

Full Length Research

Low cost growth enhancement of *in vitro* produced Pineapples (*Ananas comosus* L. Merr) propagules under nursery conditions

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Small size and slow *ex vitro* growth rate of *in vitro* produced pineapples (*Ananas comosus* L. Merr) constitute a production and marketing problem for large-scale farmers. To accomplish that we conducted a study to elucidate the effect of various soil media on growth enhancement at the nursery stage of *in vitro* produced pineapple propagules after the acclimatization phase in greenhouse. Consequently, we investigated the effect of various soil mixes namely medium A (sand), B (silt), C (1 sand and 1 silt), D (2 sand: 1 silt), and E (1 sand: 2 silt) on the growth of *in vitro* produced pineapples following acclimatization phase in greenhouse. The results revealed that the highest growth parameters in terms of leaf number, leaf length, and number of roots were associated with silt medium followed by medium D (2 sand: 1 silt) and the least values obtained from medium E (1 sand: 2 silt). Root length and leaf width were not soil type dependent. Effect of mycorrhization was only apparent in 8% of plants from the treatment B (silt) yielded signs of nodulation. Survival rate of *ex vitro* pineapple propagules regardless of soil type was almost 100% and this was attributed to the maintenance of humidity by the plastic cover (microclimate). In conclusion, the efficacy of the use of plastic bags, tunnels or greenhouse for growth enhancement should be investigated, and the optimum stage at which the propagules should be transplanted must also be determined. Thus if clonal propagation is a priority to maintain uniformity of pineapple production, field tests of *in vitro* propagated pineapple is necessary to determine the degree of genetic stability and as well avoid off-types derived from *in vitro* propagation.

Keywords: Low cost growth, *in vitro*, Pineapples *Ananas comosus* L. Merr, propagules, nursery conditions.

INTRODUCTION

Pineapple (*Ananas comosus* L. Merr.) is not only among the most important tropical fruit crop of South Sudan, but also a potential export crop. It is confined to the lateritic soils of Greater Equatoria.

History of the pineapple industry in South Sudan is very young. Potentiality of Western Equatoria for pineapple production had been fully realized only in early 1940's (DeSchlippe, 1948). Since then little effort has been done to improve the crop. Most of the early introductions which led to the existing pineapple cultivars; 'Ex-Uganda' grown in Yei and Ex-Congo' cultivated in Yambio and Maridi, were probably introduced by both Belgium and British Administration from Congo and Uganda respectively (Exans-pritchard, 1960).

The "Green Belt" of Equatoria, a major producing region has an elevation of about 1225-1500 m above sea level with annual rainfall of 900-1600 mm spread over 6-9 months.

Pineapple plant discovered 500 years ago on the Guadeloupe Island (South America), is now grown in many tropical and subtropical regions; even under protected cultivation (greenhouse): in temperate region for local consumption as well as an export crop.

The quality of pineapple rests on the amount of sugar (12%) and acid (0.6%) present in the fruit which is also a rich source of vitamin (A, B especially thiamine, and C) and minerals (calcium; potassium; phosphorus; and iron).

For traditional propagation various vegetative organs such as ground and stem suckers, slips, crowns and stumps are used. Naturally, each plant produces very few number of each type these vegetative organs. These organs from the same plant perform differently under field condition especially with respect to fruiting age. For instance suckers fruit earlier (15-18 months), slips intermediate (20-22 months) and crowns are (22-24 months). Uniformity of propagule type is an advantage. Given the fact the number of propagules required per feddan varies from 9,975-13,300 plants (Mohammed, 1983) which the traditional method could not provide for large scale production from one type of suckers (plant material), shortage of quality propagules is a major barrier to expansion pineapple production and even the establishment of new orchards.

Consequently, plant tissue culture as a tool for micropropagation has been successfully employed for pineapple multiplication. According to Sharrock (1992), the *in vitro* pineapple plant multiplication rate is much higher than those obtained from conventional propagation. Kiss *et al.* (1995) developed an *in vitro* method for pineapple clonal propagation using the concept of etiolated nodal segments.

However, the small size and slow *ex vitro* growth rate of *in vitro* produced pineapples constitute a marketing problem. Such propagules are easily transplanted to soil in greenhouse, but elongation and formation of new leaves is very slow even after transfer to the nursery. In addition to that these propagules are subject to high mortality rates and take a long period of time (4-6 months) to reach optimum size for direct field transplanting.

Therefore, successful transplanting of vigorous *in vitro* pineapple propagules assures regeneration of several thousand per year, thus making available clean planting material during the planting season.

Objectives

This study was conducted to elucidate the effect of various soil media on growth enhancement at the nursery stage of *in vitro* produced pineapple propagules after the acclimatization phase in greenhouse.

MATERIALS AND METHODS

Materials

Plant material

Ananas comosus L. Merr.

Plantlets of leaf length 3.5—8.0 cm were initially *in vitro* propagated in modified Murashige and Skoog (MS) medium containing 3.0 mg and 0.3 mg NAA; and rooted

in medium containing 1.8 mg NAA or 2.0 mg IBA per litre.

The plant materials presumably grown *ex vitro* in a V-shaped plastic container were provided as a courtesy by the Leena Tissue Culture Laboratory at Kaduru, Khartoum North.

Culture medium (soil)

The *in vitro* plants were planted in different soils; sand, silt, and combination of the two using locally made clay pots (height 21 cm; mouth diameter 25-27 cm; and bottom diameter 12 cm). Prior to filling the pots dried leaves (leaf mould) were placed on gravel stones or pieces of broken red bricks at the bottom to facilitate good drainage (FAO, 1986).

Polythene cover

Polythene bag of 27 x 45 cm size was used to cover the potted plants. The polythene bag has 2 fundamental roles; that of raising the falling night temperatures and secondly maintain sufficient humidity. Note that the polythene bag (cover) was perforated to assure proper gas exchange and to avoid excess humidity.

Experimental site

The experiment was conducted on the 20th September 1999 (terminated on the 28th April 2000) at the Sudan University of Science and Technology's College of Agricultural Studies, Department of Horticulture Nursery, Shambat, Khartoum North. The nursery is raised with metal frame and sheltered with bamboo on the top and sides.

Methods

Planting

Plants were grown under slightly modified condition whereby control measures pertained to temperature and humidity modifications were exercised. Plants were planted singly (plant/pot) 1.5-2.0 cm deep in 4.5-5.0 kg capacity pot as described earlier in materials section (3.1.2). The soil surface was raised slightly to give a dome shape to avoid waterlogging as earthening the meristem will lead to death of the plant.

Mycorrhiza treatment

During planting, all the roots of the plantlets were inoculated with a solution of fungi. Following mycorrhizal

treatment, plants were watered with about 2 litres and covered with a perforated polythene bag (2 holes of 5 cm diameter each).

Experimental design

The experiment was conducted based on randomized complete block design (RCBD) and using the following treatments; Treatment A: Sand 100%, Treatment B: Silt 100%, Treatment C: Sand + Silt (1:1), Treatment D: Sand + Silt (2:1), and Treatment E: Sand + Silt (1:2). All treatments in the experiment were replicated 5 times. Each pot was regarded a replicate and each treatment represented a block.

Nutritional requirement

Nutritional requirements were pragmatically designed with the aim of invigorating the small plants and to maximize survival rate by accomplishing the following protocol:

(i) Mycorrhiza inoculation: this will act as starter to boost vigorous absorption of nutrients already available in the soil medium.

(ii) Superphosphate: Superphosphate was provided at dose of 5g/pot incorporated into the medium (soil) before planting to promote strong root development.

(iii) Foliar fertilizer (FETRITON®--Combi)

The foliar fertilizer was applied in liquid form between 2 irrigation intervals to the lower leaf axil at the rate of 2g/litre per 25 plants i.e. 80mg/plant. This was provided simultaneously with superphosphate doses during planting, was routinely applied following 14 days either singly or together with urea. Fetriton®—Combi is a trademark of BASF Aktiengesellschaft D—6700 Ludwigshafen—Federal Republic of Germany. It consists of 4.0% zinc, 4.0% iron, 3.0% Manganese, 0.5% copper, 1.5% boron and 0.05% molybdenum as well as 2.0% magnesium oxide (MgO) and 2.8% sulphur.

(iv) Urea Urea was applied also as liquid on weekly basis 3 days after irrigation at the rate of 5g/litre per 25 plants (200g/plant). Mode of application was as described in foliar fertilizer (to the lower leaf axils). However, urea dosage was increased by 100% (after 3 months) as the plants increased in size as well as the dry matter (DW).

Irrigation and water supply

With special regard to pineapple plant at this stage of growth, survival is more irrigation-dependent. Therefore to facilitate irrigation and protect the shoot-tip, the soil surface as described earlier is raised to form a dome-shape surface so that when watering the plant the water that would mix with soil particles was not allowed to

cover the shoot tip. However, plants were irrigation automatically after planting and then later after 7 days. Low flowing was first splashed carefully at the shoot tip by a plastic pipe inserted onto a tap water control valve, directed to the plant through the hole on the plastic bag at the rate of 2.0 litre per pot.

Data collection and statistical analysis

Plant height, leaf length and width, number of leaves, number of roots and root length were recorded at the beginning and at the end of the experiment. Later the same data as before was taken as the resulting plants approaches a suitable size for direct field transplanting as well as monitoring possible phenotypic variation in terms of colour change, presence or absence of spines and including undesirable morphological characters (off type).

Statistical analysis was based on ANOVA. All treatments in the experiment were replicated 5 times. Each pot was regarded a replicate and each treatment represented a block.

RESULTS AND DISCUSSION

The statistical analysis of the data on growth enhancement of *in vitro* produced Pineapples (*Ananas comosus* L. Merr) propagules under nursery conditions revealed the following findings:

Leaf number of pineapple propagules under *ex vitro* conditions was affected by soil type. Silt (B) excelled other soil types by maximizing leaf number (figure 1). Silt (B) ranked top with significance difference over the other treatments except for treatment D (2 sand: 1silt).

It was apparent from the results obtained in this study that leaf number, length and number of roots of pineapple propagules under *ex vitro* conditions were affected by soil type while leaf width and root length were unaffected. This result is similar to the findings of Ahmed (1997) whose experiment on soil type mixes effects on three (3) different plants revealed that the greatest growth parameters were associated with silt and this may be attributed to the fact that silt is rich nutrient source, with moderate pH and high water retaining capacity.

Again, silt (B) resulted in higher leaf length compared to other treatments (figure 1). The difference was significant. Treatment D (2 sand: 1 silt) ranked second although it was not significantly different from treatments A (sand) and C (1 sand: 1 silt). The least leaf length was obtained from treatment E (1 sand: 2 silt).

Although higher leaf length was obtained by silt (B); followed by treatment D (2 sand:1 silt); and treatment E (1 sand : 2 silt) the least, there was no significant difference between treatment A (sand) and C (1 sand:1 silt). The results agree with the above explanation

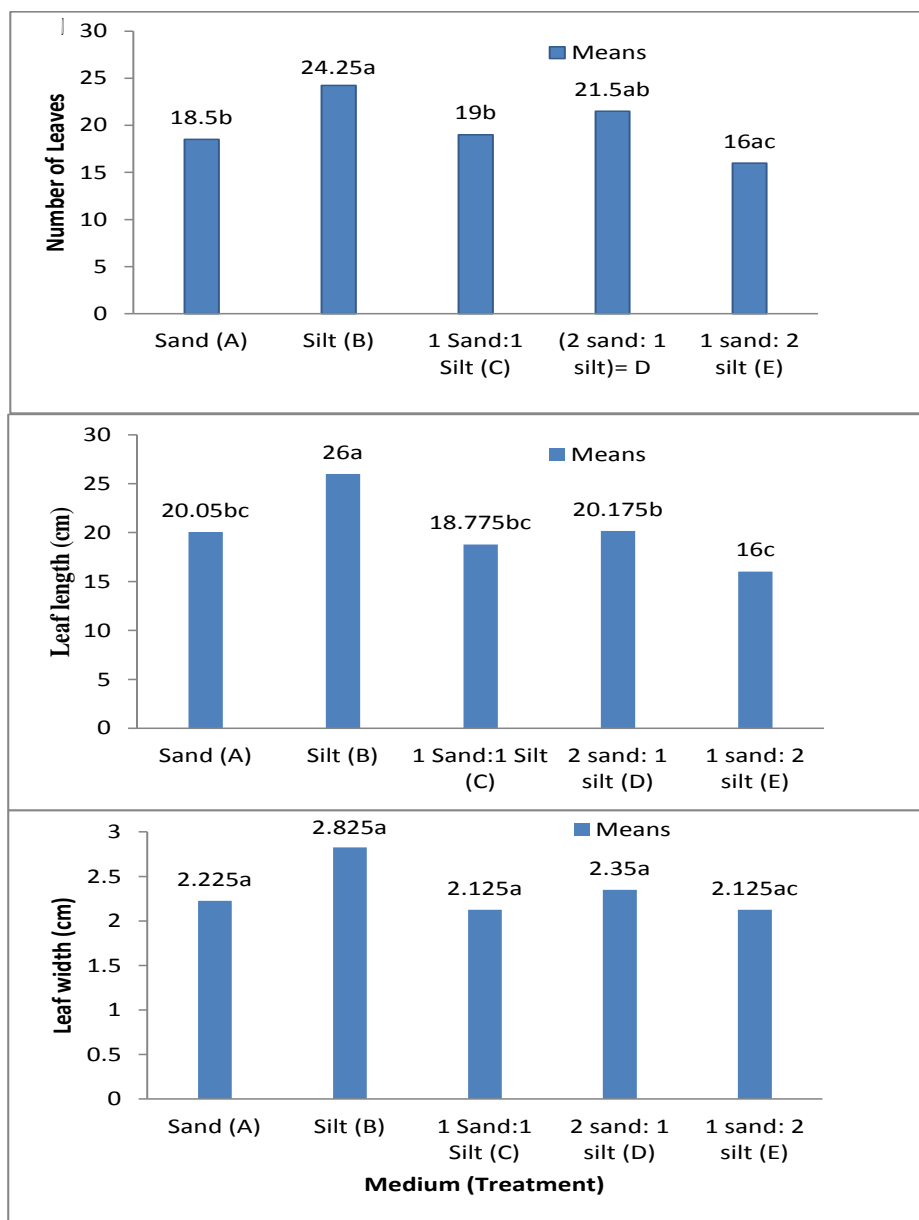


Figure 1: Effect of soil type (medium) on the number of leaves, leaf length and leaf width of ex vitro pineapple propagules). *Means are separated by LSD and means with the same letter are not significantly different.

except that treatment E should have ranked second to silt (B). But according to Campbell (1980) sand tend to cause silt to compact.

Leaf width was not affected by soil type. No significant difference was obtained among all treatment (figure 1). Lack of significance for leaf width assumes that leaf width is one of the growth components which are not considerably influenced by soil variation compared to leaf length.

Despite that sand is universally popular as a good rooting media, number of root count showed that silt (B) superseded the other treatment probably because it was found to encourage the development of roots (Garner

and Saeed, 1985). Non-significance among other treatments (A, B, C, D and E) may be likely due to the unique phenomenon associated with sand which during irrigation causes finer silt to compact or be washed down thus leaving sand soil on the top of the pot (container).

Number of roots (figure 2) was also affected by soil type. Silt (B) was also the best with significant difference superseding other treatments followed by medium A (sand) and D (2 sand: 1 silt). However, with exception of silt (B) no difference was recorded for all other treatments.

For root length (figure 2) no significant difference was obtained between all treatments. The results suggest

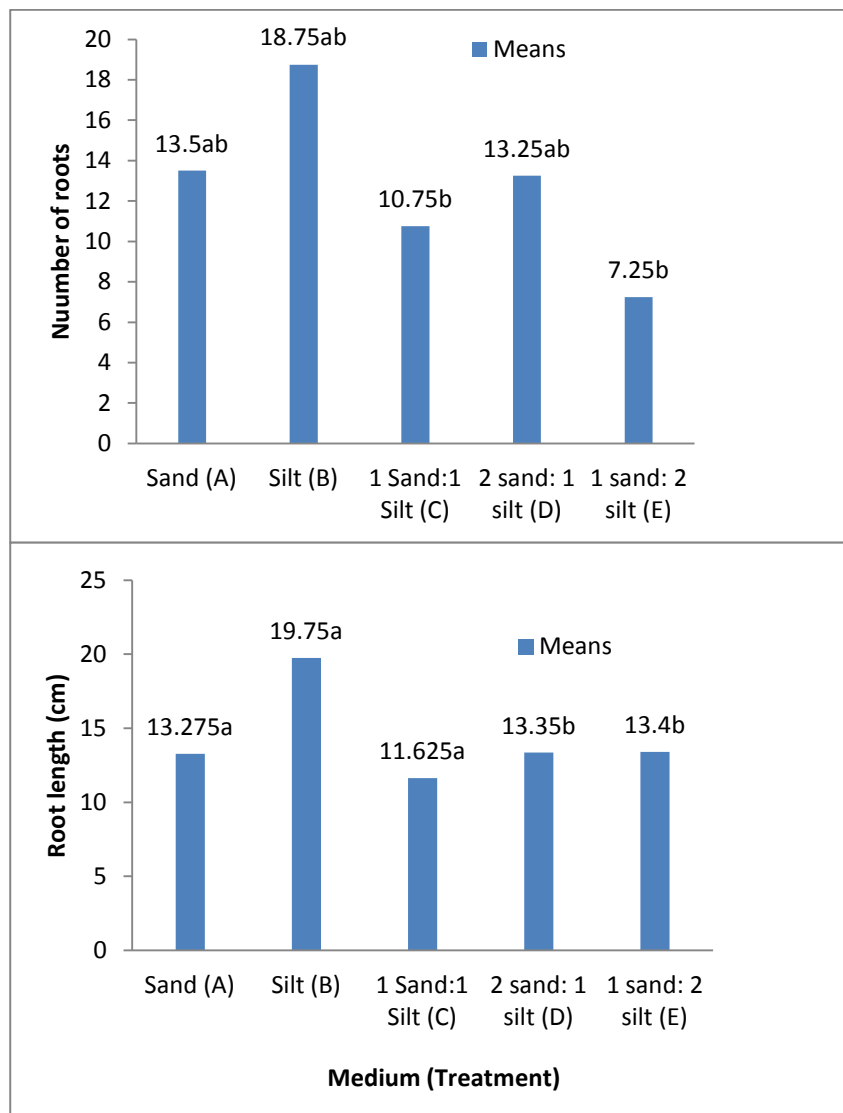


Figure 2. Effect of soil type (medium) on the number of roots and root length of ex vitro pineapple propagules. *Means are separated by LSD and means with the same letter are not significantly different.

that root length was not soil-type dependent and which is consistent with the findings of Ngugi *et al.* (1978) and Williams *et al.* (1987) who reported that under best growing media, pineapple roots grow no deeper than 30 cm. Effect of mycorrhization on root growth was not apparent although only 2 plants (8%) from the treatment B (silt) yielded signs of nodulation.

To monitor possible variation of plant morphology under *ex vitro* conditions, the following observations were reached: (i) 64% of the leaf margins were smooth-edged (spineless), (ii) 8% with spines on both sides of the leaf margin, and (iii) 28% with spines on one side of the margin. Out of the 28% one sided-spined leaves only 1-4 leaves per plant had spines. In spite of the donor parent plant possessing smooth leaves, morphological variation of 36% was recorded. This result confirms the

findings of Wakasa (1979) that variation in regenerated pineapple population was 34% for axillary bud-derived plants. Similarly, Ramcharan *et al.* (1985) reported off-type in plantain up to about 21-38% variability. However, this level of variability is unacceptable for clonal propagation but it could be an important breeding tool. But the risk of phenotypic variation could be reduced by using the concept of etiolated nodal segments (Kiss *et al.*, 1995).

Because *in vitro* propagated pineapple propagules have slow growth rate; small size; and high mortality rate, low survival rate is a major problem of maintenance under nursery conditions. But Canlas *et al.* (1994) reported that survival was 100% in *ex vitro* rooting using sand. Fortunately, this study produced encouraging

results that is survival of 100% regardless of soil type except for treatment E (1 sand: 2 silt) which was 80%).

CONCLUSIONS

So far the results reached recommends that silt is the best soil media as less fertilizer will be required during acclimatization process and its high water retaining capacity means longer irrigation interval and also low water consumption, thus, minimizing cost of maintenance.

Also the efficacy of the use of plastic bags, tunnels or greenhouse for growth enhancement should be investigated, and the optimum stage at which the propagules should be transplanted must be determined.

Furthermore, as clonal propagation is a priority to maintain uniformity of pineapple production, field tests of *in vitro* propagated pineapple is necessary to determine the degree of genetic stability and avoid off-types for further *in vitro* and *ex vitro* propagation.

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