



UNIVERSITI PUTRA MALAYSIA

**STERILIZATION TREATMENT AND PLANTLET REGENERATION
OF GUAVA (*PSIDIUM GUAJAVA* L. VAH. BEAUMONT)**

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OF GUAVA (*PSIDIUM GUAJAVA* L. VAR. BEAUMONT)**

By

TAJUL AFIF ABDULLAH

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December 2007

Chairman : Associate Professor Maheran bt. Abdul Aziz, PhD

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This study was carried out to establish an efficient regeneration protocol for mass propagation of *Psidium guajava* L. var. Beaumont planting materials and as a system for recovery of transgenic plants in the improvement programme of the crop through genetic engineering. Aseptic study on grafted nodal explant of *Psidium guajava* L. var. Beaumont taken from the field was carried out to establish an efficient sterilization protocol. It was found that Treatment 5 was most efficient in producing the highest percentage of explant survival and the lowest percentage of bacterial and fungal contamination. In Treatment 5 nodal explants were soaked in 0.5% Benlate for 1 hour and placed under running tap water plus Tween 20 for 1 hour. Next, explants were placed in beaker and rinsed with 20% Clorox plus Tween 20 for 5 minutes before it were rinse with 0.05% AgNO₃ and 0.05% HgCl₂ both for 1-2 minutes. The explants were then washed



with sterile distilled water (3 or 4 times) in order to remove the sterilizing agents used. Then the explants were soaked in liquid MS medium supplemented with 2 mg/L BAP for overnight. Next morning, the explants were blotted dry before it were cultured on MS medium plus 1 mg/L BAP and 25 mg/L ascorbic acid. However, the percentage of phenolic browning of explant was high in this treatment. To reduce percentage of phenolic browning, it is suggested if Treatment 5 are added by dipping the explant into antioxidant solution (ascorbic acid 100 mg/L and citric acid 150 mg/L) before treating it with 50g/L Calcium hypochlorite (10-40 minutes and washed 5 times with sterile distilled water. It is also suggested for Treatment 5 that the explants are cultured onto media containing activated charcoal for a few days before it is transfer to MS medium + 1 mg/L BAP + 25 mg/L ascorbic acid.

The study on shoot regeneration of *in vitro* seedling derived explants showed responses of *Psidium guajava* L. var. Beaumont to the growth regulators BAP and kinetin. In the experiment on the effect of BAP on shoot regeneration from shoot tip explant, the highest mean number of shoots proliferated (2.33) was at 1.0 mg/L BAP while the highest shoot height attained (5.56 mm) was at 0.5 mg/L BAP. By using nodal explant, the highest mean number of shoots proliferated (1.87) was also at 1.0 mg/L BAP while the highest shoot length attained (1.60mm) was at 0.5 mg/L and 1.0 mg/L BAP. It was also observed that for nodal explants MS basal medium without BAP produced significantly lower percentage of shoot formation compared to most of the other treatments containing BAP. On young leaf explants, BAP concentration at 2.0 mg/L

produced the highest percentage of explant survival (96.67%) while 1.0 mg/L BAP produced the highest percentage of explant with callus formation (70%). In the study on the effect of kinetin on shoot regeneration from shoot tip explant, MS basal medium produced the highest percentage of shoot formation (93.33%) and mean number of shoots per explant (0.93), while medium with 0.1 mg/L kinetin produced the highest shoot height of 1.45 mm. By using nodal explant, the highest percentage of shoot formation (36.67%) and number of shoots per explant (0.47) were attained at 0.2mg/L kinetin. For shoot regeneration from young leaf explant, kinetin at 0.5mg/L produced the highest percentage of explant survival (76.67%). It is suggested that treatments supplemented with 1.0 and 2.0mg/L BAP are combined with auxin such as 2,4-D and/or NAA to increase the percentage of young leaf explants to form callus. On comparing the effect of two levels of BAP (0.5 mg/L and 1.0 mg/L) on shoot regeneration from shoot tip explant using T-test, 0.5 mg/L BAP produced higher shoot height at each subculture compared to 1.0mg/L BAP except in the second subculture, while treatment 1.0 mg/L BAP produced also higher mean number of shoots per explant at each per subculture. However, the differences are non significant. It is suggested for studies on shoot proliferation of *P. guajava* L. var. Beaumont from shoot tip and nodal explant using kinetin, to be combined with supplementation of auxin. In the study on the effect of IBA and MS salt strength on rooting of guava shoots produced *in vitro*, the highest number of roots produced per explant (3.33) was attained at 0.5mg/L and 1.0mg/L IBA in full strength MS medium while full strength MS medium without IBA produced the longest root



(1.09 cm). In the study on the effect of NAA and MS salt strength on rooting of guava shoots, full strength MS medium without NAA produced the longest root (1.09cm), whereas the addition of NAA caused reduction in both parameters. In both rooting experiments, the different concentrations of NAA or IBA tested did not result in significant difference on the percentage of root formation.

Abstrak tesis yang dikemukakan untuk Senat Universiti Putra Malaysia sebagai memenuhi keperluan ijazah Master Sains Pertanian

**RAWATAN PENSTERILAN DAN REGENERASI ANAK POKOK
GUAVA (*PSIDIUM GUAJAVA* L. VAR. BEAUMONT)**

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Disember 2007

Pengerusi : Profesor Madya Maheran bt. Abdul Aziz, PhD

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Kajian ini telah dijalankan untuk menghasilkan kaedah regenerasi kultur yang efisien bagi tujuan pengeluaran *Psidium guajava* L. var. Beaumont secara besar-besaran dan sebagai sistem tumbuhan transgenik untuk meningkatkan kualiti pokok melalui kejuruteraan genetik. Kajian aseptik juga telah dijalankan menggunakan eksplan nodal pokok matang *Psidium guajava* L. var. Beaumont dari ladang bertujuan menjana protokol pensterilan yang efisien. Daripada kajian pensterilan eksplan, didapati Rawatan 5 adalah efisien di dalam menghasilkan peratus eksplan hidup yang tinggi dan peratusan pencemaran bakteria dan kulat yang rendah. Di dalam Rawatan 5 eksplan nod direndam di dalam 0.5% Benlate selama 1 jam, dan kemudian diletakkan di bawah mengalir selama 1 jam, di dalam 20% larutan pemutih Clorox dengan beberapa titis Tween 20 selama 5 minit rendaman sebelum rendaman di dalam 0.05% AgNO₃ dan 0.05% HgCl₂ masing-masing selama 1-2 minit diikuti rendaman di dalam larutan media cecair



MS + 2 mg/L BAP selama 1 malam dan eksplan akhirnya dikulturkan ke dalam media MS + 1 mg/L BAP + 25 mg/L asid askorbik). Bagaimanapun, peratusan eksplan dengan masalah pemerangan fenol menggunakan Rawatan 5 masih tinggi. Untuk mengurangkan peratus pemerangan fenol, dicadangkan agar Rawatan 5 ditambahkan dengan celupan eksplan ke dalam larutan antioksidan (100mg/L asid askorbik dan 150mg/L asid sitrik) sebelum dirawat menggunakan 50g/L (10-40 minit dan dibilas dengan air suling sebanyak 5 kali). Adalah juga dicadangkan di dalam Rawatan 5 agar eksplan dikulturkan ke media mengandungi arang teraktif selama beberapa hari sebelum dipindahkan ke media MS + 1 mg/L BAP + 25mg/L asid askorbik. Kajian penggandaan pucuk secara *in vitro* menunjukkan respon *Psidium guajava* L. var. Beaumont ke atas hormon BAP dan kinetin. Di dalam eksperimen kajian kesan BAP ke atas penggandaan pucuk daripada eksplan pucuk, min bilangan pucuk per eksplan tertinggi (2.33) adalah pada paras 1.0 mg/L BAP manakala min pucuk tertinggi (5.56 mm) adalah pada tahap 0.5 mg/L BAP. Menggunakan eksplan nodal, min bilangan pucuk per eksplan tertinggi yang terhasil (1.87) adalah pada 1.0 mg/L BAP manakala min pucuk tertinggi (1.60mm) pula adalah pada kepekatan 0.5 mg/L dan 1.0mg/L BAP. Keputusan juga menunjukkan medium MS tanpa BAP menghasilkan peratusan pucuk yang lebih rendah secara signifikan berbanding kebanyakan rawatan lain menggunakan hormon BAP. Penggunaan eksplan daun muda pada kepekatan BAP pada 2.0 mg/L BAP memberikan peratusan eksplan hidup tertinggi (96.67%) manakala kepekatan BAP 1.0 mg/L menunjukkan peratusan tertinggi eksplan berkalus (70%). Daripada kajian kesan kinetin ke

atas penggandaan pucuk menggunakan eksplan mercu pucuk, medium MS kawalan memberikan peratusan tertinggi pembentukan pucuk (93.33%) dan min pucuk per eksplan (0.93), manakala MS medium dengan 0.1 mg/L kinetin pula memberikan keputusan pucuk tertinggi (1.45 mm). Kajian menunjukkan peratusan tertinggi pembentukan pucuk (36.67%) dan min bilangan pucuk per eksplan (0.47) dicapai pada 0.2 mg/L kinetin. Di dalam kajian pembentukan pucuk menggunakan eksplan daun muda menerusi aruhan kinetin, 0.5 mg/L kinetin menghasilkan peratusan tertinggi eksplan yang masih hidup (76.67%). Adalah dicadangkan agar rawatan dengan 1.0 dan 2.0 mg/L BAP digabungkan dengan auksin seperti 2,4-D dan/atau NAA untuk meningkatkan peratus eksplan daun muda menghasilkan kalus. Di dalam kajian perbandingan dua kepekatan BAP (0.5 mg/L dan 1.0 mg/L BAP) ke atas pembentukan mercu pucuk daripada eksplan mercu pucuk menggunakan ujian-T, 0.5 mg/L BAP menghasilkan ketinggian pucuk per eksplan per subkultur yang lebih tinggi tetapi tidak signifikan pada setiap subkultur dibandingkan 1.0 mg/L BAP (kecuali pada subkultur kedua) manakala rawatan 1.0 mg/L BAP menghasilkan min bilangan pucuk per eksplan yang lebih tinggi tetapi tidak signifikan pada setiap subkultur. Sebagai cadangan, rawatan kinetin digabungkan dengan auksin untuk kajian proliferasi *P. guajava* L. var. Beaumont menggunakan eksplan mercu pucuk dan nodal. Di dalam kajian kesan IBA dan kepekatan medium MS ke atas pengakaran pucuk jambu yang dihasilkan secara *in vitro*, bilangan akar terhasil per eksplan yang tertinggi (3.33) di dapati menggunakan 0.5 mg/L dan 1.0 mg/L IBA dengan media MS manakala medium MS dengan kepekatan penuh tanpa

sebarang IBA menghasilkan akar terpanjang (1.09 cm). Di dalam kajian kesan NAA dan kepekatan medium MS ke atas pembentukan akar *Psidium guajava* L. var. Beaumont, medium MS tanpa NAA menghasilkan akar terpanjang (1.09cm), manakala penambahan NAA mengurangkan kedua-dua parameter tersebut. Di dalam kedua-dua eksperimen kajian pengakaran, rawatan berbeza menggunakan IBA dan NAA tidak memberikan perbezaan signifikan pada peratusan eksplan menghasilkan akar.



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TABLE OF CONTENTS

	Page
ABSTRACT	i
ABSTRAK	iv
ACKNOWLEDGEMENTS	viii
APPROVAL	ix
DECLARATION	xi
LIST OF TABLES	xv
LIST OF FIGURES	xvi
LIST OF PLATES	xviii

CHAPTER

1	GENERAL INTRODUCTION	
	1.1 Background	1
	1.2 Objectives	3
2	LITERATURE REVIEW	
	2.1 History and Botany of <i>Psidium guajava</i> L. var. Beaumont	4
	2.2 Problems in <i>In Vitro</i> Culture	5
	2.2.1 Phenolic browning	5
	2.2.2 Contamination	6
	2.3 <i>In Vitro</i> Plantlet Regeneration	10
	2.4 Factors Affecting Organogenesis	12
	2.4.1 Types of Explant	12
	2.4.2 Plant Growth Regulators	13
	2.5 Shoot Induction and Axillary Shoot Formation	17
	2.6 Root Induction	20
3	EFFECT OF STERILIZATION TREATMENT ON REDUCTION OF MICROBIAL CONTAMINATION ON NODAL EXPLANT OF <i>PSIDIUM GUAJAVA</i> L. VAR. BEAUMONT	
	3.1 Introduction	22
	3.2 Materials and Methods	23
	3.2.1 Preparation and culture of explant	23
	3.2.2 Effect of sterilization treatments on nodal explants of <i>Psidium guajava</i> L. var. Beaumont obtained from field	24
	3.2.3 Statistical Design and Analysis	27
	3.3 Results	31
	3.3.1 Effect of sterilization treatments on nodal explants of <i>Psidium guajava</i> L. var. Beaumont obtained from field	31



3.4	Discussion	37
3.5	Conclusion	41

4	ORGANOGENESIS : EFFECT OF PLANT GROWTH REGULATORS ON SHOOT INDUCTION AND MULTIPLICATION OF <i>PSIDIUM GUAJAVA</i> L. VAR. BEAUMONT	
4.1	Introduction	42
4.2	Materials and Methods	44
4.2.1	Preparation and culture of explant	44
4.2.2	Effect of BAP on shoot regeneration from shoot tip explant of <i>Psidium guajava</i> L. var. Beaumont	45
4.2.3	Effect of BAP on shoot regeneration from nodal explant of <i>Psidium guajava</i> L. var. Beaumont	45
4.2.4	Effect of BAP on shoot regeneration from young leaf explant of <i>Psidium guajava</i> L. var. Beaumont	46
4.2.5	Effect of kinetin on shoot regeneration from shoot tip explant of <i>Psidium guajava</i> L. var. Beaumont	46
4.2.6	Effect of kinetin on shoot regeneration from nodal explant of <i>Psidium guajava</i> L. var. Beaumont	47
4.2.7	Effect of kinetin on shoot regeneration from young leaf explant of <i>Psidium guajava</i> L. var. Beaumont	47
4.2.8	Comparison between treatment 0.5mg/L BAP and 1.0mg/L BAP on shoot regeneration from shoot tip explant of <i>Psidium guajava</i> L. var. Beaumont	47
4.2.9	Statistical Design and Analysis	48
4.3	Results	
4.3.1	Effect of BAP on shoot regeneration from shoot tip explant of <i>Psidium L. guajava</i> var. Beaumont	49
4.3.2	Effect of BAP on shoot regeneration from nodal explant of <i>Psidium guajava</i> L. var. Beaumont	53
4.3.3	Effect of BAP on shoot regeneration from young leaf explant of <i>Psidium guajava</i> L. var. Beaumont	58
4.3.4	Effect of kinetin on shoot regeneration from shoot tip explant of <i>Psidium guajava</i> L. var. Beaumont	63

4.3.5	Effect of kinetin on shoot regeneration from nodal explant of <i>Psidium guajava</i> L. var. Beaumont	67
4.3.6	Effect of kinetin on shoot regeneration from young leaf explant of <i>Psidium guajava</i> L. var. Beaumont	70
4.3.7	Comparison culture effect of treatment 0.5mg/L BAP and 1.0mg/L BAP on shoot regeneration from shoot tip of <i>Psidium guajava</i> L. var. Beaumont explant with subculture	73
4.4	Discussion	80
4.5	Conclusion	83
5	ORGANOGENESIS : ROOT INDUCTION OF <i>PSIDIUM</i> <i>GUAJAVA</i> L. VAR. BEAUMONT	
5.1	Introduction	85
5.2	Materials and Methods	87
5.2.1	Preparation of explant	87
5.2.2	Effect of IBA and MS salt strength on rooting of <i>Psidium guajava</i> L. var. Beaumont	88
5.2.3	Effect of NAA and MS salt strength on rooting of <i>Psidium guajava</i> L. var. Beaumont	88
5.2.4	Statistical Design and Analysis	89
5.3	Results	
5.3.1	Effect of IBA and MS salt strength on rooting of <i>Psidium guajava</i> L var. Beaumont	89
5.3.2	Effect of NAA and MS salt strength on rooting of <i>Psidium guajava</i> L. var. Beaumont	93
5.4	Discussion	97
5.5	Conclusion	99
6	GENERAL DISCUSSION AND CONCLUSION	
6.1	General Discussion	100
6.2	Conclusion	104
	REFERENCES	105
	APPENDICES	121
	BIODATA OF THE AUTHOR	148

LIST OF TABLES

		Page
Table	Description of treatment and stages of sterilization used.	28



LIST OF FIGURES

	Page
Figure	
Effect of sterilization treatments on (A) explant survival rate, (B) percentage of phenolic browning, (C) percentage of bacteria contamination and (D) percentage of fungi contamination of <i>Psidium guajava</i> L. var. Beaumont after 2 weeks of culture by using grafted nodal explant obtained from the field.	33
Effect of BAP on (A) percentage of shoot regeneration, (B) mean number of shoot per explant, (C) mean shoot length (mm) of <i>Psidium guajava</i> L. var. Beaumont shoot tip explant after six weeks of culture	50
Effect of BAP on (A) percentage of shoot regeneration, (B) mean number of shoot per explant, (C) mean shoot length (mm) of <i>Psidium guajava</i> L. var. Beaumont nodal explant after six weeks of culture	55
Effect of BAP on (A) percentage of survival rate, (B) mean number of callus formation of <i>Psidium guajava</i> L. var. Beaumont young leaf explant after six weeks of culture	59
Effect of kinetin on (A) percentage of shoot regeneration, (B) mean number of shoot per explant, (C) mean shoot length (mm) of <i>Psidium guajava</i> L. var. Beaumont from shoot tip explant after six weeks of culture	64
Effect of kinetin on (A) percentage of shoot regeneration, (B) mean number of shoot per explant, of <i>Psidium guajava</i> L. var. Beaumont nodal explant after six weeks of culture	68
Effect of kinetin on percentage of survival rate of <i>Psidium guajava</i> L. var. Beaumont of young leaf explant after six weeks of culture	70



Effect of 0.5mg/L BAP and 1.0mg/L BAP on (A) percentage of shoot formation from shoot tip explant of <i>Psidium guajava</i> L. var. Beaumont by first subculture until 5 th subculture.	75
Effect of 0.5mg/L BAP and 1.0mg/L BAP on (B) mean number of shoot per explant and (C) mean shoot height (mm) attained from shoot tip explant of <i>Psidium guajava</i> L. var. Beaumont by first subculture until 5 th subculture.	76
Effect of MS full strength and half strength with different IBA levels on (A) mean number of roots produced per explant (B) root length produced per explant (cm) from micro shoot of <i>P. guajava</i> L. var. Beaumont.	91
Effect of MS full strength and half strength with different levels of NAA on root length produced per explant (cm) from micro shoot of <i>P. guajava</i> L. var. Beaumont.	94



LIST OF PLATES

Plate		Page
Shoot formed of <i>Psidium guajava</i> L. var. Beaumont from nodal explant after 2 weeks of culture in (A) bacteria contamination (B)fungi contamination		35
Shoot formed of <i>Psidium guajava</i> L. var. Beaumont from nodal grafted explant after 2 weeks of culture in (A) phenolic browning (B)explant dies due to excessive strong chemicals		36
Shoots formed from shoot tip explant of <i>P. guajava</i> L. var. Beaumont on (A) MS medium + 0.5 mg/L BAP, and (B) MS medium + 1.0 mg/L BAP at week 6 of culture.		52
Shoots formed from shoot tip explant on <i>P. guajava</i> L. var. Beaumont on MS medium + 0.1 mg/L BAP at week 6 of culture		53
Shoots formed from nodal explant of <i>P. guajava</i> L. var. Beaumont on (A) MS medium + 0.5 mg/L BAP and (B) MS basal medium at week 6 of culture		57
Explant survival and shoot formation on young leaf explant of <i>P. guajava</i> L. var. Beaumont on (A) MS medium + 1.0mg/L BAP and (B) MS medium + 2.0mg/L BAP after 6 weeks of culture.		61
Shoots formed from shoot tip explant of <i>P. guajava</i> L. var. Beaumont on (A) MS basal medium and (B) MS medium + 2.0 mg/L kinetin after 6 week of culture.		66
Shoots formed from nodal explant of <i>P. guajava</i> L. var. Beaumont placed on (A) MS basal medium and (B) MS medium + 2.0 mg/L kinetin at week 6 of culture		69
Young leaf explant of <i>P. guajava</i> L. var. Beaumont remained green on (A) MS medium + 0.5mg/L kinetin and (B) MS basal medium after six weeks of culture.		71



- Young leaf explant of *P. guajava* L. var. Beaumont turned brown on (A) MS medium + 2.0mg/L kinetin and (B) MS medium + 1.0mg/L kinetin after six weeks of culture. 72
- Shoots produced from shoot tip explant of *P. guajava* L. var. Beaumont on (A) MS medium + 0.5 mg/L BAP and (B) MS medium + 1.0mg/L BAP at third subculture (week 12). 77
- Shoots produced from shoot tip explant of *P. guajava* L. var. Beaumont on (A) MS medium + 0.5 mg/L BAP and (B) MS medium + 1.0 mg/L BAP at fourth subculture (week 16). 78
- Shoots produced from shoot tip explant of *P. guajava* L. var. Beaumont on (A) MS medium + 0.5 mg/L BAP and (B) MS medium + 1.0 mg/L BAP at fifth subculture (week 20). 79
- Roots formed in (A) MS with 1.0 mg/L IBA (blue arrow), MS with 1.5 mg/L IBA (red arrow), and half MS with 2.0 mg/L IBA (yellow arrow) and (B) MS basal medium (red arrow) at week 6 of culture 92
- Roots formed in (A) MS basal medium (blue arrow) and (B) MS medium with 0.2 mg/L NAA (red arrow) at week 6 of culture. 95
- Roots formed in MS medium supplemented with 0.5 mg/L NAA (blue arrow); 1.5 mg/L NAA (red arrow) and half strength MS supplemented with 0.2mg/L NAA at week 6 of culture 96



CHAPTER 1

GENERAL INTRODUCTION

1.1 Background

Psidium guajava L. is the most vital fruit under the Myrtle family (Lim and Khoo, 1990). The guava trees may be grown under a wide range of climatic conditions that ranged from lowland tropics to an altitude of 1500m. It can also be grown in a climate with a marked dry season. The guava plant grows best in deep fertile soils even though the tree can adapt itself to a wide range of soil conditions (Bourke, 1988).

Guava tree can be found growing in the tropics and subtropics including some of the Mediterranean areas which lie within the limit of latitude 35°N and 35°S. Among the major producers of guava in the world are Brazil, South Africa, Colombia, Mexico, the Caribbean, United States (Florida and Hawaii), Australia, the Phillipines and India (Raziah and Zainal, 1992). Generally, guava fruits can be divided into two major categories, which are for fresh consumption and for processing purposes. The guava fruit is a rich source of vitamin C that makes up to 1000 mg / 100 g of fresh weight (Martin,1984). For processing purposes, guava fruits with high acid content and pink flesh are most suitable.

Three varieties of pink guava which are planted locally for processing purposes are Beaumont, GU5 and Burma Red (Zainal,1992). The largest pink guava plantation in Malaysia is located in Sungai Wangi, Sitiawan, Perak under the Golden Hope Fruit Industries Sdn. Bhd. (Golden Hope FISB). With a guava plantation of 500 hectares, and 70 percent of it planted with the Beaumont variety. Golden Hope FISB dominates almost 15 percent of the global market in producing pink guava puree. The percentage is estimated to increase to 35 percent, dominating the market by 2008, which would place the company and Malaysia as the biggest manufacturer and exporter of pink guava puree. In the global market, 90% of the product is exported to Europe, Australia and the United States (Utusan Malaysia, 2005).

Among the major problems encountered in the cultivation of guava in Malaysia is the occurrence of plant diseases such as scabby canker, stylar-end ring rot, anthracnose and root knot disease caused by nematode (Nik Masdek and Vijayasegaran, 1992; Bourke, 1988; Lim and Khoo, 1990). The diseases may cause a reduction in productivity of guava. Breeding programme such as the crossing of the Beaumont variety with other variety is needed in order to produce a new variety resistant to disease caused by nematodes. However, breeding through conventional method is slow due to the perennial nature of the crop. An alternative approach which can reduce the time taken to genetically improve the crop is through the non-conventional breeding technique of genetic engineering. However, for such technique to be implemented, an efficient *in vitro* plant regeneration protocol need to be developed.

An efficient plant regeneration protocol is also a useful system for mass production of planting materials for large scale field planting. Several efforts in micropropagating *P. guajava* L. have been carried out in several countries using their local variety, however no research has yet been published on the micropropagation of *P. guajava* L. variety Beaumont.

1.2 Objectives

The objective of this research was to establish a complete protocol of plant regeneration of *P. guajava* L. variety Beaumont through shoot multiplication and *in vitro* rooting. The specific objectives were:

1. to determine the effect of various sterilizing agents on overcoming contamination of nodal explants of *P. guajava* L. var. Beaumont obtained from field grown plants.
2. to determine the explant type and the effect of type and concentrations of growth regulators on axillary proliferation of *P. guajava* L. var. Beaumont and
3. to determine the appropriate MS salt strength and concentrations of NAA or IBA in promoting rooting of *P. guajava* L. var. Beaumont.