Evaluation of Leaf Bud Cuttings from Different Sized Crowns for Rapid Propagation of Pineapple (Ananas Comosus L. [Merr.])

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ABSTRACT
Lack of planting material is one of the constraints of pineapple production. The work aimed at maximizing the number of plantlets by using rapid multiplication method of pineapple (*Ananas comosus* L. [Merr.]) var. 'Smooth Cayenne' using crown leaf bud cuttings was conducted for one year in Bonga under a plastic shade. Effect of size of crowns were evaluated on regeneration and propagation potential of shoots. 'Smooth Cayenne' pineapple cultivar which has less yearly propagation ability was used for the experiment. The experiment consisted leaf bud cuttings from small, medium and large sized crown. Each green leaf of the crown was carefully removed along with a small piece of the stem just under the bud and the remaining top was split vertically into four parts. The cuttings were dipped in a sodium hypochlorite solution for two minutes and then rinsed with sterile tap water followed by a fungicide dip and then transferred to planting pots filled with top soil. The design was CRD. The result revealed that size of crown had a significant effect on plantlet production, number of sprouted cuttings, plantlet height, plantlet leaf length, leaf width and plantlet diameter after four months of propagation time of the cuttings. Similarly, after one year of growing time; crowns size had significant effect on plantlet number, plantlet height and plantlet leaf length. Plantlet production was increased (1:32.6.) by using leaf bud cuttings from medium sized crown. A higher (33) mean value of sprouted cuttings were obtained by using medium sized crown leaf bud cuttings. Higher mean value of plantlet height, leaf length, leaf width and diameter was obtained by using medium sized crown leaf bud cuttings. After one year of growing time, equal and higher (32.4) number of plantlets were recorded from large and medium sized crown leaf bud cuttings. Generally, significant effect was not observed on survival status of the cuttings at both propagation periods. Using of medium and large sized crowns resulted in better plantlet growth and vigor. Therefore, based on the overall result of the experiment 'Smooth Cayenne' can be propagated rapidly by using leaf bud cuttings from medium-sized crown.
Key words: Smooth Cayenne, crown, leaf bud, cuttings, propagation, rapid, regeneration

Background and Justification
Pineapple (*Ananas comosus* L. [Merr.]) is a perennial herb in the botanical family *Bromeliaceae* and native to the American tropics (Evans *et al.*, 2002). It is the second crop of importance after bananas, contributing to over 20 % of the world production of tropical fruits. Nearly 70% of the pineapple is consumed as fresh fruit in producing countries (Medina and Garcia, 2008). Globally, pineapples in both fresh and processed forms are readily available (David, 2006; Pua and Davey, 2007). According to EBDSN (2006), in Ethiopia fresh pineapple is available in market and the Red Spanish variety is good for selling fresh. In addition to this, MD2 and Smooth Cayenne varieties are currently popular in the market even though different varieties are emerging from time to time.

Pineapples are propagated vegetatively by using shoots, which are four general types: slips that arise from the stalk below the fruit, suckers that originate at the axils or leaves, crowns that grow from the top of the fruits, and ratoons that come out from the under-ground portions of the stems (CRFGI, 1996).

Lack of planting material is one of the constraints of pineapple production in the world and Ethiopia (Bartholomew, 2007). This is because the rate of propagation is slow in conventional propagation. 'Smooth Cayenne' typically provides only two propagules per plant per year hence it would take 20 years to plant one hectare from a single plant.

Shortage of planting material, production and distribution of pineapple is small and limited in many places. In special consideration of variety Smooth Cayenne, due to its significance in production and market profit farmers are seeking its planting material for production. Because the variety conventional multiplication rate is not enough to fulfill farmers demand, there is requirement for rapid multiplication of planting materials. Therefore, looking for alternative propagation methods that leads to better multiplication rate and that can be used by smallholders will have a practical importance in addressing pineapple production constraints. As a result, this project is proposed with the following objectives:

Objectives

- To develop a rapid multiplication method for pineapple using crown and stem cuttings.
- To investigate the effect of size of crown on regeneration and propagation potential of shoots.

Materials and Methods

The activity was conducted in Gimbo district in Ethiopia, on Bonga Agricultural Research center experimental site. Situated at latitude of 7^{o}19'N, longitude of 36^{o} 13'and has an altitude of 1733 m.a.s.l. It receives average annual rainfall of 2500mm, with mean maximum and minimum temperature of 35 and 20^{o}C, respectively. Pineapple cultivar "Smooth Cayenne" was used and propagation materials were obtained from pineapple orchard of Gojeb Agricultural Development Farm. The harvested crowns were sorted as small, medium and large, based on their weight diameter and length using beam balance, caliper and ruler, respectively. The design of the experiment was CRD. There were three treatments of leaf bud cutting on small, medium and large sized crowns and each of them had five crowns. The crown sizes: small sized crown (75 to 125g weight, 3 to 3.9cm diameter and 8 to 12cm length), medium (125 to 175g weight, 4 to 4.9cm diameter and 12 to 16cm length) and large (175 to 225g weight, 5 to 6cm diameter and 16 to 20cm length) were used. Materials were assigned to each size at least two of the three criteria are met. The average weight of the leaf bud cuttings taken from small, medium and large sized crowns was 3.9, 5.8 and 6.1g, respectively. Similarly, length was 4.5, 5.3 and 5.6 for small, medium and large sized crowns, respectively.

Experimental Procedure

Well-fruited crowns (fruit having single crown) were prepared after harvest of the fruit. Each crown was carefully removed from the fruits. Bottom leaves of the selected crowns were trimmed. Then they were cured in the sun for two days in upside down position. Crowns were brought to sterilized environment in laboratory with clean bags. Cutting knives were sterilized before each cutting process by alcohol (70% ethanol) and flame. The base of a crown and dry leaves were removed. Then, each green leaf of the crown was carefully removed along with a small piece of the stem just under the bud and the remaining top was split vertically into four parts. Each of the crown cuttings were tied with plastic rope prior to next treatment.

Treatment of cuttings

The cuttings were dipped in a sodium hypochlorite solution at a rate of 25ml l^{-1} for two minutes and then rinsed three times with sterile tap water followed by a fungicide dip (Redomil) as per Soler and Dole (2006) at a rate of 3g l^{-1}. Finally the treated crown leaf bud cuttings were packed with paper bag and transferred to the shade where planting was conducted.

Planting and management of cuttings

The experiment was conducted in field nursery under plastic (yellow) shade having 3mx2mx15m width, height and length, respectively. Planting medium was prepared from locally collected top soil. After disinfection, cuttings from each crown were randomly assigned to planting pots and planted in pots containing moistened soil. Crown leaf bud cuttings were planted as usual with ¼ of bottom part of the leaf buried in the soil and the remaining top was above soil with a spacing of 5x5cm apart in 25x35cm sheet pot. Manual watering was used when necessary with small sprinklers to maintain the soil wet.

Plantlets were emerged and left in the original pot until ready to be transferred to individual pots. After four months after planting (MAP) or plantlets emerged and grown, they were carefully detached from the mother plants and transplanted. All plantlets were grown separately in polyethylene bags that have a size of 10cm diameter 18cm length. A planting medium mixture was moistened top soil. The polyethylene bags were left under the shade and irrigated when it was necessary. Light fertilization of nitrogen and phosphorus was applied for rapid growth at two weeks interval as per Soler and Dole (2006), through mist at a rate of 20:20 (28.35g of fertilizer per 11.4 liter of water) as per Acquaah (2004).

Data Collection and Analysis

Data on Timing of shoot formation, Regeneration percentage (Number of cuttings that regenerated shoots), Number of plantlets produced, Propagation efficiency (plant/g), Number of leaves, Plantlet height, Leaf length, Leaf width, Plantlet diameter, Survival rate of plantlets were taken at four months and one year of propagation time. Treatment means of all the above parameters were analyzed (ANOVA) based on standard methods for CRD using computer software (SAS version 9.00) (SAS, 2002). Data of experiment were analyzed and presented on shoot regeneration capacity, and vigor and growth of regenerated shoots. Means were compared using Tukey's Honestly Significant Difference (HSD) test at a 5% probability level.

Results and Discussions

Time of sprouting

Leaf bud cuttings from large-sized crowns achieved 10 and 50% sprouting at 61 and 83.0 days after planting (DAP). Sprouting of small-sized crowns was completed at 61.4 and 88.4 days of planting. Leaf bud cuttings from medium sized achieved 10 and 50% sprouting at 61.6 and 88.2 DAP (Table 1). Based on the result, earliness was observed on large sized crowns; especially 50% emergence was 5.2 days earlier than the rest. Earliness of plantlets emergence of from large-sized crown leaf bud cuttings could be due to better food reserve in the cuttings. According to Morton (1987) and DHAOGTR (2008), size of planting material has effect on early response of the propagules. In addition to this an experiment conducted in Awada and reported by Addis (2012) showed that, plantlet emergence was earlier than this experiment. The result revealed that, in average 10 and 50% emergence was completed between 56 and 69 DAP. The current experiment result showed that, 10 and 50% emergence was completed within 61.33 and 86.53 DAP.

Table 1. Days to 10 and 50 % sprouting of leaf bud cuttings

Treatments	Percent of sprouting of plantlets in days	
	10%	50%
Small	61.4	88.4
Medium	61.6	88.2
Large	61.0	83.0

Shoot Regeneration capacity

Results of four MAP ANOVA showed that crown size had significant ($P < 0.05$) effect on mean number of cuttings emerged, total number of plantlet per crown. But regeneration percentage was not significantly ($P > 0.05$) affected by crown size. Similarly, mean number of plantlets per crown was also significantly affected by crown size after one year of growing time (Table 2).

Fig. Shoot production of leaf bud cuttings at four months after planting a, Medium sized crown cuttings b, Different size leaf bud cuttings

Emergency of leaf bud cuttings was significantly affected by crown size, cuttings from medium sized crown have got the highest (33.00) regeneration ability, although it was not significantly different from cuttings of large sized crown which has scored 31.40. Crown size had no significant effect on regeneration percentage of the cuttings and it coincides with the result of a similar experiment conducted and reported by Addis (2012). Small-sized crowns had the highest (88.23%) regeneration percentage followed by those from large (88.03%) crowns (Table 2). Leaf bud cuttings from medium-sized crown had the lowest sprouting percentage (87.78%). Even though there was no significant difference between crown sizes, higher regeneration percentage was recorded in 2012 trial. Morton (1987) and Punjab National Bank (2007) reported that 95% success in propagation is possible in a similar situation. The difference might be due to the planting medium of the propagules, because in the previous experiment sand was used as a medium instead of top soil.

After four MAP time leaf bud cuttings obtained from medium, large and small sized crowns produced mean number of 32.6, 32.4 and 23.4 plantlets, respectively (Table 1). The results are in accordance with the findings of Morton (1987) and Punjab National Bank (2007), on which one crown could give up to 20 plantlets. However, the number of plantlets produced from a single mother plant increased in this experiment. In a similar experimental setup, cuttings obtained from medium, large and small sized crowns produced mean number of plantlets 22, 21.8 and 13.2, respectively (Addis, 2012). In the current experiment, in average 10.6 more plantlets obtained from medium and large crown cuttings. Similarly, the least plantlet production was obtained from small sized crown cuttings, but it is still better than the previous one in making of plantlets. In average, a 10.2 plantlet advantage is recorded from small sized crown cuttings and it is better than the previous highest number of plantlet production by 1.4 plantlets. After one year of growing time, medium and large sized crowns showed higher number of plantlet production capability by producing 32.4 plantlets, independently. Whereas treatment of small sized crowns have produced 23.0 plantlets.

Although there was no significant difference, cuttings of crown sizes responded differently on regeneration ability. Especially, medium sized crown showed highest (8.36 plants/gram) propagation efficiency. The least (6.33 plants/gram) propagation efficiency was recorded on small sized crowns. The result is in accordance with a research result reported by Addis (2012). Based on the result of the experiments, significant number of plantlet production by large sized cuttings is explained by the increase in size of propagules used. According to DHAOGTR (2008), there is relationship between size of propagules used and leaf length, mass and number of pantlets produced. Those two treatments (medium and large sized crowns) have relatively large number of leaves and enough resource for the sprouting and growth of plantlets. Therefore, better sprouting and growth of plantlets was recorded from them than the small one.

Table 2. Comparisons of means of different sized crown leaf bud cutting effect on parameters measured at four MAP

Treatments	MNCE	RP	MNPC	PE
Small crown	24.00b	88.23	23.4b	6.33
Medium crown	33.00a	87.78	32.6a	8.36
Large crown	31.40ab	88.03	32.4a	6.79
HSD	8.05*	NS	7.756*	NS
CV %	16.19	5.16	15.6	31.73

MNCE=Mean Number of Cuttings Emerged, RP = Regeneration Percentage, MNPC = Mean Number of Plantlets per Crown, PE = Propagation Efficiency (plants/g), NS = Non Significant. * = Significant at 5% probability level

Vigor and growth of regenerated shoots

After one year of growing time, plantlet mean leaf number was not significantly ($P > 0.05$) affected by crown sizes. After four months of propagation time, plantlets of medium and large sized crowns gave 10.48 and 10.20 mean leaf number per plant, respectively. Plantlets from small sized crown leaf bud cuttings produce 9.24 leafs. Cuttings of medium, large and small sized crowns gave 16.32, 15.76 and 15.64 average leaf number after one year of propagation time, respectively.

Significant effect ($P < 0.05$) obtained on mean plantlet height, leaf length, leaf width, and plantlet diameter at four MAP (Table 3). Similarly, after one year of propagation time size of crown had significant effect on plantlet height and leaf length. However there was no significant ($P > 0.05$) difference on plantlet leaf width and diameter; and survival status of plantlets in polyethylene bags.

Leaf bud cuttings obtained from medium sized crowns produced significantly taller plantlets (7.96cm) than those from large and small sized crowns which have produced significantly shorter plantlets (15.76 and 15.64cm), respectively. In both data collection times, leaf bud cuttings obtained from medium and large sized crown produced plantlets which are significantly differ in height from small sized crown plantlets. According to DHAOGTR (2008) large planting materials produce larger plants, and it is in accordance with the findings of this experiment (Table 3).

Significantly ($P < 0.05$) longer (7.28cm) leaf was obtained from medium sized crown platelets at four MAP. During this time significantly shorter (5.70 and 5.36cm) leaf was recorded in large and small sized crown plantlets, respectively. In the next data collection time plantlets of large sized crown beat all but it (19.88cm) was not significantly different from medium sized one (18.72cm). Small sized crown plantlets scored significantly shorter (15.56cm) leaf length. Plantlet leaf width was significantly affected by the size of crowns at four MAP, but it was not observed at one year of growing time. In the first data collection time medium and large sized crowns produce plantlets with significantly wider (1.39 and 1.23cm) leaf width, respectively. With the same mean difference with large sized crowns, small sized crown plantlets gave 1.16cm leaf width. Plantlets obtained from medium sized crowns showed significantly highest (1.23cm) plantlet diameter. With the same mean difference, the next better plant diameter (1.14cm) was obtained from a large sized crown which is not statistically different (1.02cm) from small sized crowns. The wider diameter of plantlets produced by leaf bud cuttings of medium and large crown sizes can be related with the difference created by size of crown and thus to the size of leaf bud cuttings obtained from these crowns (DHAOGTR, 2008).

During the first data recording time the regeneration percentage of leaf bud cuttings of all sized crowns was not affected by the size of the propagule used. In contrast cuttings of small sized crown have shown a better survival status, even though it was not statistically different from others. Similarly, after transferring and growth of plantlets in polyethylene bags, significant difference was not observed on survival status of plantlets. Here, highest (99.34%) survival status of plantlet was recorded from large sized crown plantlets. The rest two have shown the second (99.32%) and third (98.94%) percentage according to the size of the propagules used. Unlike from the survival status of the cuttings, plantlets of large sized propagules have survived better than those from small sized ones (Table 3).

Table 3. Comparisons of means of different sized crown leaf bud cutting effect on parameters measured at four MAP and one year

Treatments	MNPC	LN	PH	LL	LW	PD	SRP
3 MAP							
Small	23.40b	9.24	5.89b	5.36b	1.16b	1.02b	
Medium	32.60a	10.48	7.96a	7.28a	1.39a	1.23a	
Large	32.40a	10.20	6.47b	5.70b	1.23ab	1.14ab	
HSD	7.756*	NS	1.3*	1.267*	0.20*	0.18*	
CV %	15.6	7.72	11.46	12.28	9.46	9.75	
One year							
Small	23.0b	15.64	18.68b	15.56b	1.68	2.08	98.94
Medium	32.4a	16.32	22.60a	18.72a	1.78	2.11	99.32
Large	32.4a	15.76	23.96a	19.88a	1.88	2.19	99.34
HSD	7.73*	NS	2.58*	2.63*	NS	NS	NS
CV %	15.67	5.60	7.05	8.65	10.35	5.68	1.85

MNPC=Mean Number of Plantlets per Crown, LN=Leaf Number, PH=Plantlet Height (cm), LL=Leaf length (cm), LW=Leaf Width (cm), PD=Plantlet Diameter (cm),
SRP=Survival Rate of plantlets (%),* = Significant at 5% probability level, NS = non significant. Values with the same letter are not significantly different.

Analysis results of Pearson correlation coefficient showed that there was strong positive relationship (0.96934) between plantlet number and number of live cuttings which gave plantlets (Mean Number of Cuttings emerged). The presence of live buds stimulates growth and production of plantlets. Plantlet production and emergence of cuttings are strongly related with propagation efficiency of the cuttings. Propagation efficiency of the cuttings was positively related with the presence of enough amount of reserve to produce large number of plantlets. Strong relationship was also observed between plant height and leaf number. Plantlet height was associated positively with the presence of many leaves. Leaf number (0.67367) and plantlet height (0.98366) also correlated with leaf length. The presence of many leaves was associated with increased leaf length; moreover leaf length was strongly affected by plantlet height. Similarly, leaf width also positively correlated with leaf number, plantlet height and leaf length, specifically leaf width is strongly associated with the presence of longer plantlets and their leaves. Generally, plantlet diameter was positively related with shoot vigor. Especially, the increase in number of leaves of a plantlet was positively related (0.86696) with the production of plantlets having wider plantlet diameter. According to DHAOGTR (2008) large planting materials produce larger plants. Therefore, the increase in plantlet height and diameter could be due to the formation of more photosynthetic organ resulting from larger planting materials used.

Table 4. Pearson Correlation Coefficients of parameters of crown leaf bud cuttings at four MAP

	MNPC	MNCE	PE	RP	LN	PH	LL	LW	PD
MNPC	1	0.96934*	0.65654*	0.09995	0.30935	0.31551	0.27945	0.35269	0.32355
MNCE		1	0.69819*	0.06429	0.28285	0.34813	0.33300	0.33141	0.34054
PE			1	0.00095	0.26075	0.12947	0.18109	0.27233	0.37565
RP				1	-0.05033	0.03032	-0.08186	-0.07328	-0.24324
LN					1	0.70354*	0.67367*	0.66873*	0.86696*
PH						1	0.98366*	0.84124*	0.70311*
LL							1	0.86579*	0.72017*
LW								1	0.77448*
PD									1

MNPC = Mean Number of Plantlets per Crown, MNCE=Mean Number of Cuttings Emerged, PE = Propagation Efficiency, RP = Regeneration Percentage. LN = Leaf Number, PH = Plantlet Height, LL = Leaf length, LW = Leaf Width, PD = Plantlet Diameter, * = significant at P=0.05.

Conclusion and Recommendation

Leaf bud cuttings of different sized of crowns were evaluated to observe multiplication rate of pineapple variety - Smooth Cayenne which is conventionally gives only one shoot per crown basis. With the techniques of detaching buds with small sized stem part of crown with leaf, increased propagation capacity of pineapple in reasonable state.

Based on the sizes of crown used, there was a significant increase in plantlet production, number of sprouted cuttings, plantlet height, leaf length, leaf width and plantlet diameter after four months of growing time. After one year of propagation time, plantlet number, height and leaf length had significantly affected by the size of crowns used. Plantlet production was increased (1:32.6.) by using leaf bud cuttings from medium sized crown. After one year of propagation time, equal and higher number (32.4) of plantlets was recorded from large and medium sized crown leaf bud cuttings. Generally, crown size does not have significant effect on survival status of cuttings and plantlets. Cuttings of medium and large sized crowns showed better plantlet growth and vigor.

With the ever-increasing demand of planting materials of pineapple especially Smooth Cayenne variety at small scale level, looking for alternative propagation methods that leads to better multiplication rate and that can be used by smallholders have a practical importance. This experimental result showed that propagation capacity of pineapple was increased within one year of propagation time. Better propagation rate and efficiency of propagules were identified; however there might be gaps that have to be clear further. Reports suggested that large number of plantlet could be obtained, therefore more works need to be done to fill the gap. Based on the overall result obtained from the experiment, 'Smooth Cayenne' can be propagated rapidly by using leaf bud cuttings from medium-sized crown.

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