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RESEARCH ARTICLE

SPACING EFFECT ON MACRO- AND MICRO-PROPAGATED PINEAPPLE PLANTLETS

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ABSTRACT

*Productivity of pineapple (*Ananas comosus *L. Merr) is primarily limited by unavailability of* planting material and improved management practices. This study was conducted to determine effect of spacing on growth and development of macro- and micro-propagated pineapple. Micro- and macro-propagated pineapple plantlets were evaluated at three spacings at RAB Rubona station located in mid-altitude zone of Rwanda during 2011/2012 season; the experimental design used was a randomized complete block (RCBD) with three replications. Data were collected on monthly basis by measuring plant height, length and width of longest leaf and counting the number of functional leaves. The data were analyzed by Microsoft excel and Genstat Discovery Edition 4 Software package, least significant difference test were used to determine if there is any significant difference among micro and macro-propagated pineapple plantlets in their growth resulting from spacing effect. Data were analyzed from 258th to 379th day after transplanting; At 379th day after transplanting, highly significant ($P<0.001$) differences among propagation modes were found for number of functional leaves, micro-propagated pineapple plantlets were found to have more number of functional leaves than macro-propagated pineapple plantlets. Significant ($P<0.05$) differences among macro- and micro-propagated were observed also for plant height and length of the longest leaf at 379th day after transplanting. At 379th day after transplanting, there were no significant ($P>0.05$) differences among micro- and macro-propagated pineapple plantlets for width of the longest leaf. Analysis of variance showed that there were no significant ($P>0.05$) differences among spacings for plant height, number of functional leaves, length and width of the longest leaf. Interaction between propagation modes and planting densities were found to have no significant ($P>0.05$) differences for all vegetative parameter. The experiment needs to be continued and repeated in other agro-ecological zones to confirm these findings.

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INTRODUCTION

Horticulture production and consumption dates far back in the pre-colonial era during which native fruits and vegetables formed a considerable proportion of Rwandan's diets (Rwanda Horticulture Development Authority - RHODA, 2011). New horticultural crops such as pineapple (*Ananas comosus* L. Merr) were however later introduced by missionaries during the colonial period (Nyabyenda, 2010). Rwanda needs to diversify its export commodities but farmers are facing problems that limit production, these problems include: lack of high yielding and early maturing planting materials, and in-sufficient information about modern agricultural practices (RHODA, 2011). *Pineapple crop has a very low natural multiplication rate;* the need to solve this problem has led to the development of tissue culture (micro-propagation) and macro-propagation techniques for the pineapple (Rwanda Agriculture Research Institute - ISAR, 2010). *The ability of pineapple to tolerate prolonged absence of rain and its preference of acid soils makes it an attractive horticultural crop for many parts of Rwanda* (Izamuhaye, 2010 and Raemaeker, 2001). Considering that horticulture has great potential to alleviate poverty, create employment, improve nutritional status, or improves the farming and export capacities its development should be put high on the development agenda of Rwanda (RHODA, 2011 and Ministry of Finance and Economic Planning - MINECOFIN, 2009). *Pineapple crop has a very low natural multiplication rate, about 2 per year, and field grown suckers have a potential risk of propagating diseases (ISAR, 2010 and Ministry of Agriculture and Animal Resources - MINAGRI, 2009). An unequalled alternative for production of planting materials is*

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*tissue culture, a technology which has led to the production of large numbers of disease-free planting materials in a short period, and independently of the season (Hawkins et al., 2007). Micro-propagation is used or the establishment of multiplication blocks which then provide conventional planting material for larger production blocks, but macro-propagation will provide ten to 15 planting materials per month (Fitchet, 1990). Farmers need earlier yielding planting material that will continue to give better economic yield;however pineapple is a perennial crop, so optimum spacing between or among plants and better crop management will ensure sustainability in pineapple production that is why it is critically important to evaluate spacing effect on the growth and development of micro-propagated and macro-propagated pineapple plantlets.*The goal of this work was to provide recommendations for using the right pineapple planting material at optimum planting density combined with better farming practice.

MATERIALS AND METHODS

Experimental site description

The experiment was carried out at ISAR Rubona station, the actual Rwanda Agricultural Board (RAB) Rubona station, located in Rusatira Sector, Huye District in the Southern Province of Rwanda. It is about 15 km from Huye town along Huye-Kigali road. Rubona station is situated in the mid-altitude zone with the Geographic coordinates of 2°29'07" S altitude and 29°47'49" E longitude and about 1650m of altitude. The daily average temperature is 18°C with annual rainfall of 1117 mm. The climate is tropical of AW_3 type according to Koppen classification. Most of the soil at RAB Rubona station has been classified as clay loam (ISAR, 2010).

Table 1. Meteorological data of RAB Rubona station

Month	Rainfall(mm)	Temperature (°C)		
		Tmax	Tmin	Average
January	11.7	25.7	15.1	20.40
February	74.8	25.4	14.2	19.76
March	77.4	25.3	14.3	19.8
April	214.2	23.8	14.1	18.97
May	241.4	23.3	14.4	18.84

Source: RAB - Rubona Meteological station

Planting material

The tissue culture pineapple plants used for this experiment were obtained from the Rubona tissue culture laboratory, while the macro-propagated plantlets were obtained from a nursery at Rubona. The pineapple cultivar used was *smooth cayenne.* Fertilizer used in the experiment was NPK $_{(17\text{-}17\text{-}17)}$ applied at a rate of20g per plant. The experimental field was weeded regularly to avoid the development of weed using hand and hoe. Watering and mulching were also done at the beginning and during dry season. A ruler was used to measure the plant height, length and width of the longest leaf. Whereas cablesand bundle wires were used to mark plants sample picked randomly.

Experimental design

Randomized Complete Block Design (RCBD) was used to evaluate treatments. Two propagation modes and three spacings were evaluated in a 2x3 factorial experiment with three replications at one location. Propagation modes were micro- and macro-propagated pineapple plantlets coded as T and M respectively; and the three spacings weresymbolized by S1 (60x40cm), S2 (45x30cm) and S3 (30x30cm) respectively. Treatment combinations were TS1, TS2, TS3, MS1, MS2, and MS3; Plot size was 1.8 by 4.4m, space between plots was 1m, and 2m between replicates (Table 2).

Table 2. Field layout of 6 treatments randomized in 18 plots

Replication I		Replication II		Replication III	
1001	004	2007	2010	3013	3016
MS1	TS2	TS3	MS1	MS1	TS2
1002	1005	2008	2011	3014	3017
TS1	MS2	TS1	MS3	TS3	MS3
1003	1006	2009	2012	3015	3018
MS3	TS3	MS2	TS2	MS2	TS1

S: spacingsTS1,2,3 and MS1,2,3: Treatment combination

Parameters measured

Variables used to evaluate growth and development of pineapple plantlets were: Plant height (PHT), number of functional leaves (NOFL), length of longest leaf (LLL), and width of longest leaf (WLL). Data were collected on a monthly basis from 10 randomly selected plants from each plot from December to April. Plant height was measured from the soil surface up to the highest leaf tip by straightening all leaves, width was measured at the widest part of the longest leaf. Leaves counted were considered healthy and more than three quarters of the leaf area was green as opposed to yellow and brown leaves.

Pineapple trial at RAB Rubona station during 2011/2012 Season

Data analysis

Data were analyzed using Microsoft excel (2007) and Genstat discovery software. Analysis of variance were computed for each trait or parameter and the data were summarized as means of each parameter and presented in figures and tables. Means comparison and separation were done using Least significant difference (LSD at 5% level of probability) to identify significant differences among treatments for the parameters measured.

RESULTS AND DISCUSSION

The analyses of variance indicated that at 258th day after transplanting there were no significant ($p>0.05$) differences among micro and macro-propagated pineapple plantlets for plant height and width of the longest leaf; However, significant ($P<0.05$) differences were observed for number of functional leaves and length of longest leaf (Table 3).

Table 3. Mean squares and their significance levels from the ANOVA for four vegetative parameters evaluated in micro- and macro-propagated plantlets at three planting density at Rubona station during 2011/2012 season

		258 th day after transplantation				289 th day after transplantation			
Source variation	df	Plant height (cm)	Number of functional leaves	Length of the longest leaf(cm)	Width of the longest leaf(cm)	Plant height (cm)	Number of functional leaves	Length of the longest leaf(cm)	Width of the longest leaf(cm)
Replication	2	20.0	2.3	14.1	0.3	22.3	0.2	183.4	0.3
Spacing	2	38.9	22.2	84.5	0.4	52.2	5.3	81.4	0.6
Propagation mode	1	7.7	2.4*	3.3*	0.2	1.7*	1.3*	0.6	0.02
Propagation mode× Spacing	2	2.1	1.7	1.2	0.1	1.3	2.1	1.4	0.05
Error	10	9.9	2.4	7.2	0.2	5.7	0.8	6.3	0.15
Total	17	198.5	59.6	193.9	4	160.3	20.6	17.3	3
CV (%)		10.8	9.8	10.1	16.3	7.4	7.6	8.5	12.1

df: degree of freedom*, **: Significant at 5% and 1% levels of probability, respectivelyCV: Coefficient of variation

Table 4. Mean squares and their significance levels from the ANOVA for four vegetative parameters evaluated in micro- and macro-propagated plantlets at three planting density at Rubona station during 2011/2012 season

		318 th day after transplantation				348 th day after transplantation			
Source variation	df	Plant height (cm)	Number of functional leaves	Length of the longest leaf(cm)	Width of the longest leaf(cm)	Plant height (cm)	Number of functional leaves	Length of the longest leaf(cm)	Width of the longest leaf(cm)
Replication	2	35.3	1.2	17.3	0.3	25.7	0.8	7.9	0.2
Spacing	2	102.6	28.3	106.8	1	119.3	18.3	163.8	0.9
Propagation mode	1	2.4*	0.6**	1.6*	0.0032*	4*	0.067*	0.07*	0.11*
Propagation mode× Spacing	2	0.5	1.2	0.1	0.02	3.8	1.4	1.67	0.006
Error	10	14.1	1.2	13.4	0.13	13.8	1.5	9.3	0.07
Total	17	321.2	46.5	279.3	2.8	325.1	38	276.4	2.4
CV (%)		10.5	8.5	11.3	11.2	9.8	9.4	9	8.1

df: degree of freedom*, **: Significant at 5% and 1% levels of probability, respectivelyCV: Coefficient of variation

Table 5. Mean squares and their significance levels from the ANOVA for four vegetative parameters evaluated in micro- and macro-propagated plantlets at three planting density at Rubona station during 2011/2012 season

		379 th day after transplantation			
Source variation	df	Plant height (cm)	Number of functional leaves	Length of the longest leaf(cm)	Width of the longest leaf(cm)
Replication	2	29.6	0.7	13.0	0.2
Spacing	2	98.6	31.2	167.2	0.02
Propagation mode	1	2.2*	0.115**	3.8*	1.1
Propagation mode× Spacing	2	8.4	0.08	7.0	1.2
Error	10	17.7	0.7	18.4	1.0
Total	17	356.6	40.5	399.5	15.7
CV (%)		10.5	6.5	11.8	27.9

df: degree of freedom*, **: Significant at 5% and 1% levels of probability, respectivelyCV: Coefficient of variation

Table 6. Means of four vegetative parameters evaluated in micro- and macro-propagated pineapple at Rubona station at 258th day after transplantation during 2011/2012 season

Propagation mode	Plant height (cm)	Number of functional leaves	Length of the longest leaf(cm)	Width of the longest leaf(cm)
Micro-propagated pineapple	27.7	17.1	24.3	2.7
Macro-propagated pineapple	30.6	14.9	28.7	3.0
LSD (at 5% level)	NS	1.6	2.8	NS
CV%	10.8	9.8	10.1	16.3

CV: Coefficient of variation NS: no significance at 5% level of probabilityLSD: Least significant difference

Table 7. Means of four vegetative parameters evaluated in micro- and macro-propagated pineapple at Rubona station at 289th day after transplantation during 2011/2012 season

Propagation mode	Plant height(cm)	Number of functional leaves	Length of the longest leaf(cm)	Width of the longest leaf(cm)
Micro-propagated pineapple	30.6	12.4	27.5	3.0
Macro-propagated pineapple	34.0	11.3	31.8	3.4
LSD (at 5% level)	2.52	0.941	NS	NS
CV%	7.4	7.6	8.5	12.1

CV: Coefficient of variation NS: no significance at 5% level of probabilityLSD: Least significant difference

Table 8. Means of four vegetative parameters evaluated in micro- and macro-propagated pineapple at Rubona station at 318th day after transplantation during 2011/2012 season

Propagation mode	Plant height(cm)	Number of functional leaves	Length of the longest leaf(cm)	Width of the longest leaf(cm)
Micro-propagated pineapple	33.6	14.1	29.9	2.9
Macro-propagated pineapple	38.4	11.6	34.8	3.5
LSD (at 5% level)	3.9	1.1	3.8	0.4
CV%	10.5	8.5	11.3	11.2

CV: Coefficient of variation LSD: Least significant difference

Table 9. Means of four vegetative parameters evaluated in micro- and macro-propagated pineapple at Rubona station at 348th day after transplantation during 2011/2012 season

Propagation mode	Plant height(cm)	Number of functional leaves	Length of the longest leaf(cm)	Width of the longest leaf(cm)
Micro-propagated pineapple	35.4	14.1	30.9	3.1
Macro-propagated pineapple	40.5	12	37.0	3.5
LSD (at 5% level)	3.9	1.3	3.2	0.3
CV%	9.8	9.4	9	8.1

CV: Coefficient of variation LSD: Least significant difference

Table 10. Means of four vegetative parameters evaluated in micro- and macro-propagated pineapple at Rubona station at 379th day after transplantation during 2011/2012 season

Propagation mode	Plant height(cm)	Number of functional leaves	Length of the longest leaf(cm)	Width of the longest leaf(cm)
Micro-propagated pineapple	37.9	14.7	33.3	3.7
Macro-propagated pineapple	42.6	12.1	39.4	3.7
LSD (at 5% level)	4.4	0.9	4.51	NS
CV%	10.5	6.5	11.8	27.9

CV: Coefficient of variation LSD: Least significant difference

Table 11. Means of four vegetative parameters measured in the evaluation of spacing effect on micro- and macro-propagated pineapple at Rubona station at 258th and 289th day after transplantation during 2011/2012 season

	258 th day after transplantation				289 th day after transplantation			
Spacing	Plant height(cm)	Number of functional leaves	Length of the longest leaf(cm)	Width of the longest leaf(cm)	Plant height(cm)	Number of functional leaves	Length of the longest leaf(cm)	Width of the longest leaf(cm)
S1(60x40cm)	27.9	15.3	25.7	2.7	32.3	12.1	29.7	3.2
S2(45x30cm)	29.4	16.6	26.6	2.8	31.8	12.1	29.3	3.2
S3(30x30cm)	30.1	16.0	27.2	3.1	32.8	11.3	30	3.3
LSD (at 5% level)	NS	NS	NS	NS	NS	NS	NS	NS
CV%	10.8	9.8	10.1	16.3	7.4	7.6	8.5	12.1

LSD: Least significant differenceCV: Coefficient of variation NS: No significant difference at 5% level of probabilityS1,2,3: Spacing

Table 12. Means of four vegetative parameters measured in the evaluation of spacing effect on micro- and macro-propagated pineapple at Rubona station at 318th and 348th day after transplantation during 2011/2012 season

	318th day after transplantation				348th day after transplantation			
Spacing	Plant height(cm)	Number of functional leaves	Length of the longest leaf(cm)	Width of the longest leaf(cm)	Plant height(cm)	Number of functional leaves	Length of the longest leaf(cm)	Width of the longest leaf(cm)
S1(60x40cm)	36.7	12.6	31.9	3.2	37.0	12.9	34	3.1
S2(45x30cm)	35.4	13.2	32.2	3.2	38.3	13.2	33.9	3.4
S3(30x30cm)	35.9	12.8	33	3.2	38.5	13.0	34.1	3.4
LSD (at 5% level)	NS	NS	NS	NS	NS	NS	NS	NS
CV%	10.5	8.5	11.3	11.2	9.8	9.4	9	8.1

LSD: Least significant differenceCV: Coefficient of variation NS: No significant difference at 5% level of probabilityS1,2,3: Spacing

Table 13. Means of four vegetative parameters measured in the evaluation of spacing effect on micro- and macro-propagated pineapple at Rubona station at 379th day after transplantation during 2011/2012 season

	379 th day after transplantation			
Spacing	Plant height(cm)	Number of functional leaves	Length of the longest leaf(cm)	Width of the longest leaf(cm)
S1(60x40cm)	39.7	13.3	35.5	3.3
S2(45x30cm)	40.2	13.5	37	4.2
S3(30x30cm)	40.9	13.3	36.6	3.5
LSD (at 5% level)	NS	NS	NS	NS
CV%	10.5	6.5	11.8	27.9

LSD: Least significant differenceCV: Coefficient of variation NS: No significant difference at 5% level of probabilityS1,2,3: Spacing

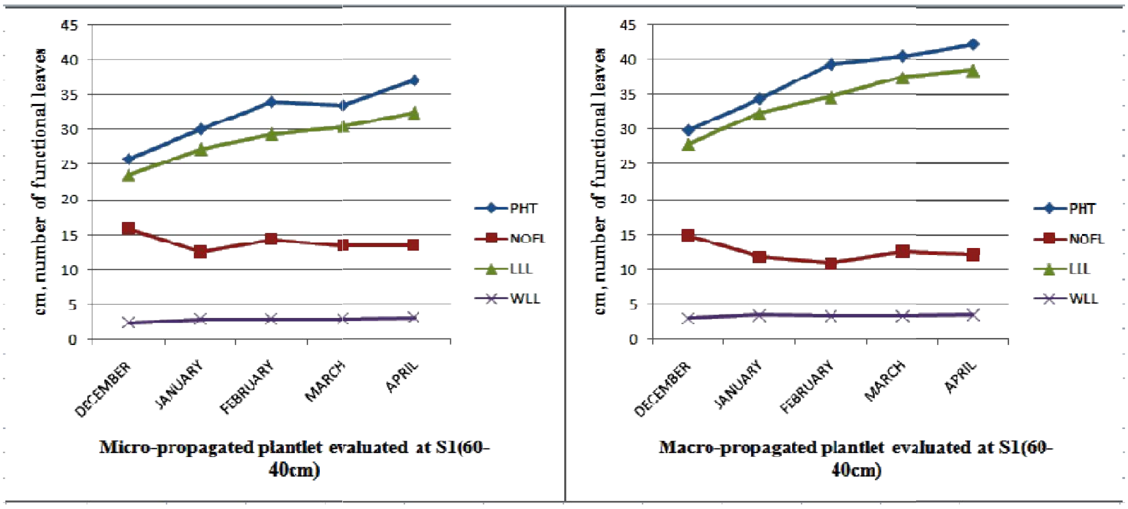

Figure 1. Variation of plant height, number of functional leaves, length and width of the longest leaf in micro- and macro-propagated pineapple plantlets evaluated at S1 (60x40 cm) planting density from December 2011 to April 2012 at RAB Rubona station

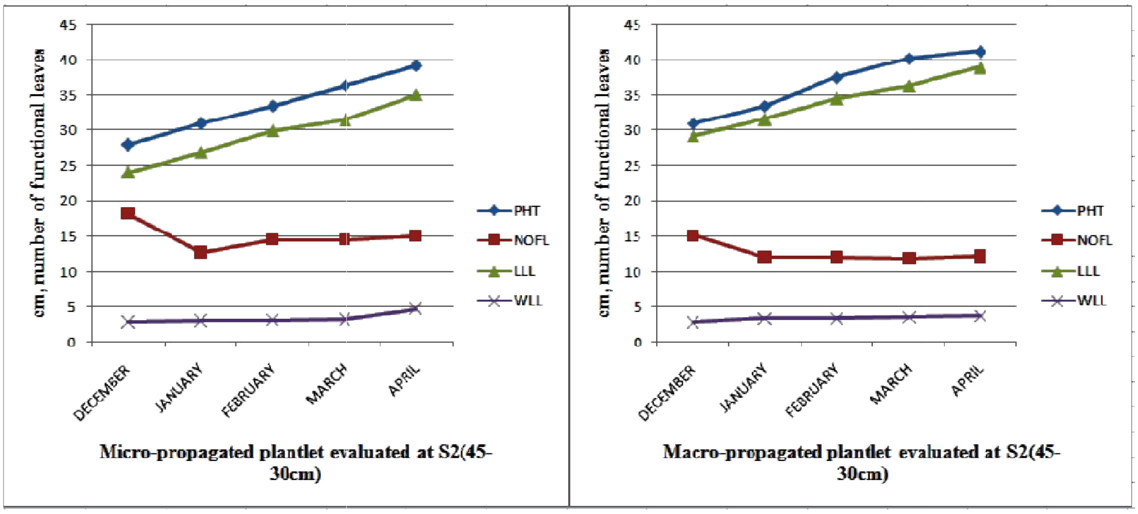

Figure 2. Variation of plant height, number of functional leaves, length and width of the longest leaf in micro- and macro-propagated pineapple plantlets evaluated at S2 (45x30 cm) planting density from December 2011 to April 2012 at RAB Rubona station

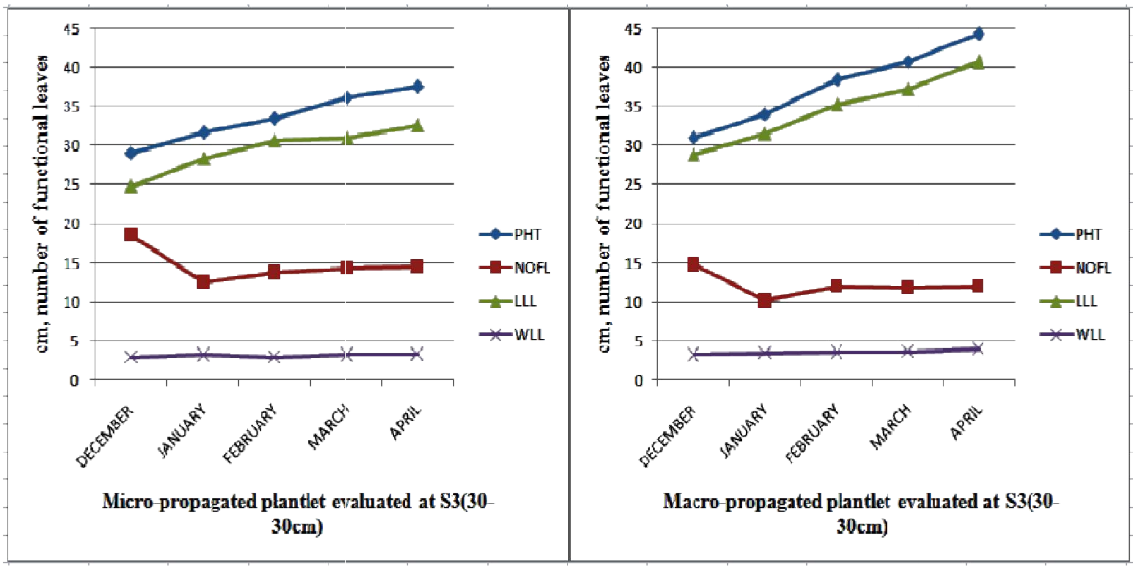

Figure 3. Variation of plant height, number of functional leaves, length and width of the longest leaf in micro- and macro-propagated pineapple plantlets evaluated at S3 (30x30 cm) planting density from December 2011 to April 2012 at RAB Rubona station

However, micro-propagated had more number of functional leaves (17) than macro-propagated pineapple plantlets while macro-propagated plantlets were found to have longer leaves (28.7cm) than micro-propagated pineapple at 258th day after transplanting (Table 6). Though, Munyaneza (2011) and Bartholomew *et al.*(2003), reported that macro-propagated pineapple plantlets were found to perform better than micro-propagated at early stage after transplanting. Furthermore, at 289th days after transplanting, analyses of variance revealed that there were significant ($p<0.05$) differences among the micro and macro-propagated plantlets for plant height and number of functional leaves (Table 3). Thus from laboratory to experimental field, micro-propagated pineapple plantlets were exposed to harsh environmental condition while macro-propagated were already adapted to field environmental condition this is why at early stage after transplanting macro-propagated were likely to perform better than micro-propagated pineapple. Similar results were obtained by Martha *et al.* (2011) and Nguyen *et al*. (2010), for banana production. Macro-propagated pineapple height was 34cm while micro-propagated had 30.6cm (Table 7). However, there were no significant ($P>0.05$) differences for length and width of longest leaf at 289th day after transplanting (Table 3). The analyses of variance indicated that at 318th day after transplanting, there were highly significant ($p<0.001$) differences among the micro and macro-propagated pineapple for number of functional leaves (Table 4). Pineapples plantlets produced from tissue culture technology were found to have more numbers of functional leaves than macro-propagated pineapple (14.1and11.6 leaves respectively) (Table 8). Significant ($P<0.05$) differences were found for plant height, length and width of longest leaf at 318th and 348th day after transplanting (Table 4). Micro-propagated were found to have more number of functional leaves while macro-propagated pineapple plantlets had taller plant height, longer length and wider width of the longest leaf (Table 8 and 9).

At 379th day after transplanting, significant ($p<0.05$) differences were observed among micro and macro-propagated pineapple for plant height, length of the longest leaf however highly significant ($P<0.001$) differences were found for number of functional leaves; However, no significant ($P>0.05$) differences were observed for width of the longest leaf (Table 5). Taller plant height was found in macro-propagated pineapple (42.6cm) while micro-propagated had more number of functional leaves (14.7) (Table 10). At early stage after transplanting, macro-propagated plantlets perform better than micro-propagated plantlets; but at full vegetative development micro- and macro-propagated plant have no significant difference in their growth and development. Therefore, tissue culture technology can be used in harsh environment and for intensive cropping at initial stage where there is a limited supply of vegetative propagation materials (Turinzwenayo, 2012). Differences observed among planting densities were not significant ($p>0.05$) neither for plant height, number of functional leaves nor for the length and width of the longest leaf at 258th, 289th ,318th ,349th and 379th day after transplanting (Table 3, 4, 5). Nguyen *et al.* (2010), reported that increasing planting density tended to increase plant height. Spacing of 30cm between rows and 30cm among pineapple plants produced 55,500 plants per hectare. According to Lacoeuilhe (1974), cited by NARI, (2010), a farmer can produce a maximum of pineapple per hectare (77,000/ ha) if he uses 20cm spacing between plants and 40cm spacing among rows. The difference in number of plants produced per hectare is obvious because high planting density increase number of plants produced per hectare. The analyses of variance indicated that from 258thto 379th day after transplanting, there were no significant ($p>0.05$) differences among propagation modes and planting density interactions for plant height, number of functional leaves, length and width of the longest leaf (Table 3, 4, 5). Consequently, the start of vegetative growth, statistical analysis also indicated that number of functional leaves decreased, while other vegetative parameters increased linearly from 258th to 379th day after transplanting for both micro- and macro-propagated pineapple plantlets (Figure 1, 2, 3). In this study, it was observed that number of functional leaves decreased from 258th to379th for both micro and macro-propagated pineapple.

Conclusions and Recommendations

In general, taller plants heights were observed in both micro- and macro-propagated pineapple plantlets at a spacing of 30x30cm (55,500 plants/ha). Micro-propagated pineapple plantlets were found to have more number of functional leaves than macro-propagated ones. Plant vigor was almost the same in micro- and macro-propagated pineapple plantlets; plant height, length and width of the longest leaf increased linearly from 258th to379th day after transplanting, while number of functional leaves decreased from earlier stage after transplanting up to 12 months after transplanting. Therefore, Macro-propagated pineapple plantlets should be used as planting material after further evaluation of planting density effect on growth and development of micro-and macro-propagated pineapple. For the fact that pineapple (*Ananas comosus* L. Merr) is a perennial crop this research should be repeated in different agro-ecological zones in order to confirm these results and formulate more valid conclusions. This research should also be continued until the harvesting time when it will be possible to determine the best performing plantlets on growth and yield.

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