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

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By

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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% $AgNO_3$ and 0.05% $HgCl_2$ 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 minutesand 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 onshoot 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)

TAJUL AFIF ABDULLAH

Disember 2007

Pengerusi : Profesor Madya Maheran bt. Abdul Aziz,PhD

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Kajian ini telah dijalankan untuk menghasilkan kaedah regenerasi kultur yang efisen 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 effisien 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% $AgNO_3$ dan 0.05% $HgCl_2$ 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 antioksida (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 harisebelum 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 mengunakan 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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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 Carribean, 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, antrachnose and root knot disease caused by nematode (Nik Masdek and Vijaysegaran, 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.