UNIVERSITI PUTRA MALAYSIA

POLYEMBRYONY ABILITY OF JACKFRUIT (ARTOCARPUS HETEROPHYLLUS) SEED

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FACULTY OF AGRICULTURE

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POLYEMBRYONY ABILITY OF JACKFRUIT (*ARTOCARPUS HETEROPHYLLUS)* SEED

BY

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A project report submitted to Faculty of Agriculture, Universiti Putra Malaysia, in fulfillment of the requirement of PRT 4999 (Final Year Project) for the award of the degree of Bachelor of Agricultural Science

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CERTIFICATION

This project entitled “Polyembryony Ability of Jackfruit (*Artocarpus heterophyllus)* Seed”, is prepared by Nur Farah Dinah Bt Muhamad and submitted to the Faculty of Agriculture in fulfillment of the requirement of PRT 4999 (Final Year Project) for the award of the degree of Bachelor of Agricutural Science.

Student’s name: Student’s signature

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LIST OF ABBREVIATIONS

BAP	6-benzylaminopurine
BA	benzyladenine
GA_3	gibberellic acid
NaOH	sodium hydroxide
HCl	hydrochloric acid
RCBD	randomize complete block design
MS	Murshige and Skoog
PGR	plant growth regulator
LAR	laminar air flow

ABSTRACT

Polyembryony is common in jackfruit but which part of the seed responded to polyembryony is the main issue. The development of multiple embryos within the same seed is defined as polyembryony. The objectives of the experiment were to compare the ability of different structural forms of seed and the effect of different concentration of benzylaminopurine (BAP) on shoot induction through polyembryony in *Artocarpus heterophyllus* seed while the different structural forms of seed tested were complete seed, seed without minor cotyledon and seed without minor cotyledon and zygotic embryo. The different concentrations of BAP were 0 mg/L, 4 mg/L and 8 mg/L. The experiment was arranged in a Randomized Complete Block Design (RCBD) with 9 treatments and 10 replications for each treatment. Each replication contained one explant. The different seed structures were cultured inside jars containing half strength MS (Murshige and Skoog) medium with different concentrations of BAP. After 12 weeks of culture, it was observed that complete seed cultured on medium supplemented with 4 mg/L BAP showed the best response producing the highest number and percentage of shoot induction.

ABSTRAK

Poliembrio adalah biasa pada nangka tetapi bahagian biji manakah yang memberi respon kepada poliembrio menjadi isu utama. Perkembangan embrio berganda dalam kalangan biji didefinisikan sebagai poliembrio. Objektif eksperimen ini adalah untuk membandingkan keupayaan struktur yang berbeza pada biji dan kesan kepekatan benzylaminopurine (BAP) yang berbeza untuk pertumbuhan pucuk melalui poliembrio pada biji *Artocarpus heterophyllus*. Perbezaan struktur biji terdiri daripada biji lengkap, biji tanpa kotiledon minor serta zigot embrio manakala perbezaan kepekatan BAP pula adalah diantara 0 mg/L, 4 mg/L dan 8 mg/L. Eksperimen ini diatur dalam Rekabentuk Blok Lengkap (RCBD) dengan 9 rawatan dan 10 replikasi bagi setiap rawatan. Setiap replikasi mengandungi satu eksplan. Biji yang berbeza struktur telah dikulturkan di dalam balang jar yang mengandungi media MS separuh (Murshige dan Skoog) dengan kepekatan BAP yang berbeza (0, 4 dan 8 mg/L). Selepas dikultur selama 12 minggu, pemerhatian telah dijalankan terhadap biji lengkap yang dikultur dalam media yang ditambah dengan 4 mg/L BAP menunjukkan kesan yang paling baik dalam memberi respon untuk penghasilan jumlah pucuk dan peratusan bagi pertumbuhan pucuk yang tinggi.

CHAPTER 1

INTRODUCTION

According to Love and Paull (2011) jackfruit is said to have originated from Southwest India, where it can be found growing wild in the rain forest of the Western Ghats, India. The jackfruit plants were then grown widely throughout India mostly in West Bengal, Bihar, Kerala, Tamilnadu and Karnataka. Then, the fruit was introduced throughout the tropical countries of Southeast Asia including Malaysia and Indonesia, and tropical Africa. Now, jackfruit becomes common in Bangladesh, Burma, Sri Lanka and Thailand, where the fruit is highly esteemed (Jaiswal and Amin, 1992). In South India, jackfruit is among the popular fruit ranking next to mango and banana (El-Zaher, 2008). In Bangladesh, jackfruit is known as their national fruit. The jackfruit plays an important role in the economy of Bangladesh because it is a multipurpose tree that provides food, fodder, timber and fuel (Khan *et al*., 2010).

Barrau (1976) suggested that jackfruit originated in Malaysia due to the great diversity of jackfruit cultivars in Malaysia. Jackfruit is widely cultivated in Malaysia, because of this it was said to have likely been introduced and never found in the wild (Jarrett 1959). According to the statistics by Department of Agriculture Malaysia, in 2011 the planted area of jackfruit in Malaysia is about 4260.8 hectare with harvested area of 2895.4 hectare as shown in Figure 1. The most planted area of jackfruit in Malaysia is in Pahang with 1745.9 ha, mostly in

Temerloh (546.8 ha) followed by Negeri Sembilan with 463.1 ha and Johor with 404.8 ha. Estimation on the production of jackfruit is about 23 735 mt in 2011 with a production value of more than RM 55,000,000.

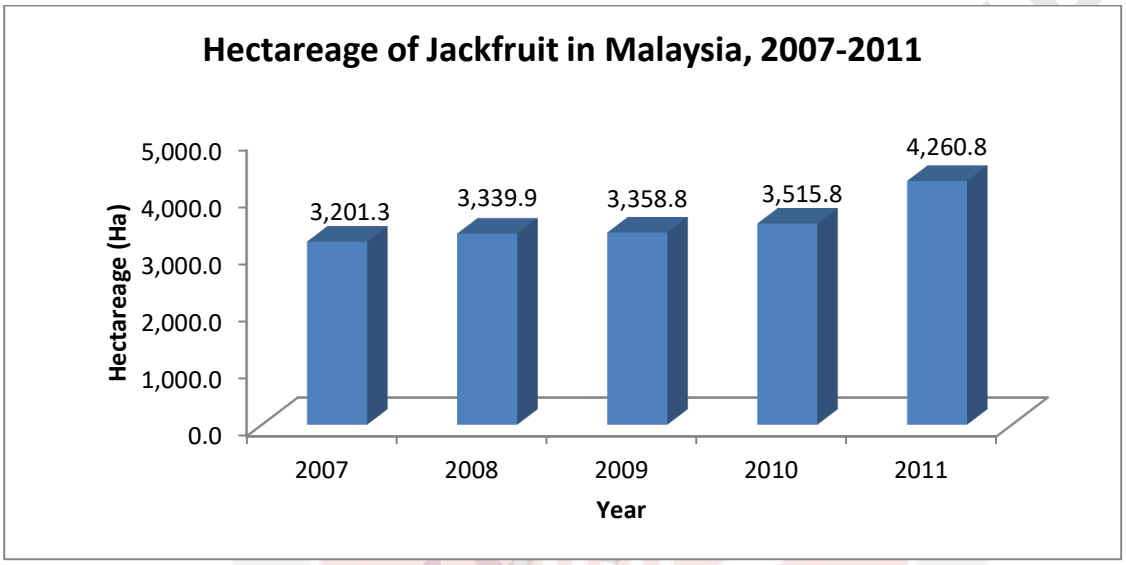

Figure 1 : Hectarage of Jackfruit in Malaysia from 2007-2011.

(Source : Agriculture Department Peninsular Malaysia, 2012)

In Malaysia, jackfruit is being eaten fresh because the ripe flesh having crunchy and juicy texture with sweet tasted is much favoured by the people. This tropical fruit is highly demanded during the fruit season. More than 25 clones of jackfruit were registered in Malaysia. However, there are five varieties of jackfruit which are popular and mostly planted by the local farmers, which are J29, J31, Mantin (J32), Tekam Yellow and Mastura. These

varieties are chosen as the best because their fruits are of very high quality in term of the colour, texture and taste. Moreover, these jackfruit trees are capable of producing high yield per season and resistance towards diseases.

Jackfruit tree is considered as an economic crop which can give bundles of benefits. Besides, growing jackfruit is considered as profitable if the tree can last for more than 8 to 10 years old. Moreover, this plant is regarded as a species which is worthy of research attention because of its wider potential use in nutrition and potential to increase local incomes when grown in agroforestry and home garden system (Haq, 2006). Therefore, because of the benefits, the improvement in propagation of jackfruit through in vitro method is very important. Studies on polyembryony under in vitro condition allow high rate of multiplication which lead to the production of large number of progeny compared with the polyembryony of jackfruit under natural condition in the field. Due to that reason, this study was carried out to meet the objectives which are:

- To determine the polyembryony ability of *Artocarpus heterophyllus* under in vitro condition.

- To determine the effect of different concentrations of BAP on shoot induction from different structural forms of *A. heterophyllus* seed.

CHAPTER 2

LITERATURE REVIEW

2.1 *Artocarpus heterophyllus*

The scientific name for jackfruit is *Artocarpus heterophyllus* Lamarck. Jackfruit belongs to the family Moraceae which is in the same family as other fruit bearing plants such as fig (*Ficus carica*) and mulberry (*Morus indica*) (Jaiswal and Amin, 1992). Jackfruit is a perennial medium sized tree that can grow to 10 to 25 meter in height, with trunk diameter from 30 to 60 cm (Lim, 2012). The jackfruit is a monoecious evergreen latex producing tree with dark green leaves (Nakasone and Paull, 1998). Lim (2012) stated that the leaves are spirally arranged and borne on 1-3 cm long petiole. The seedling trees form a vigorous tap root system. Jackfruit tree has milky white and very sticky latex on all parts of the plant. Flowers of jackfruit are borne in inflorescences on the trunk and older branches (Love and Paull, 2011). The flowers are tiny and green in colour (Plate 1). The fruit is a compound or aggregate fruit.

Normally, the fruit can have up to 500 seeds with the size between 2-4 cm long and each seed is surrounded by a horny endocarp and subgelatinous exocarp (Nakasone and Paull, 1998). The seeds are brown and rounded shaped enclosed in a thin, whitish membrane (Lim,

2012). The seed of jackfruit is firm and waxy, oval, oblong or oblong ellipsoid in shape with a coriaceous testa. The testa is thin and leathery and is rather thick, tough and crinkly when dry. The inner seed coat is a thin, brownish membrane. Jackfruit seed is thickened at the hilum, which is situated with the micropyle in the distal end or near the reticular end of the seed. The fleshy cotyledons of jackfruit seed are unequal, with one cotyledon (minor cotyledon) only about one-third to one-half the size of the other cotyledon (major cotyledon) as shown in Plate 2. If there is a present of endosperm, the size is very small (Haq, 2006).

Plate 1 : Flower inflorescence of *A. heterophyllus*
(Source : http://www.indianaturewatch.net/displayimage.php?id=144032)

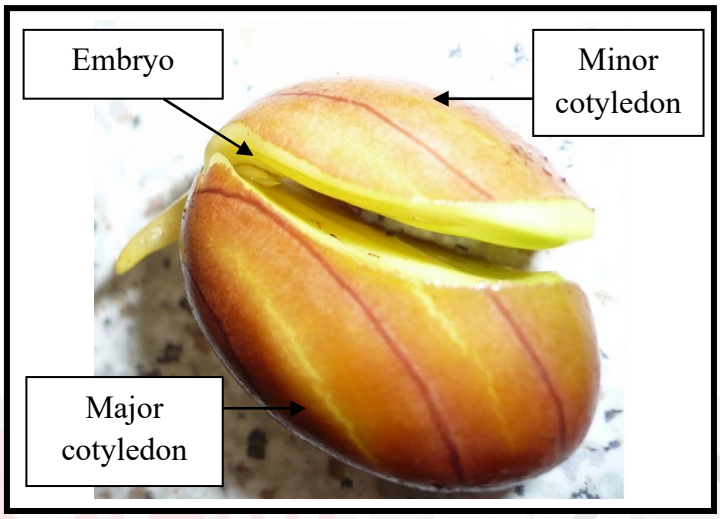

Plate 2 : Parts on *A. heterophyllus* seed

(Source : http://www.flickr.com/photos/39398826@N03/5267886719)

Love and Paull (2011) mentioned that the indication of fruit maturity starts at about 6 to 8 months after flowering. Then, the maturity of fruit usually takes about 3 months before it can be harvested. This depends on rainfall, irrigation and tree age. Jackfruit has the ability to produce around 20 to 250 fruits per year, sometimes up to 500 fruits on old growing trees. The environment which favours the growth of jackfruit tree is the tropical warm and humid frost-free climates at elevations below 5000 feet. Jackfruit trees have salinity tolerance but poor drought and flooding tolerance. It needs proper irrigation during drought in order to produce fruits. The trees will grow well when planted in well-drained soils with pH between 5 and 7.5. However, jackfruit trees are unable to grow well in exposed locations with strong and drying winds. Jackfruit has high value of nutrients which is good for health. Ripe fresh pulp contains good quantities of protein, carbohydrates, calcium, phosphorus, iron, sodium, potassium, vitamin A, niacin and ascorbic acid. The seeds are said to have high amount of starch and good sources of vitamin B (El-Zaher, 2008). SCUC (2006) stated that jackfruit is

rich in calories (84%), carbohydrates (18.9%), proteins (1.9%), vitamin A (540 IU), iron (500.1mg) and potassium (350 mg).

Usually, the fleshes of ripe jackfruit are eaten fresh. However, nowadays, people are getting innovative by creating varieties of delicious foods using jackfruit as the based ingredients. The full grown and unripe flesh including the seeds can be cooked green and served as vegetables. The ripe pulps has been used to make ice cream, jam, jelly and paste, powder, dried and fried in oil and salted for eating like potato chips. Jackfruit seeds can be eaten after it is boiled or roasted, and dried seeds are ground to make flour for baking (El-Zaher, 2008). Jaiswal and Amin (1992) also mentioned that the leaves and rind of the fruit can be fed to livestock. In Sri Lanka, Bangladesh and India, the wood of jackfruit tree is used to make furniture and musical instruments.

Jackfruit is highly variable in fruit shape, size and quality as well as in productivity and fruiting season because of open pollination and conventional seed propagation (Jaiswal and Amin, 1992). Propagation of jackfruit trees is commonly by using the seed. Usually, seeds germinate within 3 to 8 weeks. Other propagation methods that are said to be successful are by root cuttings, stem cuttings and air layers. In India and Southeast Asia, grafting and budding are now widely used to propagate jackfruit trees (Love and Paull, 2011).

2.2 Polyembryony

Polyembryony is the term used to describe the development of multiple embryos within the same seed (Hartmann *et al*, 2011). This polyembryonic seed was discovered in plants by Leeuwenhoek in 1719. In other words, the seed is able to produce several number of seedlings. The processes constituting polyembryony cause the appearance of different genotypes in the same seed and enchancing the genetic heterogeneity of seeds. Polyembryony may be caused by hybridization, pollination, physical, chemical and other factors. At present, the morphophysiological and genetic reasons for polyembryony are still unknown (Batygina, 2009). According to Rangaswamy (1982) 16 plant families were identified containing polyembryonic species. Among the plants are mostly tropical fruit species such as *Citrus* sp., *Garcinia mangostana*, *Lansium domesticum*, *Mangifera indica* and *Syzygium* sp.

2.3 In vitro propagation

Plant propagation through tissue culture or micropropagation is to propagate plants that are true-to-type or known as clones. Cultures are started with very small pieces of plant tissue either shoot tips, root tips, embryos, other parts of seeds, stems, callus or single cells. In vitro propagation requires small amount of space for the explants to greatly increase their number. This propagation method must be carried out under aseptic condition to avoid contamination. Normally, the methods involve three distinct steps, namely shoot proliferation,

rooting of regenerated shoots and establishment of rooted plantlets in the nursery (Haq, 2006). Advantages of micropropagation is the rate of propagation is much greater than macropropagation. Large number of plants can be produced in a given time. At the same time, newly selected varieties can be made available quickly and widely (George *et al.*, 2008). Micropropagation also may help to produce clones of some species of plants that are slow or difficult to propagate vegetatively.

Studies on in vitro propagation of jackfruit mostly has been done using shoot tips and nodal segments. Ashrafuzzaman *et al.* (2012) indicated that regeneration of shoots using healthy and juvenile shoot tips, increased comparatively when MS medium was enriched with 2 mg/L of BAP. Adiga *et al.* (1998) stated that sucrose was a good carbon source, as cultures supplemented with 3% or 4% of sucrose were able to produce more shoots while 6 mg/L of GA_3 promoted increase in shoot length. Lee and Keng (2005) reported that the apical shoots cultured on MS medium supplemented with 4.5 mg/L BA produced the most number of shoots after three to four weeks of culture.

2.4 Browning and contamination problem under in vitro condition

Some plants, particularly tropical species contain high concentrations of phenolic substances that are oxidised when cells are wounded or senescent. The cultured explant then becomes brown or black and fails to grow (George *et al*., 2008). Besides, contamination usually caused by bacteria and fungi commonly occurs under in vitro condition. These problems come from various possible source of infection such as the explants used in culture, poorly sterilized culture media, operator's negligence and contamination introduced by frequent movement in and out of the culture room without taking adequate precautions to maintain the aseptic conditions. Sometimes, contamination is not evident in the first phases of culture, either because the culture medium does not readily support microbial growth or because endogenous microorganisms that are not eliminated by the surface sterilization of the explants occur within the tissue. These are frequently caused by bacilli and can sometimes be treated (Munoz, 1999).

2.5 Plant Growth Regulator (PGR)

George *et al.* (2008) defined plant growth regulator as synthetic chemicals with similar physiological activities to plant growth substances or compound which have the ability to modify plant growth. The concentration of a PGR is very important. If PGR is too little, there

will be less or no response towards the plant growth. If PGR is too much, there will be an unwanted response to the plant growth (Gaba, 2005).

There are five major groups of PGR, which is auxins, cytokinins, gibberellins, abscisic acid and ethylene. These PGRs have multiple roles to promote plant growth and development, especially in tissue culture to manipulate the plants. In tissue culture, they regulate the initiation and development of shoots and roots on explants cultured in semisolid or liquid medium cultures. Most important PGRs used in tissue culture are the auxins and cytokinins. The concentration of auxin and cytokinin is important and play a major role according to the plant growth requirement (Gaba, 2005).

2.5.1 Cytokinin

Sathyanarayana (2007) stated that cytokinin are a group of phenyl urea derivatives of adenine. Kinetin is the first cytokinin to be discovered and isolated in Professor Skoog's laboratory, following experiment to promote continuing growth of the callus which formed on tobacco stem sections on nutrient media. Zeatin is a cytokinin identified from corn in 1963. The most frequently used cytokinins are benzylaminopurine (BAP) or 6-benzyladenine (BA), 6-β-β-dimethylaminopurine (2-ip), Kinetin and Zeatin. There are two types of cytokinin

which is naturally occuring cytokinins (Zeatin and 2-ip) and synthetically derived cytokinins (BA and Kinetin).

Mainly, the function of cytokinin is for cell division. This cell division for in vitro plant lead to shoot regeneration by stimulating the formation of shoot apical meristems and shoot buds. Sometimes, cytokinin can caused the production of undifferentiated callus. High concentration of cytokinins will able to block the root development. Cytokinin can induces formation of multiple shoot, by causing the release of shoot apical dominance which stimulate the growth of lateral buds (Gaba, 2005). One of the most commonly synthetically-derived cytokinin used by commercial laboratories is the benzylaminopurine (BAP). This synthetic 6-substituted aminopurine cytokinin give an important role in plant growth, as it can accelerate plant cell growth and division.

CHAPTER 3

MATERIALS AND METHODS

3.1 Research location

The experiment was conducted at the Agrobiotechnology Laboratory, Department of Agriculture Technology, Faculty of Agriculture, Universiti Putra Malaysia, Serdang, Selangor.

3.2 Source of Explant

The explants used in this experiment were the seeds of jackfruit (*A. heterophyllus*) variety Madu Klon J33 (Plate 3) obtained from the Department of Agriculture, Negeri Sembilan.

Plate 3 : The jackfruit from clone J33

3.3 Experimental Design and Treatment

The experiment was conducted using Randomized Complete Block Design (RCBD). There were nine treatments consisting of combinations of different structural forms of seed and concentration of BAP ranging from 0 to 8 mg/L. Each treatment had 10 replications with one explant per replication. The treatments are shown in Table 1.

Table 1 : Treatment application

Treatments	Structural Form of Seed	Concentration of BAP (mg/L)
T1	Complete seed	0
T2	Complete seed	4
T3	Complete seed	8
T4	Seed without minor cotyledon	0
T5	Seed without minor cotyledon	4
T6	Seed without minor cotyledon	8
T7	Seed without minor cotyledon and zygotic embryo	0
T8	Seed without minor cotyledon and zygotic embryo	4
T9	Seed without minor cotyledon and zygotic embryo	8

3.4 Methodology

3.4.1 Stock solution

Stock solutions of macronutrients, micronutrients, vitamins and irons were prepared separately for preparation of Murashige and Skoog (1962) culture medium. Plant growth regulator (PGR) stock solution for BAP at the concentration of 1.0 mg/ml was also prepared. The components used for each stock solution were weighed and dissolved in distilled water by using magnetic stirrer. Then, the stock solutions were labelled and stored in the refrigerator before use.

3.4.2 Media preparation

Half strength of MS media with different concentrations of BAP (0 mg/L, 4 mg/L and 8 mg/L) were prepared. All the stock solutions, myo-inositol, sucrose and BAP were added and dissolved into half the volume of required distilled water. The pH of the media was adjusted to a range of 5.6 to 5.8 using 0.1N NaOH or 0.1N HCl as needed. Then, the final volume of the media was adjusted by adding distilled water. Gelrite was added into the media and the media was heated in the microwave for proper dissolution of the gelrite.

After that, the media were poured into sterilized culture jars. About 50 ml of medium was dispensed into each jar. Next, the media were sterilized using autoclave at 121^{o}C and 105 kPa for 20 minutes. After sterilization, the media were allowed to cool at room temperature and then stored at room temperature.

3.4.3 Explant sterilization

After the removal of the seed testa, the explants were washed with distilled water. The explants were sterilized using 0.1% of mercuric chloride ($HgCl_2$) for 10 minutes followed by rinsing with sterilized distilled water three times. Next, the explants were sterilized with 20% Clorox with a few drops of a wetting agent, Tween 20 for 20 minutes and then the explants were rinsed with sterilized distilled water three times. The whole sterilization procedure was carried out inside the laminar air flow (LAR) cabinet.

3.4.4 Culturing

To avoid contamination, the culturing of explants was carried out inside the LAR cabinet. Before culturing, the LAR cabinet was sprayed with 70% ethanol to avoid contamination. All the equipment used for culturing was sprayed with 70% ethanol before placing them inside the LAR cabinet.

First, the explants were placed in a petri dish and excision was made accordingly following the treatments in Table 1. Then, the explants were placed in the culture jars containing half strength MS medium with different concentrations of BAP. The jars were closed tightly and then sealed with parafilm. The cultures were then incubated in the culture room under controlled environment of 25±2ºC and 16 hour illumination from white fluorescent light with a light intensity of 30 µmol m^{-2} s^{-1}.

3.5 Data collection

Data were collected accordingly based on the following parameters:

i. Number of shoots produced per seed explant

ii. Mean shoot length per seed explant (cm)

iii. Mean percentage of shoot regeneration per seed explant

3.6 Statistical Analysis

All data were analyzed using the Analysis of Variance (ANOVA). The treatment means separation was determined using Duncan Multiple Range Test (DMRT).

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Percentage of shoot induction.

The data of this experiment were recorded every week until the week 12 of culture. Figure 2 shows the percentage of shoot induction per treatment after 12 weeks of culture. Based on the result, the highest percentage of shoot induction was obtained in treatment 2 (complete seed + 4 mg/L BAP) (Plate 4) with 90% of shoot induction after 12 weeks of culture followed by treatment 1 (complete seed + 0 mg/L BAP) and treatment 3 (complete seed + 8 mg/L BAP) with 80% and 60% of shoot induction respectively. Treatment 5 (seed without minor cotyledon + 4 mg/L BAP) (Plate 5) and treatment 6 (Seed without minor cotyledon + 8 mg/L BAP) resulted in the same percentage of 20% of shoot induction while treatment 4 (seed without minor cotyledon + 0 mg/L BAP) resulted in only 10% of shoot induction. However, in treatment 7 (seed without minor cotyledon and zygotic embryo + 0 mg/L BAP), treatment 8 (seed without minor cotyledon and zygotic embryo + 4 mg/L BAP) and treatment 9 (seed without minor cotyledon and zygotic embryo + 8 mg/L BAP), no shoot induction occurred.

The percentage of shoot induction on seed without minor cotyledon was lower compared to the percentage of shoot induction on complete seed. Hence, the removal of minor cotyledon affected the percentage of shoot induction. When cotyledons are removed from seeds, it affects their growth and survival (Lamont and Milberg, 1997; Moles and Westoby, 2004). Investigation by Iortsuun *et al*. (2008) revealed that cotyledon removal reduced seedling establishment rate and performance of *Telfaria occidentalis*. Removal of cotyledons results in reduction in growth performance of seedlings but not necessarily the ultimate death of the seedling at its early stage of development depending on the degree of removal or damage. Besides, the percentage of shoot induction was affected by bacteria and fungus contamination that existed in the seed itself and also from the environment during culturing activity (Plate 6). Bacterial contaminants are often difficult to detect because they remain mostly within the plant tissue (Debergh and Vanderschaeghe, 1988; De Fossard and De Fossard, 1988; Viss *et al*., 1991). Besides, the other problem that influenced shoot induction from the jackfruit seed was browning caused by phenolic compound (Plate 7). The freshness of fruits was also another factor that could have influenced the rate of shoot induction.

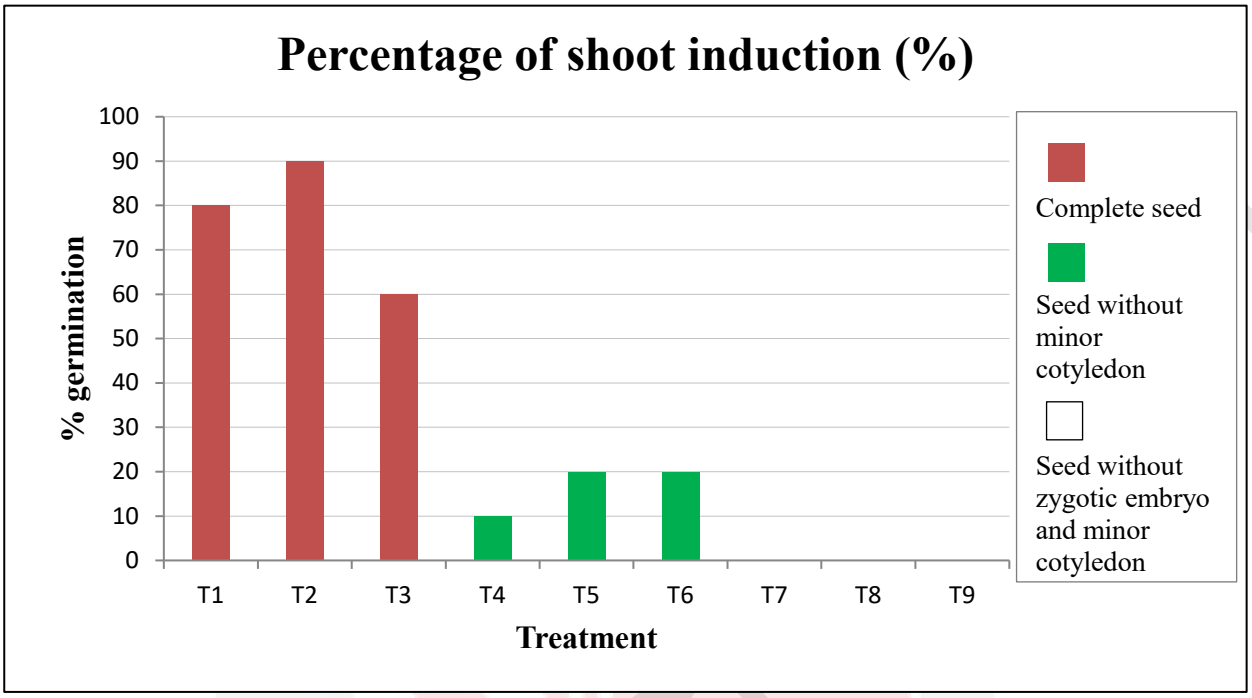

Figure 2 : Percentage of shoot induction from different structural form of *A. heterophyllus* seed placed on different BAP concentration after 12 weeks of culture

Treatments :

T1 : Complete seed + 0 mg/L BAP (control)

T2 : Complete seed + 4 mg/L BAP

T3 : Complete seed + 8 mg/L BAP

T4 : Seed without minor cotyledon + 0 mg/L BAP (control)

T5 : Seed without minor cotyledon + 4 mg/L BAP

T6 : Seed without minor cotyledon + 8 mg/L BAP

T7 : Seed without minor cotyledon and zygotic embryo + 0 mg/L BAP (control)

T8 : Seed without minor cotyledon and zygotic embryo + 4 mg/L BAP

T9 : Seed without minor cotyledon and zygotic embryo + 8 mg/L BAP

Plate 4 : Initial growth of shoot from complete seed placed on 4 mg/L BAP after 3 weeks of culture

Plate 5 : Initial growth of shoot from seed wihout minor cotyledon placed on 4 mg/L BAP after 3 weeks of culture

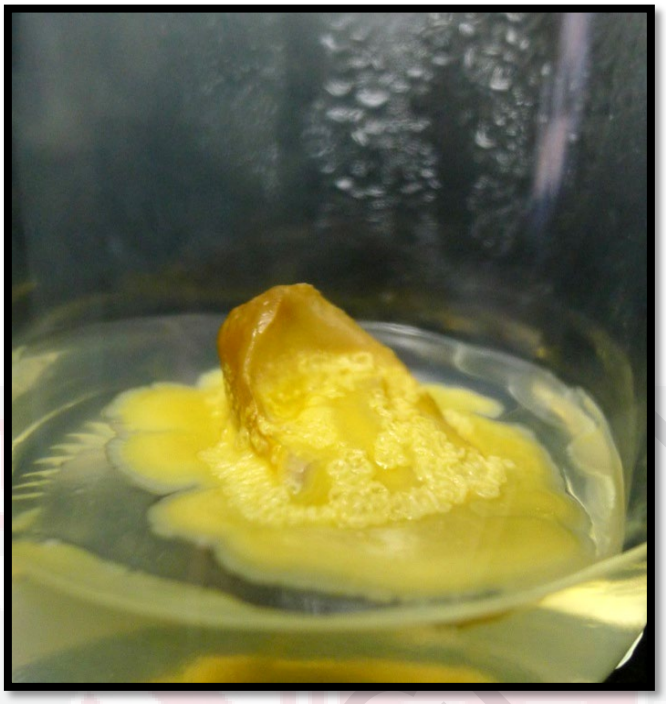

Plate 6 : Contamination of *A. heterophyllus* seed caused by bacteria

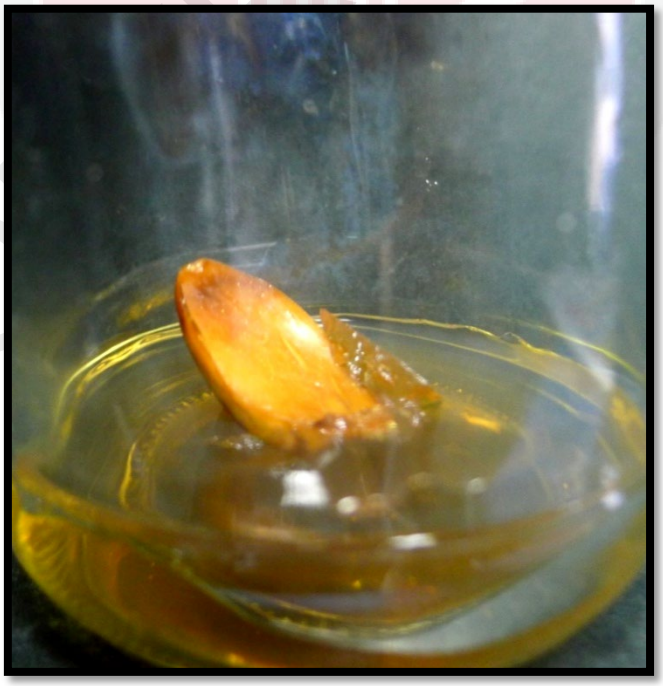

Plate 7 : Browning of *A. heterophyllus* seed due to exudation of phenolic compound

4.2 Mean number of shoots produced per seed explant.

Figure 3 shows the mean number of shoots formed per seed using different structural forms of the seeds placed on different concentrations of BAP. From the result, treatment 2 (complete seed with 4 mg/L BAP) (Plate 8) showed the highest mean number of shoots formed per seed at 5.9 shoots followed by treatment 3 (complete seed with 8 mg/L BAP) (Plate 9) and treatment 1 (complete seed with 0 mg/L BAP) at 4.2 and 1.3 shoots per seed, respectively. For seeds without minor cotyledon, those placed on 4 mg/L BAP (treatment 5) showed the highest mean number of shoots per seed (0.9 shoots) compared with treatment 6 (8 mg/L BAP) and treatment 4 (0 mg/L BAP) which resulted in 0.3 shoots and 0.1 shoots per seed respectively. However, in treatment 7 (seed without minor cotyledon and zygotic embryo + 0 mg/L BAP), treatment 8 (seed without minor cotyledon and zygotic embryo + 4 mg/L BAP) and treatment 9 (seed without minor cotyledon and zygotic embryo + 8 mg/L BAP) no shoot induction occurred.

Based on the ANOVA (Appendix 2), there was no significant difference between the treatments using complete seed (T1, T2 and T3) and treatments using the seed without minor cotyledon (T4, T5 and T6) on the mean number of shoots formed per seed after 12 weeks of culture. Therefore, the mean number of shoot production requires moderate level of BAP only. Based on the result, the mean number of shoots produced in treatment using 4 mg/L

BAP was higher compared to treatment using 0 mg/L BAP (control). The mean number of shoot induction on seed without minor cotyledon was lower compared to the mean number of shoot induction on complete seed. Hence, the removal of minor cotyledon affected the mean number of shoot induction. This was supported by the reports of Bonfil (1998) who showed that in *Quercus laurina*, cotyledon removal severely reduced the growth and survival rates of seedlings.

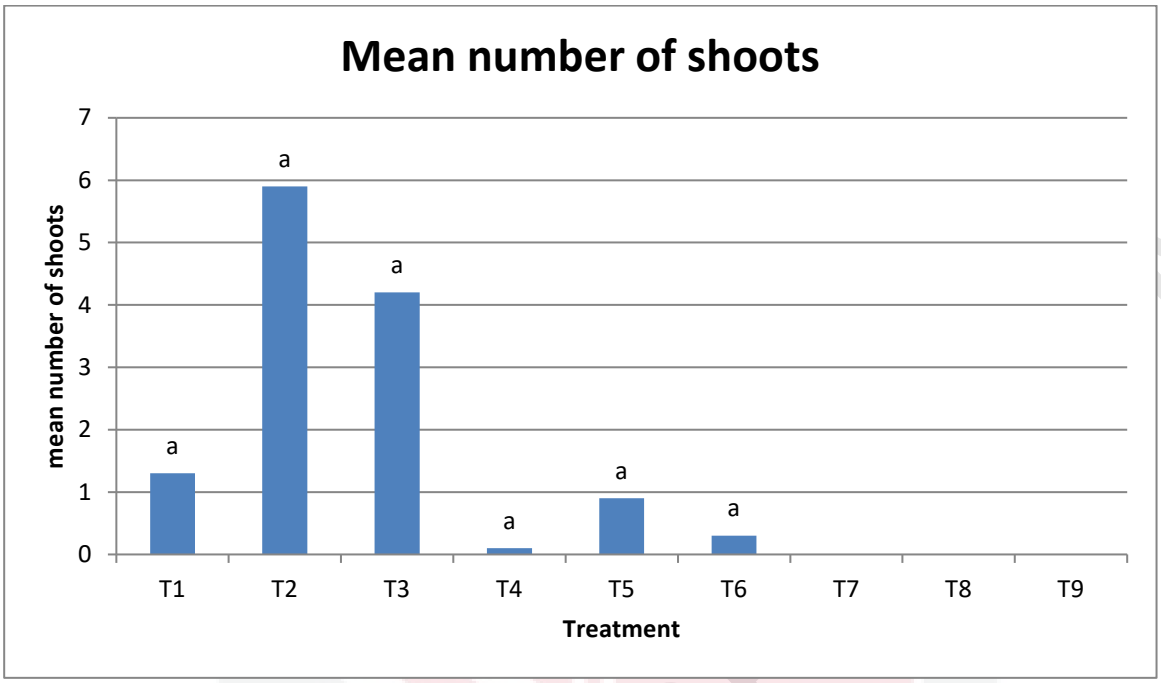

Figure 3 : Mean number of shoots formed per seed in *A. heterophyllus* after 12 weeks of culture.

Treatment:

T1 : Complete seed + 0 mg/L BAP (control)

T2 : Complete seed + 4 mg/L BAP

T3 : Complete seed + 8 mg/L BAP

T4 : Seed without minor cotyledon + 0 mg/L BAP (control)

T5 : Seed without minor cotyledon + 4 mg/L BAP

T6 : Seed without minor cotyledon + 8 mg/L BAP

T7 : Seed without minor cotyledon and zygotic embryo + 0 mg/L BAP (control)

T8 : Seed without minor cotyledon and zygotic embryo + 4 mg/L BAP

T9 : Seed without minor cotyledon and zygotic embryo + 8 mg/L BAP

Plate 8 : Multiple shoot formation on complete seed of *A. heterophyllus* placed on 4 mg/L BAP after 12 weeks of culture

Plate 9 : Multiple shoot formation on complete seed of *A. heterophyllus* placed on 8 mg/L BAP after 12 weeks of culture

4.3 Mean shoot length attained

Figure 4 shows the mean shoot length attained using different structural form of seeds placed on different concentrations of BAP. From the result, the treatment which showed the highest mean shoot length per seed (2.63 cm) was treatment 2 (complete seed with 4 mg/L BAP) followed by treatment 3 and treatment 1 with mean shoot length of 1.75 cm and 2.19 cm, respectively. For seed without minor cotyledon, those placed on 4 mg/L BAP (treatment 5) showed the highest mean shoot length (0.3 cm) compared with the mean shoot length attained in treatment 6 and treatment 4 at 0.25 cm and 0.15 cm, respectively. However, for treatment 7, treatment 8 and treatment 9, there were no shoots produced.

The result from ANOVA (Appendix 3), no significant difference between the treatments using complete seed (T1, T2 and T3) and treatments using the seed without minor cotyledon (T4, T5 and T6) on the mean shoot length after 12 weeks of culture. Treatments using seed without zygotic embryo (T7, T8 and T9) differed with treatments (T1, T2, T3, T4, T5 and T6) because in those treatments no shoot induction occurred. Therefore, shoot induction is impossible to occur when there is absence of zygotic embryo. Besides, the use of different concentration of BAP at 4 mg/L and 8 mg/L did not influence rate of shoot induction. Ashrafuzzaman *et al*. (2012) reported that regeneration of shoots from shoot tips of

jackfruit increased comparatively when MS medium was enriched with 2 mg/L of BAP. Amany *et al.* (2007) indicated that MS medium at half strength supplemented with 3.0 mg/L BAP gave the best shoot growth on shoot tip of jackfruit. Therefore, concentration of BAP below 4 mg/L is adequate for shoot induction in *A. heterophyllus*.

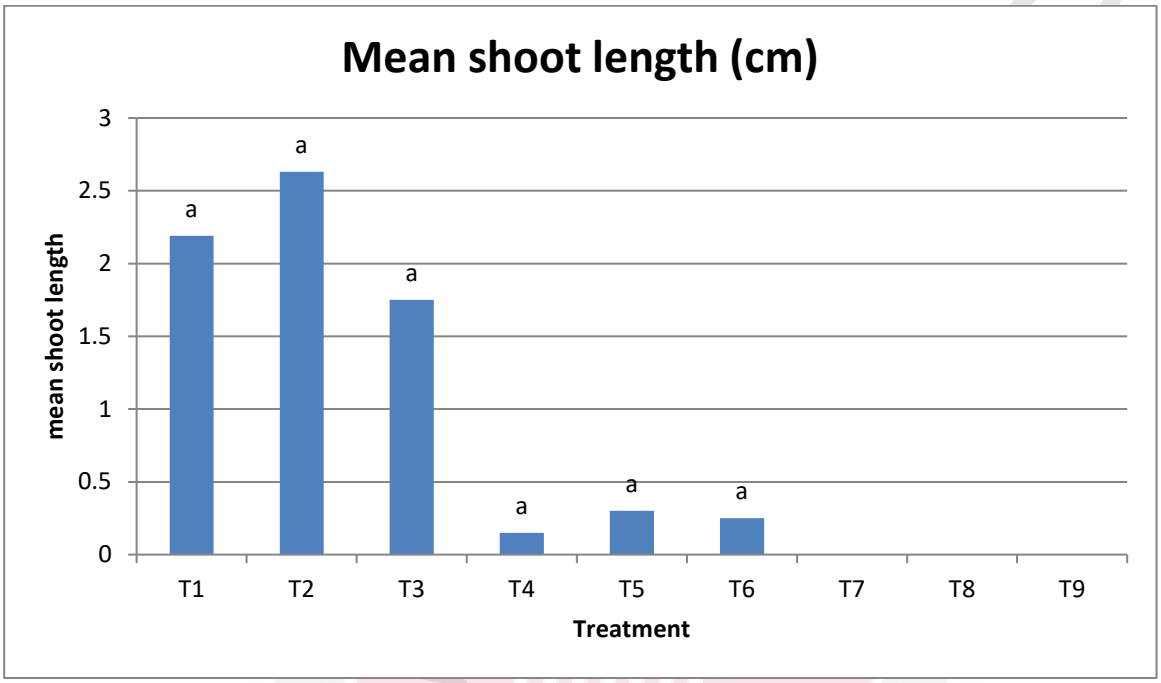

Figure 4 : Mean shoot length per seed per treatment at different concentration of BAP.

Treatment:

T1 : Complete seed + 0 mg/L BAP (control)

T2 : Complete seed + 4 mg/L BAP

T3 : Complete seed + 8 mg/L BAP

T4 : Seed without minor cotyledon + 0 mg/L BAP (control)

T5 : Seed without minor cotyledon + 4 mg/L BAP

T6 : Seed without minor cotyledon + 8 mg/L BAP

T7 : Seed without minor cotyledon and zygotic embryo + 0 mg/L BAP (control)

T8 : Seed without minor cotyledon and zygotic embryo + 4 mg/L BAP

T9 : Seed without minor cotyledon and zygotic embryo + 8 mg/L BAP

CHAPTER 5

CONCLUSION

In this study, the experiment was carried out to determine the ability of different structural forms of seed and the effect of different concentrations of BAP on shoot induction of *Artocarpus heterophyllus*. From the experiment, it was observed that the percentage of shoot induction from *A. heterophllyus* seed was influenced by the different structural form of seed. The percentage of shoot induction from complete seeds was higher compared to seeds without the minor cotyledon. Shoot induction was impossible when the zygotic embryo was totally removed. Complete seed cultured on 4 mg/L BAP was most effective in inducing the highest number and length of shoots.

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APPENDICES

APPENDIX 1

Murashige and Skoog (1962) Medium Formulation

No	Elements	Amount (mg/L)	Amount (g/L)
1	**Macro elements**		**10X**
	Calcium chloride, CaCl2	332.02	3.3202
	Potassium Dihydrogen Phosphate KH2PO4	170.00	1.7
	Potassium Nitrate, KNO3	1900.00	19
	Magnesium Sulphate, MgSO4	180.00	1.8
	Ammonium Nitrate,NH4NO3	1650.00	16.5
2	**Micro elements**		**100X**
	Cobalt Chloride,CoCl26H2O	0.025	0.025
	Cuprum Sulphhate, CuSO45H2O	0.025	0.025
	Boric Aid, H3BO4	6.20	6.2
	Potassium Iodide, KI	0.83	0.83
	Manganese Sulphate, MnSO44 H2O	16.90	16.9
	Sodium Molybdate, NaMoO42 H2O	0.25	2.5
	Zinc Sulphate, ZnSO47 H2O	8.60	8.6
3	**Vitamins**		**100X**
	Glycine, C2H5NO2	2.00	0.2
	Nicotinic Acid, C6H5NO2	0.50	0.05
	Pyridoxide, C8H11NO3	0.50	0.50
	Thiamine, C2H7CIN4O5	0.10	0.01
4	**Iron**		**100X**
	Disodium ethylenediaminetetraacetic acid, Na2EDTA	37.25	3.725
	Ferrous sulphate FeSO47 H2O	27.85	2.785
5	**Other**		
	Myo-inositol	100	0.1
	Sucrose	30000	30
	Gelrite	400	0.4

APPENDIX 2

The SAS System 21:33 Saturday, May 4, 2013 1

Obs	trt	blk	x	y
1	1	1	1	1.00000
2	1	2	.	.
3	1	3	1	1.00000
4	1	4	1	1.00000
5	1	5	5	2.23607
6	1	6	.	.
7	1	7	1	1.00000
8	1	8	.	.
9	1	9	1	1.00000
10	1	10	3	1.73205
11	2	1	7	2.64575
12	2	2	1	1.00000
13	2	3	9	3.00000
14	2	4	7	2.64575
15	2	5	4	2.00000
16	2	6	10	3.16228
17	2	7	12	3.46410
18	2	8	.	.
19	2	9	1	1.00000
20	2	10	8	2.82843
21	3	1	1	1.00000
22	3	2	16	4.00000
23	3	3	10	3.16228
24	3	4	11	3.31662
25	3	5	.	.
26	3	6	.	.
27	3	7	.	.
28	3	8	.	.
29	3	9	3	1.73205
30	3	10	1	1.00000
31	4	1	.	.
32	4	2	.	.
33	4	3	.	.
34	4	4	.	.
35	4	5	.	.
36	4	6	.	.
37	4	7	.	.
38	4	8	1	1.00000
39	4	9	.	.
40	4	10	.	.
41	5	1	.	.
42	5	2	4	2.00000
43	5	3	.	.
44	5	4	.	.
45	5	5	.	.
46	5	6	.	.
47	5	7	.	.
48	5	8	.	.
49	5	9	.	.

Obs	trt	blk	x	y
50	5	10	5	2.23607
51	6	1	.	.
52	6	2	.	.
53	6	3	.	.
54	6	4	2	1.41421
55	6	5	.	.
56	6	6	.	.
57	6	7	.	.
58	6	8	.	.
59	6	9	.	.
60	6	10	1	1.00000
61	7	1	.	.
62	7	2	.	.
63	7	3	.	.
64	7	4	.	.
65	7	5	.	.
66	7	6	.	.
67	7	7	.	.
68	7	8	.	.
69	7	9	.	.
70	7	10	.	.
71	8	1	.	.
72	8	2	.	.
73	8	3	.	.
74	8	4	.	.
75	8	5	.	.
76	8	6	.	.
77	8	7	.	.
78	8	8	.	.
79	8	9	.	.
80	8	10	.	.
81	9	1	.	.
82	9	2	.	.
83	9	3	.	.
84	9	4	.	.
85	9	5	.	.
86	9	6	.	.
87	9	7	.	.
88	9	8	.	.
89	9	9	.	.

The ANOVA Procedure

Class Level Information

Class	Levels	Values
trt	9	1 2 3 4 5 6 7 8 9
blk	10	1 2 3 4 5 6 7 8 9 10

Number of Observations Read 89
Number of Observations Used 27

The ANOVA Procedure

Dependent Variable: y

Source	DF	Sum of Squares	Mean Square	F Value	Pr> F
Model	14	14.04726431	1.00337602	1.14	0.4153
Error	12	10.57494686	0.88124557		
Corrected Total	26	24.62221117			

R-Square	CoeffVar	Root MSE	y Mean
0.570512	48.20893	0.938747	1.947247

Source	DF	Anova SS	Mean Square	F Value	Pr> F
blk	9	5.84591625	0.64954625	0.74	0.6712
trt	5	8.20134807	1.64026961	1.86	0.1753

The ANOVA Procedure

Duncan's Multiple Range Test for y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	12
Error Mean Square	0.881246
Harmonic Mean of Cell Sizes	2.478689

NOTE: Cell sizes are not equal.

Number of Means	2	3	4	5	6
Critical Range	1.837	1.923	1.975	2.010	2.033

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	trt
A	2.4163	9	2
A			
A	2.3685	6	3
A			
A	2.1180	2	5
A			
A	1.2812	7	1
A			
A	1.2071	2	6
A			
A	1.0000	1	4

APPENDIX 3

The SAS System 15:45 Sunday, May 5, 2013 1

Obs	trt	blk	x
1	1	1	2.5
2	1	2	.
3	1	3	1.0
4	1	4	3.2
5	1	5	2.5
6	1	6	.
7	1	7	0.7
8	1	8	.
9	1	9	5.2
10	1	10	6.8
11	2	1	4.0
12	2	2	2.0
13	2	3	5.0
14	2	4	.
15	2	5	3.0
16	2	6	5.5
17	2	7	3.0
18	2	8	.
19	2	9	0.8
20	2	10	3.0
21	3	1	2.8
22	3	2	2.7
23	3	3	5.0
24	3	4	4.0
25	3	5	.
26	3	6	.
27	3	7	.
28	3	8	.
29	3	9	1.5
30	3	10	1.5
31	4	1	.
32	4	2	.
33	4	3	.
34	4	4	.
35	4	5	.
36	4	6	.
37	4	7	.
38	4	8	1.5
39	4	9	.
40	4	10	.
41	5	1	.
42	5	2	1.5
43	5	3	.
44	5	4	.
45	5	5	.
46	5	6	.
47	5	7	.
48	5	8	.
49	5	9	.

Obs	trt	blk	x
50	5	10	1.5
51	6	1	.
52	6	2	.
53	6	3	.
54	6	4	1.3
55	6	5	.
56	6	6	.
57	6	7	.
58	6	8	.
59	6	9	.
60	6	10	1.2
61	7	1	.
62	7	2	.
63	7	3	.
64	7	4	.
65	7	5	.
66	7	6	.
67	7	7	.
68	7	8	.
69	7	9	.
70	7	10	.
71	8	1	.
72	8	2	.
73	8	3	.
74	8	4	.
75	8	5	.
76	8	6	.
77	8	7	.
78	8	8	.
79	8	9	.
80	8	10	.
81	9	1	.
82	9	2	.
83	9	3	.
84	9	4	.
85	9	5	.
86	9	6	.
87	9	7	.
88	9	8	.
89	9	9	.
90	9	10	.

The ANOVA Procedure

Class Level Information

Class	Levels	Values
trt	9	1 2 3 4 5 6 7 8 9
blk	10	1 2 3 4 5 6 7 8 9 10

Number of Observations Read 90
Number of Observations Used 26

The ANOVA Procedure

Dependent Variable: x

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	14	27.81286172	1.98663298	0.55	0.8554
Error	11	39.81675366	3.61970488		
Corrected Total	25	67.62961538			

R-Square	Coeff Var	Root MSE	x Mean
0.411253	68.04176	1.902552	2.796154

Source	DF	Anova SS	Mean Square	F Value	Pr > F
blk	9	15.19961538	1.68884615	0.47	0.8689
trt	5	12.61324634	2.52264927	0.70	0.6369

The ANOVA Procedure

Duncan's Multiple Range Test for x

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	11
Error Mean Square	3.619705
Harmonic Mean of Cell Sizes	2.464548

NOTE: Cell sizes are not equal.

Number of Means	2	3	4	5	6
Critical Range	3.772	3.946	4.049	4.117	4.163

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	trt
A	3.288	8	2
A			
A	3.129	7	1
A			
A	2.917	6	3
A			
A	1.500	1	4
A			
A	1.500	2	5
A			
A	1.250	2	6