

## Original Research Article

# An Alternative Low Cost Multiplication Technique: Natural Substances for Regenerating Plantlets from Mini tubers of Sweet Potato (*Ipomoea batatas*, L.)

### ABSTRACT

**Aims:** This study was conducted specifically to develop a low cost rapid plantlet multiplication process easily affordable for farmers to enable them speedily generate plantlets for their farms from mini tubers of sweet potato variety, TIS 87/0087

**Study design:** The experimental design was a completely randomised design with three replications. Analysis of variance was used ( $P=0.05$ ) to test treatment effects in a completely randomised design and mean comparison was by LSD.

**Place and Duration of Study:** The study was carried out between February and April 2019 in the plant culture laboratory of the Department of Plant Science and Biotechnology, Rivers State University, South-South, Nigeria.

**Methodology:** Mini tubers of sweet potato (*Ipomoea batatas* L.) were preconditioned by soaking in a 1:10 ratio of natural substance to water for 12 hours before planting in soil. The natural substances comprised coconut water, grapefruit juice and honey with water as control.

**Results:** Mini tubers primed with grapefruit juice and coconut water sprouted significantly earlier ( $P=0.05$ ) (7days and 10days respectively) than the control (water) which sprouted after 19days. However, there was no significant difference in time to sprouting between mini tubers primed with honey (16days) and the control (19days). Mini tubers primed with grapefruit juice, coconut water and the control did not differ significantly ( $P=0.05$ ) in the number of initial plantlet sprouts but mini tubers primed with honey had significantly lower initial sprouts than others. On average, coconut water primed mini tubers produced significantly higher ( $P=0.05$ ) total number of regenerated plantlets compared to the other treatments and continued regeneration of plantlets longer than other treatments. Coconut water regenerated almost 3times the number of plantlets regenerated by mini tubers soaked in water. Mini tubers primed with honey stopped sprouting after 29days which was significantly earlier ( $P=0.05$ ) than those primed with grapefruit juice (38days), water (46days) and coconut water (51days).

**Conclusion:** This study found that mini tubers of sweet potato after a preconditioning treatment by soaking in dilute coconut water (1:10 coconut water:water ratio) for 12hrs before planting regenerated almost 3times the number of plantlets regenerated by mini tubers soaked in water for the same period.

**Keywords:** [Sweet potato, Mini tubers, Natural substances, Preconditioning, Regeneration]

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16 **1. INTRODUCTION**

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18 Sweet potato (*Ipomoea batatas* L) is a vegetatively propagated crop which is commonly  
19 propagated by farmers from the root tuber, or from the vines and by breeders from the seed.  
20 While developing countries account for over 95% of the world's sweet potato production  
21 [1,2], in Europe, the biggest producers are Portugal, Spain, Italy and Greece [3,2]. Although  
22 sweet potato is a source of food, animal feed and industrial raw material for production of  
23 sugar syrups, ethanol and flour, etc for confectionaries [4,5] one major constraint facing  
24 sweet potato growers worldwide is shortage of clean planting materials at the beginning of  
25 every planting season. It is cultivated by recycling planting materials from old fields; is locally  
26 sourced, and must be fresh to be viable and therefore is not sold in local markets or by  
27 traders like seed propagated crops. The problem is more severe for farmers in the drought  
28 prone and high disease pressure areas. For instance, it was reported [6] that farmers in  
29 Bukedea and Soroti districts in Uganda normally experience 3–4 months of dry weather  
30 between mid-November and March. During this dry period, vegetation of the sweet potato  
31 crop is completely desiccated, leading to difficulties in securing vines as planting material at  
32 the onset of the rains. About 58% of the farmers interviewed in Soroti claimed that their  
33 failure to plant was due to lack of planting material. In some sub-Saharan African regions,  
34 the dry season extends even more to 5-7 months later, especially in those with prolonged  
35 drought periods further compounding the problem [7]. Elsewhere, it was stated [8] that  
36 availability of quality planting material on a sustainable basis is a major challenge for the  
37 farming communities of Odisha state of east India. In order to overcome these challenges,  
38 farmers in many areas try to solve this problem by conserving planting materials near water  
39 sources, or in the home garden, or by storing roots which they sprout at the onset of rains, or  
40 do sequential planting immediately after onset of rains to get enough materials for field  
41 expansion [8,6]. Most farmers lose upwards of 4-6 weeks or more of the growing season at  
42 the beginning of the rains while they re-establish sufficient vine production for planting. They  
43 obtain initial limited planting material from residual plants, re-sprouting roots, or secondary  
44 growth of harvested fields, thereby limiting the sweet potato production areas [7]. The  
45 immediate obvious solution to the problem of limited planting material of course, is rapid  
46 multiplication either by tissue culture, sand hydroponics or aeroponics techniques [9,10].  
47 However, the main disadvantages of these rapid multiplication techniques in developing  
48 economies are that they require special skills, extra manpower, sophisticated equipment,  
49 high capital outlay and high production costs [11,12] that are not readily available to farmers.  
50 Plant tissue culture is essentially rapid multiplication of tiny shoot tips/apical meristems,  
51 axillary buds, and sometimes of somatic embryos and cell clumps, etc in suspension  
52 cultures and bioreactors. What developing economies need is a technique to do the same at  
53 minimal cost. Besides, it has been stressed time and again that in the long-term, agriculture  
54 needs to be sustainable, use little or no crop-protection chemicals, have low energy inputs  
55 and yet maintain high yields, while producing high quality material, and also saving land  
56 resources [13]. Bearing these in mind therefore, an alternative cheaper, efficient, effective  
57 and simple rapid multiplication technique with low energy inputs designed to provide planting  
58 material for mass propagation of sweet potato other than by use of tissue culture technique  
59 has become imperative. This means that farmers must be able to apply the technique  
60 themselves while avoiding high technology and building of complex infrastructure especially  
61 during the release of improved varieties. Such a technique must not be too complicated, nor  
62 labour intensive and must be within a short duration in limited spaces and should not require  
63 farmers to learn multiple new tasks so it can be readily adopted by resource poor farmers. In  
64 fact, some farmers may choose to specialise solely in the production of planting materials in  
65 desired quantities timed to match peak demands and thus establish a new line of business.  
66 One of such techniques is the use of mini-tubers as has been done for yams through use of  
67 mini setts [14]. The other is by a preconditioning of the mini tubers in natural substances to  
68 act as growth primers for regenerating plantlets. Both techniques combined could provide a

69 substantial increase in the number of plantlets regenerated for planting and expand farmers'  
70 fields beyond the practices earlier described. To the best of my knowledge, there is no  
71 scientific literature available on the use of the following natural substances; grapefruit juice,  
72 honey and coconut water as growth primers in a preconditioning technique for rapid  
73 regeneration of sweet potato plantlets from mini tubers.

74 Grapefruit juice contains in addition to myo-inositol also known as inositol, carbohydrates,  
75 proteins, fat, and vitamins B1, B2, and B9, vitamin C, and vitamin P (bioflavonoid). It has  
76 also a plethora of minerals like iron, iodine, potassium, calcium, cobalt, magnesium,  
77 manganese, copper, sodium, phosphorus, fluoride and zinc [15]. Most of these are essential  
78 for plant cell and tissue growth [16]. Honey is composed of sugar (about 76% - fructose,  
79 glucose and sucrose), water (18%) and minerals: potassium, chlorine, sulphur, calcium,  
80 sodium, phosphorus, magnesium, silicon, iron, manganese and copper; proteins, acids and  
81 vitamins: vitamin C and some B complex vitamins - riboflavin, pantothenic acid, pyridoxine,  
82 biotin, nicotinic acid (niacin) (6%), and inositol [17,18,19,20,21]. The constituents of coconut  
83 water are water 94%, sugars such as glucose, fructose and sucrose around 5%, proteins  
84 around 0.02% and lipids only about 0.01%. It is rich in minerals such as potassium, calcium,  
85 magnesium and manganese, and low in sodium. Amino acids include glutamic acid,  
86 asparagine, proline, and glycine; and organic acids particularly malic acid  
87 [22,23,24,25,26,27]. In addition to inositol [28] coconut water contains auxin, various  
88 cytokinins, and gibberellins [29,30] which are all plant growth hormones that support cell  
89 division and promote rapid growth.

90 The main objective of this combined mini tuber and natural substance primers is to produce  
91 elite planting material irrespective of season, for the rapid regeneration of sweet potato  
92 plantlets as planting materials for farmers. The method would also be useful for introduction  
93 of new improved varieties to farmers' fields. The ease of application of the method at  
94 relatively low cost and the avoidance of any use of chemicals is an added advantage.

95 This study was therefore conducted specifically to develop a low cost rapid plantlet  
96 multiplication process easily affordable for farmers to enable them speedily generate  
97 plantlets for their farms from mini tubers of sweet potato.

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## 2. MATERIAL AND METHODS / EXPERIMENTAL DETAILS / METHODOLOGY

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101 This study was carried out in the plant culture laboratory of the Department of Plant Science  
102 and Biotechnology, Rivers State University, Port Harcourt, south-south, Nigeria.

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### Preparation of Natural Substances as Primers

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106 The following natural substances were obtained: fresh coconut water, fresh grapefruit juice,  
107 fresh honey and water (control). These were prepared as primers by adding 50ml of each  
108 substance to 500ml of water in a 1:10 ratio of natural substance to water.

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### Preparation of propagules (mini tubers)

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112 The tubers of purple skinned/white fleshed sweet potato (*Ipomoea batatas* L) variety TIS  
113 87/0087 (TIS 87/0087-registered variety from the National Root Crop Research Institute,  
114 Umudike, Abia State, Nigeria) were washed under running water after which the tubers were  
115 cut into pieces (mini tubers) of approximately 7cm X 7cm size or 25g weight.

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### Treatment Applications (Preconditioning Technique) and Experimental Design

Each mini tuber was soaked in the prepared natural substance primers for 12hours (soaking  
beyond 12hours caused the mini tubers to rot) before planting in soil in a plastic germination

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118 tray with watering done as required for multiplication of plantlets. Treatments were the four  
119 natural substance primers described earlier with water as control in a completely randomised  
120 design with 3 replications. The regenerated plantlets were transplanted from the mini tubers  
121 to the field every 2weeks.  
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#### 123 **Data Collection and Statistical Analyses**

- 124 (1) Number of days taken from planting to first sprouting of plantlets from mini tuber;
- 125 (2) Number of initial plantlets sprouted from mini tuber;
- 126 (3) Total number of plantlets regenerated from mini tuber
- 127 (4) Length of time the mini tuber continued to produce plantlets until the last plantlet;
- 128 (5) Physical appearance and quality of sprouted plantlets

129 The treatment effects were subjected to analysis of variance (ANOVA) using the GLM  
130 procedure of Statistical Analyses Software (SAS) version 9.1 [31] and any effects found to be  
131 significant were tested at a significance level of 5% while means were compared using LSD  
132 at  $P = 0.05$ .  
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### 134 **3. RESULTS AND DISCUSSION**

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#### 136 **Effects of preconditioning on time taken to first sprouting of plantlets from mini** 137 **tubers**

138 The effect of the natural substance primers on the time taken to first sprouting in each  
139 treatment is shown in Figure 1. Grapefruit juice and coconut water treated mini tubers  
140 produced the first plantlets 7days and 10days after priming respectively while the control  
141 (water) sprouted after 19days. Thus grapefruit juice and coconut water treated mini tubers  
142 sprouted significantly earlier ( $P=.05$ ) (almost 3times and 2times faster respectively) than the  
143 control. However, there was no significant difference in time to sprouting between mini  
144 tubers treated with honey (16days) and the control (19days). Coconut water, grape fruit juice  
145 and honey are natural sources of myo-inositol also known as inositol, a plant growth factor  
146 although grapefruit juice has a much higher content than coconut water and honey [28,32].  
147 The high viscosity of honey may have made its low inositol content less effective. In addition,  
148 coconut water contains auxin, various cytokinins, and gibberellins [29,30] which are all plant  
149 growth hormones that support cell division and promote rapid growth. These growth  
150 promoters could account for the early sprouting by mini tubers treated with grapefruit juice  
151 and coconut water.  
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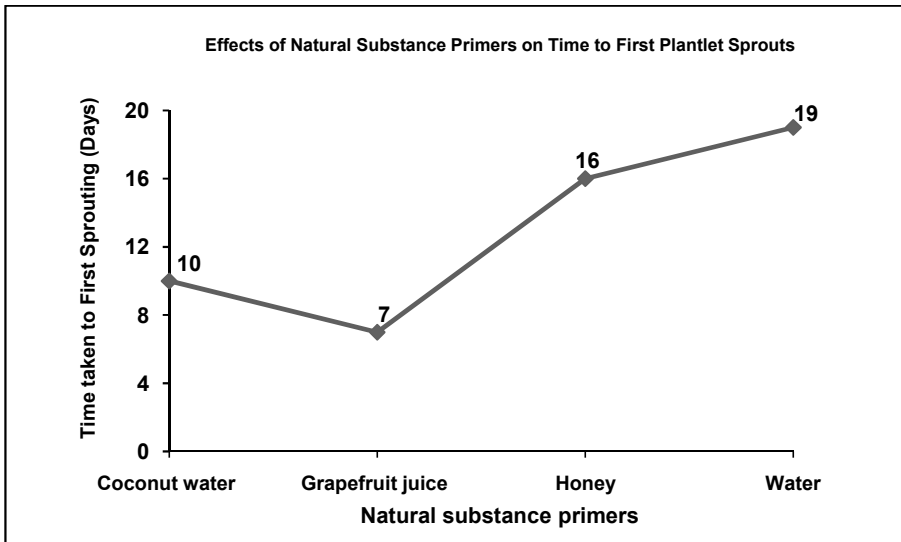


Fig. 1. Effects of natural substance primers on length of time from planting to first plantlet sprouts from mini tubers

**Effects of natural substance primers on number of initial plantlet sprouts**

In Figure 2, the numbers of initial plantlet sprouts are presented. There were no significant differences ( $P=0.05$ ) in the number of initial plantlet sprouts between grapefruit juice and coconut water treated mini tubers and the control. The initial plantlet sprouts from mini tubers treated with honey was significantly less than all other treatments. Honey being more viscous than the other natural substances [17] could have resulted in a slower effect on mini tubers than other liquid substances despite its inositol content compared to water.

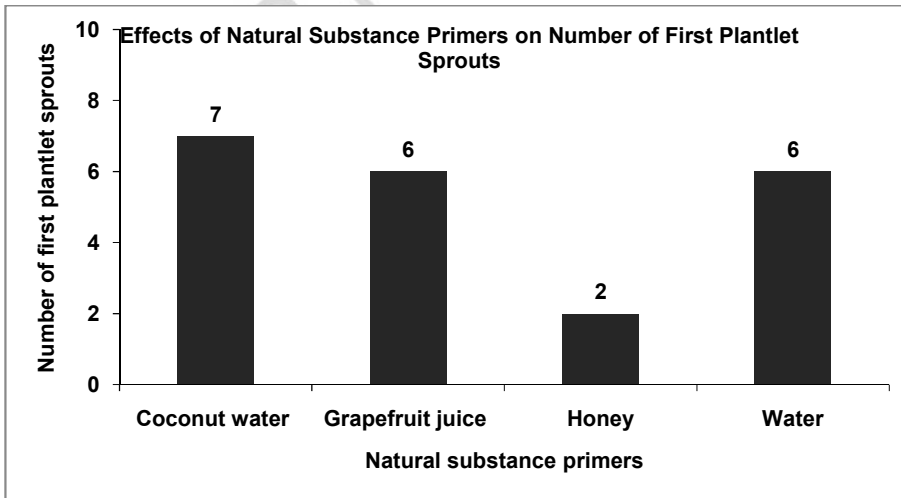
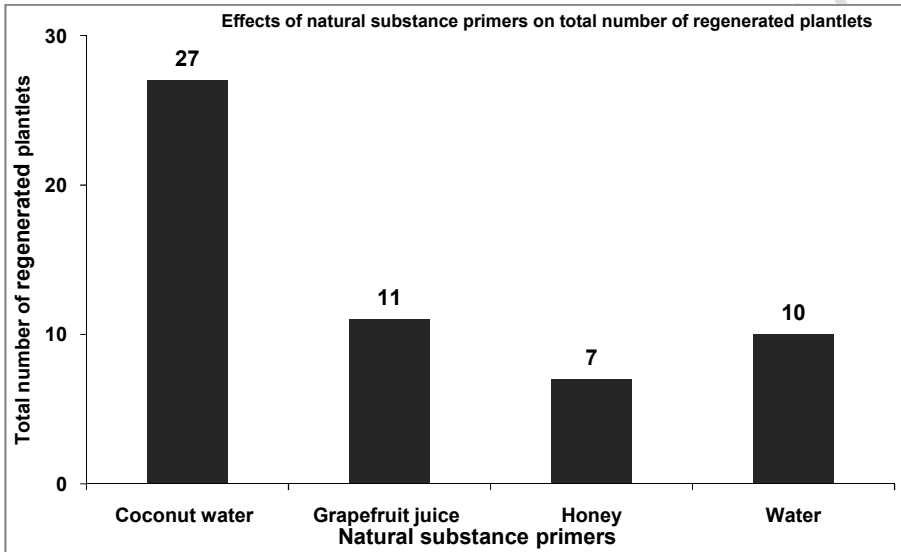


Fig. 2. The effects of natural substance primers on number of first plantlet sprout from mini tubers

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**Effects of natural substance primers on total number of plantlets regenerated**

The total numbers of regenerated plantlets in all treatments are shown in Figure 3. Coconut water produced significantly higher ( $P=.05$ ) total number of regenerated plantlets than all other treatments. The vitamins, minerals, and amino acids present in coconut water [22,23,24,25,26,27] and the auxin, various cytokinins, and gibberellins which are all plant growth hormones that support cell division and promote rapid growth [29,30] all working in synergy could explain the sustained regeneration of plantlets that almost quadrupled, tripled and more than doubled the number of plantlets produced by mini tubers primed in coconut water compared to the honey, water (control) and grapefruit juice respectively [33,34].

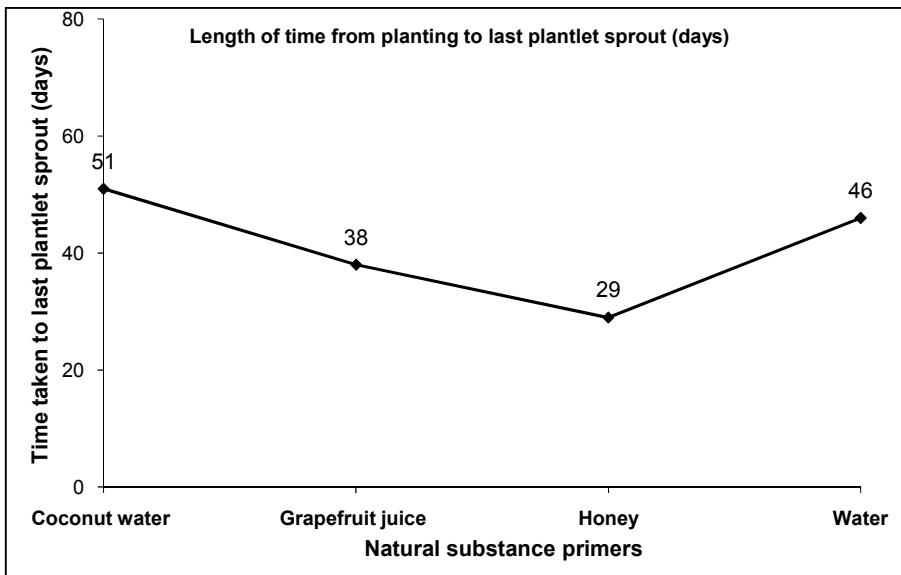


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**Fig. 3.** Effects of natural substance primers on the total numbers of regenerated plantlets from mini tubers

**Effects of natural substance primers on time taken to last sprouting of plantlets from mini tubers**

185 In Figure 4, the number of days to last sprouting in each treatment is shown. Mini tubers  
186 treated with honey stopped sprouting significantly earlier ( $P=05$ ) than those primed with  
187 grapefruit juice (38days), water (46days) and coconut water (51days). Coconut water primed  
188 mini tubers continued regeneration of plantlets longer than other treatments. Perhaps the  
189 high content of vitamins, minerals, amino acids and plant growth hormones in coconut water  
190 could explain the prolonged healthy shoot regeneration over a longer period of time than the  
191 other substances.  
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194 **Fig 4:** Effects of natural substance primers on length of time from planting to last plantlet  
195 sprout from mini tuber

#### 196 **Physical appearance and quality of sprouted plantlets**

197 Plantlets from the mini tubers primed with coconut water, grapefruit juice and honey were  
198 normal and of good quality not requiring any hardening period and easily withstood  
199 transplanting to the field without any adverse effects as is often needed for tissue cultured  
200 plantlets. However, plantlets produced with water priming had small juvenile leaves, with  
201 pale yellow colour and reduced photosynthetic capacity. This meant that they took much  
202 longer to recover when transplanted to the field than plantlets of other primers.  
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#### 204 **4. CONCLUSION**

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206 This study found that mini tubers of sweet potato after a preconditioning treatment by  
207 soaking in dilute coconut water (1:10 coconut water :water ratio) for 12hrs before planting  
208 regenerated almost 3times the number of plantlets regenerated by mini tubers soaked in  
209 water for the same period.  
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#### 211 **CONFLICT OF INTEREST**

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213 Author has declared that no conflict of interests exists  
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