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Plant Responses on Elective Gels for in Vitro Shoot Multiplication and Root Elongation 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 utmost common coagulating agents in plant in vitro clonal propagation. Pure grade agar has high value price and fear of over manipulation of its resources affected searching for low cost options. Therefore, liquid medium supplement with silica gel, glass beads and sands substratum and agar in two steps of micro propagation (seedling 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 none significant difference among 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 half strength 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. These silica gel, glass beads and sands used as agar gels to support and aeration plantlets.
Keywords: Elective gels, low cost, shoots multiplication and root elongation.
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Introduction

Pineapple (*A. comosus*) is registered as a chief tropical fruit in biosphere production (FAOSTAT, 2010). Plants associated with soil epochs back to the basis of land plants having early rhizoidal roots attached to the bedrock. Thus was certainly unknown to mankind that roots could be perceiving gravity (Perrin *et al*., 2005). Soil is demarcated as a thin layer of earth crust which obliges as a natural media for plant growth. Mineral matter that has been subjected to absorb by plants due to genetic factor, environmental factors, micro climate, organisms and topography were acting over a period of time. Some of soils differences from the related material in the morphological, physical, chemical and biological possessions. Therefore, soils vary between themselves in some or all the possessions, depending on the differences in the genetic and ecological factors. Hence some soils are red, black, yellow, verity in color and also deep and shallow surface, in texture some are coarse textured and fine textured. Rooted plants, the "hidden half" of plants, which remain underground in the ground, oblige a multitude of functions. These have been liable for anchorage, absorb water, macro and micro nutrients, and interchange various growth materials with the vascular tissue of the plant system. The roots achieve the basic tasks in most herbs and in all abundant plants. The root and soil interface is the site where the most exchangeable between plants and their environment occur. Roots constitute a major source of organic material for the soil and thus affect its arrangement, aeration and biological activities. Whereas organic chemicals move out of the roots into the soil, inorganic ions move in, some of the entering materials are necessary for normal metabolism of the plants and are vigorously required. Inadequate or too much accumulation of elements would harm to plant growth and thus, uptake of nutrients has been affected in root surface (Waisel *et al*., 2002). However, examine for appropriate alternative of soil maybe dates back to the time of plant parts have been first grown *in vitro* techniques of plant tissue culture. The 1990s saw continued expansion in the application of the *in vitro* knowledge and skills to an accumulative increasing number of plant species and which in recent times have capped in research in plant transgenic (Thorpe, 2007). The widespread and almost common application of agar powder with a combination of polysaccharides derivative from red algae as the gelling material in culture medium for all the above mentioned branches of plant tissue culture is due to its following advantage. In addition to this, agar gels have been high clarity, stability, nontoxic and resistance to variation during inoculation (MacLachlan, 1985; Henderson and Kinnersley, 1988). On the other hand, wide spread usage of agar gels in plant clonal propagation via tissue culture technics has definite limitations as well in many plant growth systems. In our present trial, we have to substitute agar gels via silica gels, glass beads and sands as alternative matrices in liquid medium and compared to *in vitro* shoot clonal multiplication and rooting response of pineapple.

Materials and Methods

MS Stock solution preparation

Murashige and Skoog (1962) medium were used during this study. Fortunately, stock solutions of macronutrients, micronutrients, iron, sodium EDTA and vitamins were prepared distinctly. These recommended MS nutrient was measured by volume for liter of stock solutions 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, these MS stock solution was poured into cleaned flasks and kept at 5°C till to consume.

Preparation Plant Hormones as solution forms

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 meditation of 1:1 ratio that mines 1mg solute dissolved in1ml solvent 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 5°C until use.

Mixtures of growth cultures

Inoculation media was ready taking the recommended volumes of MS (Murashige and Skoog, 1962) solutions enhanced with 30g/L sugar as energy source plus 7g/L agar as solidifying agents. Designed for each experiment the desired concentrations and combinations of auxins and cytokinins were supplementary to the inoculation medium accordingly. Previous to adding 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 50 ml of inoculation media was dispensed into 300 ml jam jar and slid with a plastic film. Finally, these growth medium was autoclaved at 121°C for 21 min. After cooling, the entire autoclaved medium was maintained at media storage room for minimum of three days to check contamination over the growth 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). Thus sprout explants were socked under running water for 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 washed with 70% ethanol alcohol for a minute and followed by a superficial disinfection using 5% active chlorine commercial bleach of 300 ml added plus 3 drops of Tween-20 for 15 minutes in laminar air flow hood. Finally, the explants were thoroughly cleaned three times using sterile distilled water, and the dead and damaged tissues by chemicals were trimmed off and discarded. Surface disinfected explants consisting of a sucker with a small portion of the slips 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. Sucker, slip and crown of *Ananas comosus* have been examined for the fungal and bacterial contagion as well as for sprouting of seedlings. 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 incubation room with 16 hours light/ 8 hours dark photoperiod at 25 ± 3°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 full strength MS media intended for four weeks for initiation. Similar to above, the fungal and bacterial contamination was monitored to obtain aseptic culture. Successfully initiated aseptic buds were cultured on the MS media supplemented with 2.5mg/L BA and 0.5mg/L KN aimed at 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 have been analyzed through analysis of variance (ANOVA) by General leaner Model (GLM) of SAS version 9.3. The mean variability among treatments was compared by least significant difference (LSD) value

and P<0.05 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 hormones of 2.5 mg/l BA combination plus 0.5 mg/l KN harvested more seedlings per explant in six weeks duration. For root elongation and induction in vitro shoots transplanted to ½ strength MS media, 30g/l, 7g/l agar plus 1mg/l NAA produced root per micro shoots in a month. MS media ingredients combined with 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 none significant difference among shoot number and shoot length on the agar and sands on shoot multiplication and plant morphology, 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 inside a column are none significant difference @ p<0.05 ANOVA table and 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 seedlings that have been appropriately healthy with fast growing (Figure 2d) and afterwards transmitted to try holes for gradually acclimatized in greenhouse condition. Growing of seedlings in liquid media is a common exercise for various plant clonal propagation and more suitable than agar gel media morphologically as we observed. These silica gel, glass beads and sands used as agar gels to support and aeration. These milieus has been easily distant and re-used again and again after autoclaved. This incubation reduce root damage and maintained from unwanted contamination of microbes. 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).

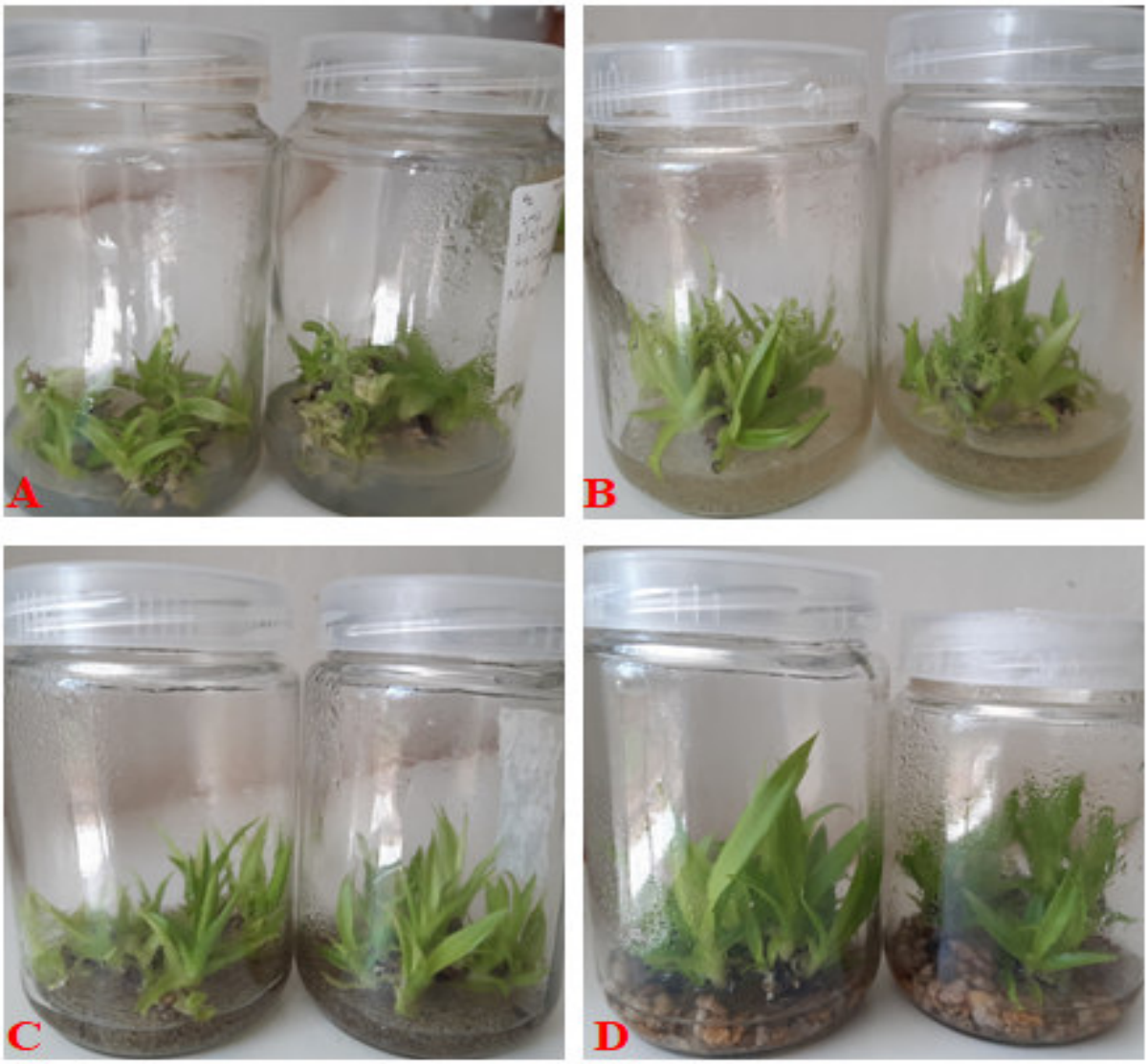

Figure 1: *In Vitro* shoot multiplication of pineapple; A) shoot in agar, B) shoot in silica gels C) shoot in glass beads D) shoot in sands.

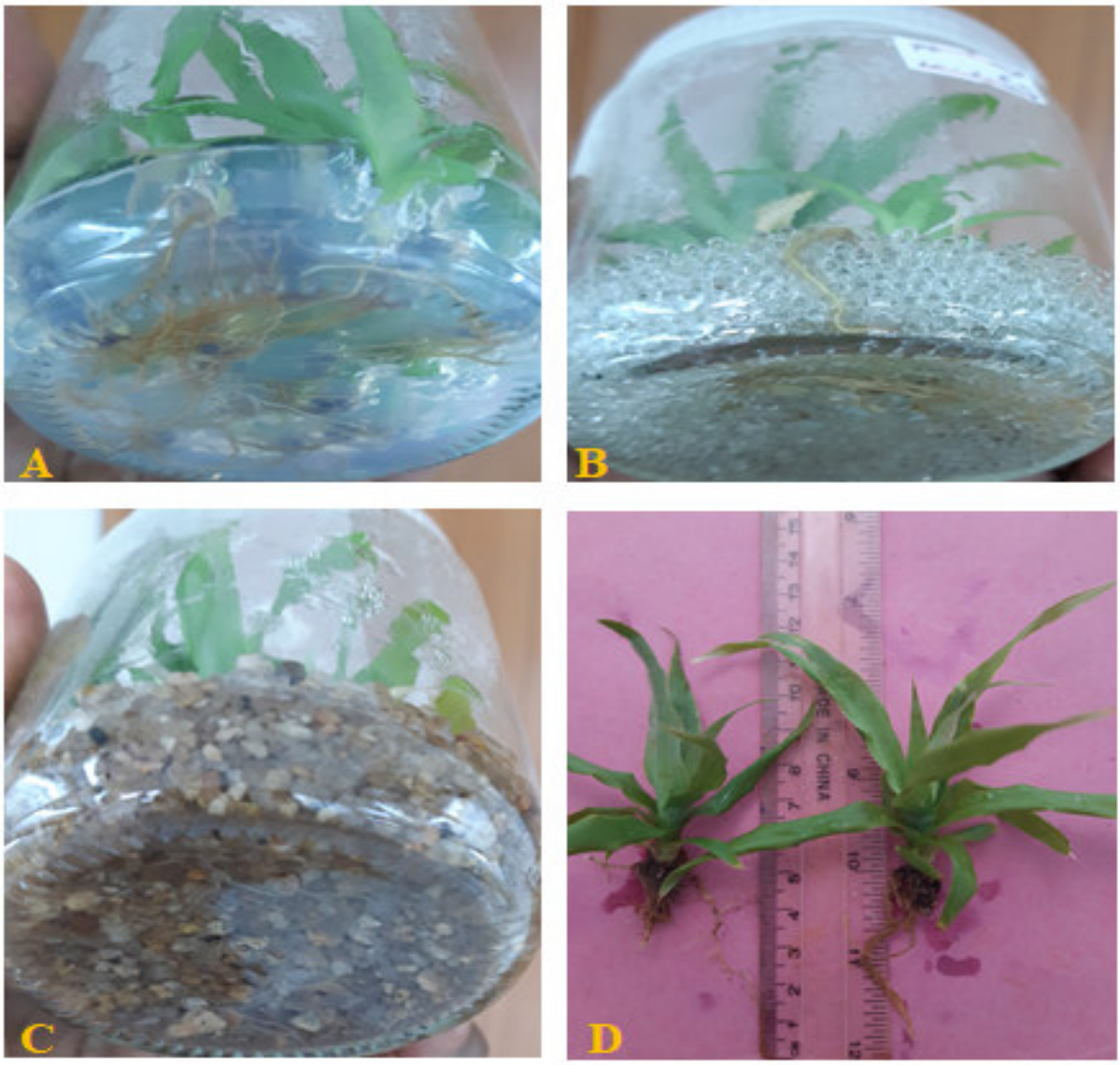

Figure 2: *In Vitro* root induction of pineapple; A) root in agar, B) rooting in glass beads C) rooting in sands D) rooting for acclimatization.

Conclusion

The protocol has been optimized to substitute agars with glass beads, silica gels and local sands for clonal propagation and root induction of pineapple. Silica gel, glass beads and sands used as substituted gels of basal medium was initiated to produce healthier grades in shoot multiplication and root induction for cost-effective. Thus protocol has been applied for large scale production of disease free pineapple seedlings.

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