue culture is a continuing process. Agar is one of the most popular solidifying agents in plant tissue culture. High price of pure grade agar and fear of over exploitation of its resources caused searching for low cost alternatives. In this study, liquid medium with silica gel, glass beads and sands substratum and agar in two steps of micro propagation (shoot proliferation and root induction) were investigated. The shoot multiplication in the cytokinins hormone of 2.5mg/l BA and 0.5mg/l KN supported by 7g/l agar and alternative matrices has been produced 33.1 shoots and 5.3 shoot length in sands and agar. There is no significant difference in shoot number and shoot length on the agar and sands on shoot multiplication and plant, and there is significant difference in glass beads and silica gel compared with sand/agar in the shoot multiplication, and there is highly significance in shoot length in the matrices of plant support. Pineapple root induced in auxins hormone *in vitro* culture of plant propagation in ½ MS media, 30g/l sugar, 1mg/l NAA and 7g/l agar. The higher root number in sands 10.6 and followed 10 in silica gel and agar supported matrices. The root length also none significant difference among means treatments in agar, glass beads and sands. However, there is a significance difference with silica gel in root length. The use of silica gel, glass beads and sands as a support matrix to the explants affords better aeration.

Keywords: Alternative matrix, low cost, shoots multiplication and root induction.

INTRODUCTION

Pineapple is listed as a major tropical fruit in world production (FAOSTAT, 2010). Plant's association with the soil dates back to the origin of land plants having early rhizoidal roots anchoring the substratum. It was indeed unknown to mankind that roots could perceive gravity (Perrin. *et al*., 2005). Soil may be defined as a thin layer of earth's crust which serves as a natural medium for growth of plants. It is the unconsolidated mineral matter that has been subjected to, and influenced by, genetic and environmental factors-parent material, climate, organisms and topography all acting over a period of time. Soil differs from the parent material in the morphological, physical, chemical and biological properties. Also, soils differ among themselves in some or all the properties, depending on the differences in the genetic and environmental factors. Thus some soils are red, some are black; some are deep and some are shallow; some are coarse textured and some are fine textured. Roots, the "hidden half" of plants, which remain underground in the soil, serve a multitude of functions. They are responsible for anchorage, supply the plants with water, nutrients, and exchange various growth substances with the shoots. Roots perform the basic functions in most ferns and in all seed plants, whereas additional traits (e.g. formation of storage organs, determination of the depth of the regenerating buds, or aeration of inundated organs) are characteristic of roots of exclusive groups of plants. The root-soil interface is the site where the most interactions between the plants and their environment occur. Roots constitute a major source of organic material for the soil and thus affect its structure, aeration and biological activities. While organic chemicals move out of the roots into the soil, inorganic ions move in: some of the entering materials are needed for normal metabolism of the plants and are actively sought. Insufficient or excessive accumulation of most elements would damage plants, and therefore, their uptake is controlled at the root surface (Waisel. *et al*., 2002). The search for suitable alternative of soil probably dates back to the time when plants or plant parts were first grown *in vitro*, that is, advent of the techniques of plant tissue culture. The early studies led to root cultures, embryo cultures and the first true callus/tissue cultures. The period between the 1940s and the 1960s was marked by the development of new techniques and the improvement of those that were already in use. The 1990s saw continued expansion in the application of the *in vitro* technologies to an increasing number of plant species, which in recent times have culminated in research in plant transgenic (Thorpe, 2007). The wide and almost universal application of agar powder, a mixture of polysaccharides derived from red algae as the gelling material in culture media for all the aforesaid branches of plant tissue culture is due to its following advantage: high clarity, stability (after autoclaving), nontoxic nature and resistance to alteration during culture (MacLachlan, 1985; Henderson and Kinnersley, 1988). However, wide

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spread use of agar in plant tissue culture media has certain limitations too in many plant systems, which in the long run affects plant survival after transfer to soil from culture vessels. In our present experiment, we have replaced agar with glass beads and sands as an alternative support matrix in liquid medium, and compared the *in vitro* shoot multiplication and rooting response of pineapple.

MATERIALS AND METHODS

MS Stock Solution Preparation

Murashige and Skoog (1962) basal medium was used throughout this study. Initially, full strength stock solutions of macronutrients, micronutrients, iron and vitamins were prepared separately. The recommended amount of each component nutrient was measured in volume for a liter of stock solutions (macronutrients, micronutrients, iron and vitamins), and it was dissolved in distilled water using magnetic stirrer and adjusted to final volume with distilled water. Exceptional, potassium nitrate (KNO_3) was dissolved with hot water before mixing with other components of the macronutrient stock. After all the component nutrients of each stock were completely dissolved, the solution of each stock was poured into plastic conical flasks and stored at 4°C until use.

Plant Growth Regulators Stock Solution Preparation

The plant growth regulators (PGRs) employed for this study was N-benzyladenine (BA), kinetin (KN), α-naphthalene acetic acid (NAA) and indol-3-butyric acid (IBA). Each of these growth regulators stock was prepared at a concentration of 1.0 mg/ml by weighting the required amount using precision balance followed by dissolving in few drops of 1.0 M NaOH and it was adjusted to final required volume with distilled water. After complete dissolution, the solution was transferred to bottle and stored at 4°C until use.

Preparation of Growth Culture Conditions

Culture medium was prepared taking the recommended amounts of MS (Murashige and Skoog, 1962) stock solutions supplemented with 3% (w/v) sugar as a carbon source, and 0.7% (w/v) agar as solidifying agents. For each experiment the desired concentrations and combinations of auxins and cytokinins was added to the culture medium accordingly. Prior to addition of agar, the medium were adjusted to the final volume and the pH of the culture medium was adjusted to 5.8 with either 1% N HCl or 1% N NaOH, and 7g of agar was added. Then to melt the agar and dissolve the whole solution, it was boiled using boiler before dispensing to culture jar. Then, about 40-50 ml of the media was dispensed into 350 ml jar and covered with a plastic cap.

Finally, the medium was autoclaved at 121°C for 20 min. After cooling, the entire autoclaved medium was maintained in the media storage room for a minimum of four days prior to use in order to check contamination over the media.

Plant Material Preparation and Sterilization

Auxiliary Bud Preparation and Sterilization

Sprouting slip/sucker of pineapple having intact buds was collected from the garden of the horticulture division of Jimma Agricultural Research Center (JARC, Ethiopia). The buds were kept under running tap water for half an hour to remove the soil and other dirt. Then the buds were thoroughly washed with tap water using laboratory detergent. Then the outer bud scales were removed until the bud with single shoot tip remains, which was followed by washing using detergent. The cleansed buds were transferred to laminar air flow hood and it was rinsed with 70% ethanol for 1 min, followed by a surface sterilization using 5% active chlorine commercial bleach of 30% (v/v) added with 3 drops/l Tween-20 for 15 min in laminar air flow hood. Finally, the explants were thoroughly rinsed three times using sterile distilled water, and the dead and damaged tissues by chemicals were trimmed off and discarded. Surface sterilized explants consisting of a bud with a small portion of the rhizome were cultured on a basal MS medium for initiation.

Establishment of Aseptic Culture and Initiation

For establishment of cultures with *in vitro* regenerated shoot tip explants, seeds were sown on the basal MS solid media. Seeds and seedlings of *Ananas comosus* were monitored for the fungal and bacterial contamination as well as for germination of the seeds. The culture jars with cultured seeds were sealed properly with plastic cap, labeled for cultivars and placed under dark growth room until the seeds are germinated. After the seeds are germinated, the cultures were transferred to the growth room with 16/8 hours photoperiod and a temperature of 25 ± 2°C. The seedlings were maintained to grow until the shoot tip is well off for the subsequent use as explants.

In case of axillary bud explants, the bud explants were cultured on the basal MS media for four weeks for initiation. Similar to above, the fungal and bacterial contamination was monitored to obtain aseptic culture. Successfully initiated

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aseptic buds were cultured on the MS media supplemented with 2.5mg/l BA and 0.5mg/l KN for six weeks. Then its response in terms of shoot multiplication was compared to cultures containing the same PGRs (2.5mg/l BA and 0.5mg/l KN) *in vitro* regenerated seedlings' shoot tip explants. Root inductions also respond at 1mg/l NAA for a month.

Data Analysis

Data were analyzed using the analysis of variance (ANOVA) using General leaner Model (GLM) of SAS statistical software version 9.3. The mean variability among treatments least significant difference (LSD) at 5% level of significance used.

RESULTS AND DISCUSSION

Clean explants of pineapple have been applied on full strength MS medium, 30g/l sugar, 7g/l agar and Plant growth regulators of 2.5 mg/l BA combination with 0.5 mg/L KN produced the highest number of micro shoots per explant in six weeks. For root induction all the explants treated with half MS media strength, 30g/l, 7g/l agar and 1mg/l NAA produced root per micro shoots in a month. There were differences among media based on solidified agents. Media with composition of agar, silica gel, glass beads and sand were solid and semi liquid (Table 1). The shoot multiplication in the cytokinins hormone supported by agar and alternative matrices has been produced 33.1 shoots and 5.3 shoot length in sands and agar. There is no significant difference in shoot number and shoot length on the agar and sands on shoot multiplication and plant, and there is significant difference in glass beads and silica gel compared with sand/agar in the shoot multiplication, and there is highly significance in shoot length in the matrices of plant support saw in (Table 1 and figure 1). The supported agent of silica gel, glass beads and sands were effected in shoot proliferation liquid MS media and cost effective in tissue culture industry. The morphological growth of *in vitro* plantlets supported by the supported gel was incubated in six weeks.

Table 1: the plant responses for alternative matrices on shoot multiplication and root induction

Treatments	Shoot number	Shoot length(cm)	Root number	Root length(cm)
Agar	32.8^{a}	3.1^{d}	10.0^{ab}	7.13^{a}
Silica gel	31.5^{b}	3.4^{c}	10.0^{ab}	6.5^{b}
Glass beads	31.3^{b}	4.1^{b}	9.8^{b}	7^{ab}
Sands	33.1^{a}	5.3^{a}	10.6^{a}	7.06^{ab}
Mean	32.2	3.9	10.1	6.9
F-test	**	**	*	*
LSD @ 0.05	0.67	0.24	0.66	0.6
CV%	1.12	3.24	3.5	4.7

**significant at P<0.001 and *significant at p<0.01, Means with same letter within a column are not significantly different at $p<0.05$ ANOVA at least significance difference (LSD). CV% = coefficient variation.

Root Induction

Pineapple root induced in auxins hormone *in vitro* culture of plant propagation at 1mg/l NAA. In this experiment there is no significance difference root number among all means treatment except glass beads in root number. The higher root number in sands 10.6 and followed 10 in silica gel and agar supported matrices. The root length also none significant difference among means treatments in agar, glass beads and sands. However, there is a significance difference with silica gel in root length. The plantlets that were sufficiently healthy with new growth (Figure 2d) were subsequently transferred to larger pots and gradually acclimatized to outdoor conditions. Maintenance of cultures in liquid media is a common practice for many plant systems and has been found to be more convenient than agar gel media. The use of silica gel, glass beads and sands as a support matrix to the explants affords better aeration. These matrices can be easily removed and re-used after sterilization. The chances of root damage or the presence of agar that remains on the roots, leads to unwanted bacterial and fungal contamination. MacLeod and Nowak (1990) reported no differences in regeneration capability and observed a 60 % saving on media components by replacing agar with glass beads. Though, it may be debated, whether media cost really contributes significantly to the total cost (George 1996), however it may be noted that the agar powder ideally used in plant tissue culture media, is an expensive commodity.

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Figure 1: *In Vitro* shoot multiplication of pineapple; A) shoot in agar, B) shoot in silica gels C) shoot in glass beads D) shoot in sands.

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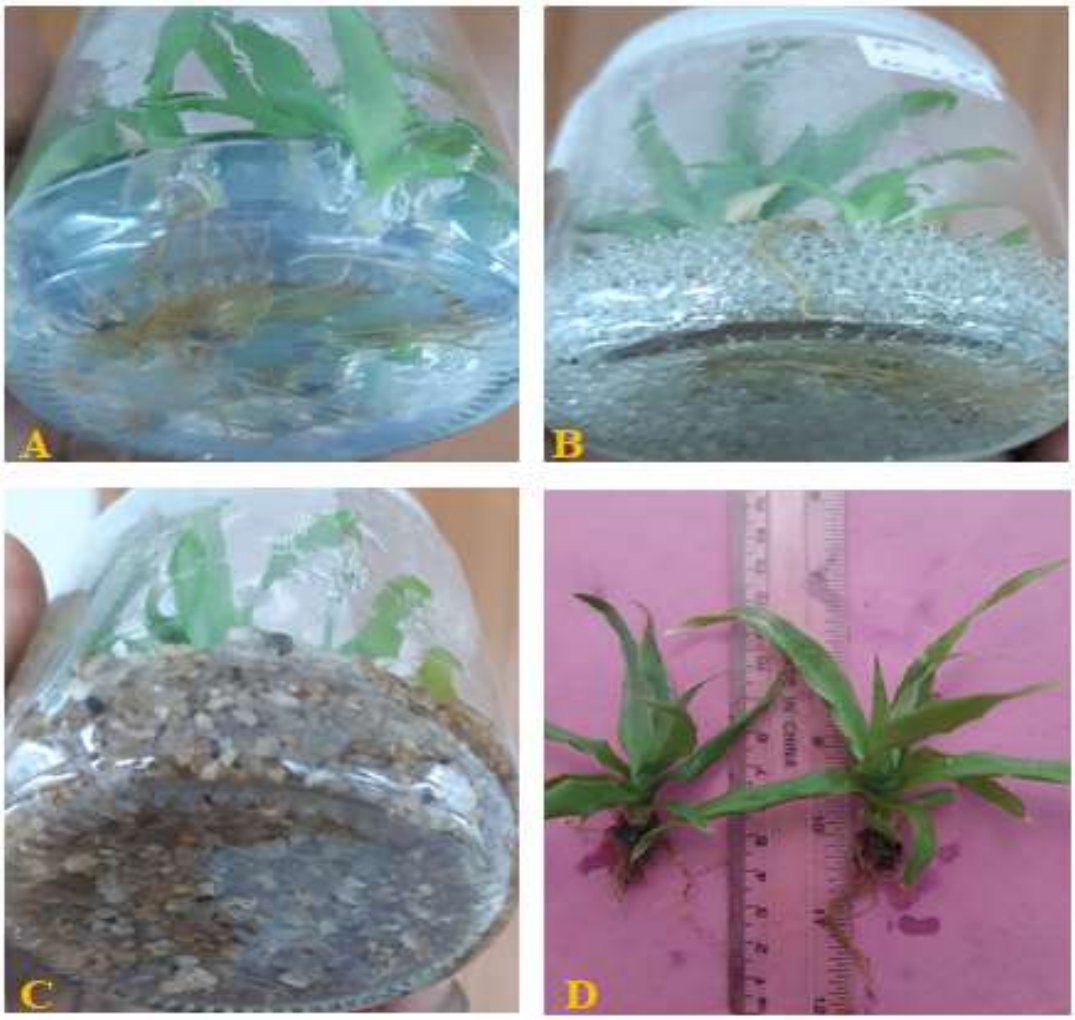

Figure 2: *In Vitro* root induction of pineapple; A) root in agar, B) rooting in glass beads C) rooting in sands D) rooting for acclimatization.

CONCLUSION

A protocol has been developed to replace the agar with glass beads and local sands for *in vitro* rooting of *A. comosus* plantlets. Silica gel, glass beads and sands as a support matrix of liquid medium was found to produce better results in shoot multiplication and root induction for cost-effective and large scale production of disease free pineapple plants for commercial cultivation.

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