The Effect of Packaging and Storage Periods on Germination and Vegetative Growth G2 Seedling of Sugarcane (*Saccharum Officinarum* L.) from Tissue Culture

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ABSTRACT

A field experiment to study the effect of packaging and storage periods on germination and vegetative growth G2 seedling of sugarcane (Saccharum officinarum L.) from tissue culture, has been conducted at Indonesian Sugar Research Institute Experimental (P3GI) , Pasuruan, ± 4 m asl, average temperature 24-32^{o}C, since August up to December 2011. The experiment was designed in a Randomized Block Design (RAK) non factorially with three replicates. The treatment were of : packaging method (i.e. M1 = vacuum plastic, M2 = without vacuum plastic, and M3 = "waring"); storage periods (i.e. L0 = unstorage, L1 = storage periods with 2 days, L2 = storage periods with 4 days L3 = storage periods with 6 days, L4 = storage periods with 8 days, and L5 = storage periods with 10 days). The result show that the combined treatment packaging method with "waring" and storage periods with 10 days increase to the observed variables germination percentage, stalk high, stalk diameter, leaf number, and the number of tiller.

Key word : *G2 seedling of sugarcane, packaging method, storage periods*

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INTRODUCTION

The concept of distribution of micro-generation mules budset two (G2) sugarcane tissue culture results taken for the supply of sugarcane planting material in bulk, and fulfilling aspects of quality, pure, and healthy. Seed is the seed of captive G2 G1 stem diameters of 1-2 cm and bred to the nursery ready. G1 seed is the seed from captive breeding of G0 (plantlets). Hence G2 seed cane diameter is relatively small compared to the conventional mules, it is necessary to study ways of packing a mule support for micro-G2 seed stays fresh during shipping process. In this study will be assessed the use of plastic bags and vacuum and without vacuum "waring". During this "waring" used in the delivery of conventional mules. The use of vacuum bags is expected to reduce the respiration of the seeds that the seeds remain fresh during delivery. In addition to packaging, storage time should be studied as well as both factors affect seed germination and vegetative growth of sugarcane tissue culture G2.

The purpose of this study is to determine the effect of the old ways of packaging and storage of seed germination and vegetative growth of sugarcane G2 and to find out how packaging and storage time as a good simulation of the delivery of seed cane.

The hypothesis proposed in this study is the way of packaging and storage time can affect seed germination and vegetative growth of sugarcane G2 and packaging using a vacuum bag can result in seed germination and vegetative growth of sugarcane G2 is better than the plastic bag without vacuum packaging and "waring ".

MATERIALS AND METHODS

The experiment was conducted in August-December 2011 in the Garden Experiments Pasuruan Indonesian Sugar Research Center (P3GI) Pasuruan. The tools used in research is a vacuum sealer, hoe, drill tools, yells, rulers, and the long slide. The materials used are the seeds of varieties of tissue culture origin G2 PS 862, 12.5 x10cm size polybag ¸ 40x35cm size baskets, ropes, "waring" size 30x25cm, 25x20cm size clear plastic bag, and mix the soil and planting medium sand. The draft study was prepared according to the Simple Random Group. The treatment is a combination of the old ways of packaging and storage. Way packaging is made up of three treatments. (M1 = plastic vacuum, M2 = plastic without vacuum, and M3 = "waring"), while the storage time of 6 treatments is (L0 = not recorded, L1 = storage time to 2 days), L2 = length of storage in 4 days), L3 = storage time to 6 days), L4 = storage time to 8 days), and L5 = storage time to 10 days). There are 18 treatment was repeated 3 times, in order to obtain 54 experimental plots. Observations made at the time of seed cane has been packaged and stored. Observations include depreciation of seed weight, percentage of moldy seeds, the percentage of normal eyes on, the percentage of roots that grow, and change the color on the surface of the seed pieces. Observations made during germination when the plants were 14, 28, and 42 dap. Observation parameters included the percentage of germination and shoot length. Observations made at the time the plants were 8, 10, 12.14, and 16 MST (weeks after planting). The parameters observed include stem height, stem diameter, number of leaves, number of tillers, and number of segments. Observational data obtained were analyzed using a variety of analysis (F test) at the level of 5%. When the test results obtained real difference then followed by a test comparison between treatment with the Smallest Real Differences (LSD) at the level of 5%.

RESULTS AND DISCUSSION

A. Packaging and storage

1.1 Depreciation of weight during storage of seed cane G2

The longer the seed cane G2 is stored, both are packed with plastic bags or plastic bags divakum without vacuum and "waring" cause shrinkage of the weight percentage of the higher seed. Shrinkage percentage weight of seed stored for 10 days with a plastic bag divakum packaging, plastic bags without vacuum and "waring" by 13.7%, 21.48% and 28.81%. Depreciation stored seed weight by using plastic bags and plastic bags divakum without vacuum started in the seeds stored for 4 days ie by 5.9% and 11.9%, while the seeds are packed with "waring" severe shrinkage of the seed has occurred in 2 days storage time is equal to 15.5%. Depreciation is the weight of seed cane G2 can be seen in Figure 1

1.2 The percentage of seed cane is moldy during storage

From these experiments, in general, only moldy seed cane sugar cane seeds are packed with "waring" starting storage time of 4 to 10 days with a percentage of 100%. Cane seeds are packed in plastic bags or without vacuum divakum either showed no moldy seed. The percentage of seeds that moldy during storage can be seen in Figure 2.

1.3 The percentage of normal seed cane buds during storage G2

The percentage of normal seed cane buds are packed with plastic bags and plastic bags divakum without vacuum began to be recorded are not recorded for 4 days and 10 days that is equal to 100%, but decreased the percentage of normal buds seen in seeds stored for 6 up to 8 days of 85%. The percentage of normal buds on the seeds are packed with "waring" the longer it will be kept declining. Seeds are not stored on all normal buds or buds may be said the percentage of normal is 100%. But the seeds stored for 2 to 10 days decreased the percentage of normal buds, but the percentage decrease in normal buds occurs slowly. The percentage of normal buds on seed cane G2 can be seen in Figure 3.

1.4 The percentage of roots that grow on the seed cane G2 during storage

Roots growing in G2 seed during storage occurs only in the seeds are packed with "waring". The longer the seed is stored then the percentage of roots that grow higher and higher, ie 100% of the storage time of 8 to 10 days. Roots begin to grow on the seeds stored for 2 days but the percentage of root growth is not very high at 13%. Seeds are packed with sugar cane using either a plastic bag and without vacuum divakum not show the roots that grow on the seed cane G2. Roots of sugar cane grown on the seed during storage G2 is displayed in Figure 4.

1.5 Changes in the color of the surface of the seed cane pieces during storage G2

Discoloration of the cut surface of seed cane G2 is influenced by a variety of seed packaging and storage time. Not all the effects of packaging treatments and storage duration causes the color change. On the data made clear that pengemasn seed cane with a plastic bag divakum with different levels of storage time does not cause discoloration of the cut surface of the seed. While the seeds by using plastic packaging without vacuum only storage time 10 days the color changes to red pieces. While the seeds are packed with sugar cane using the "waring" at all levels of storage time to change the color of the seed piece G2, the average color of the seed pieces turn red.

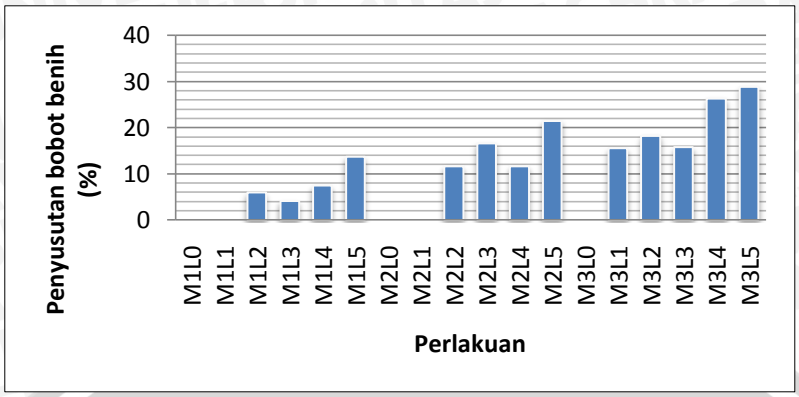


Figure 1. Histogram weight shrinkage during storage of seed cane G2

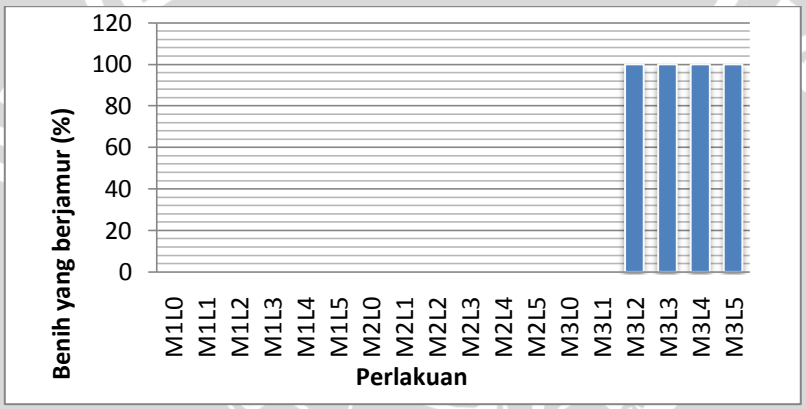


Figure 2. Histogram of seed cane G2 moldy during storage

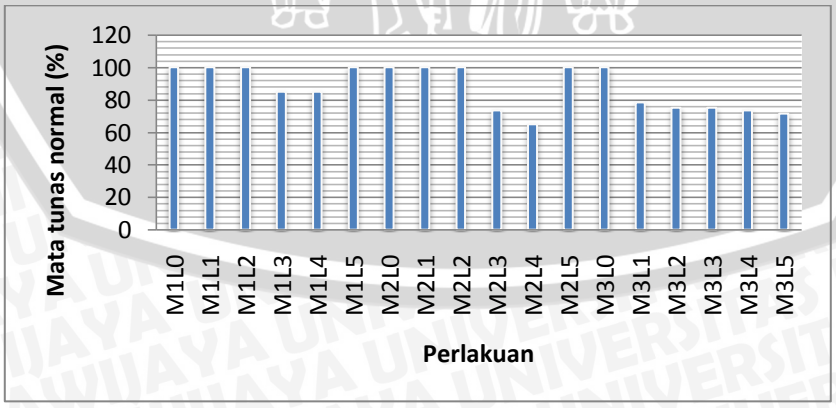


Figure 3. Histogram normal buds during storage of seed cane G2

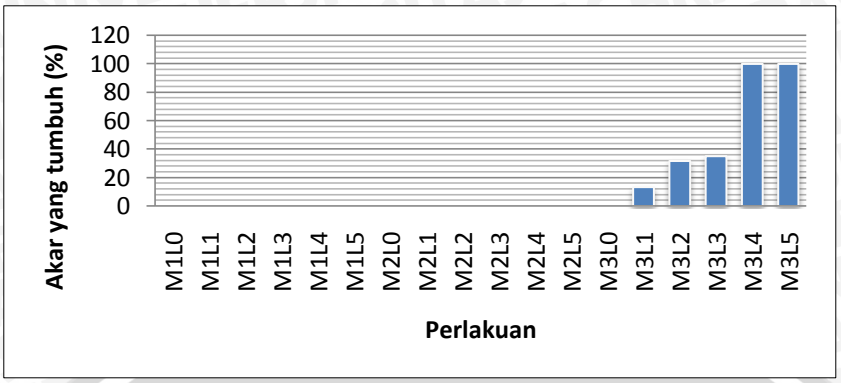


Figure 4. Histogram root sugarcane grown on the seed during storage G2

2. Germination

2.1 The sprouts

G2 germination of seed cane are packed in plastic bags and "waring" on a variety of storage time varies. In general, the longer it is stored germination decreased. Germination rate of decline varies, depending on a variety of packaging and storage time. In Figure 5 can be explained that the seeds are packed with sugar cane using a plastic bag divakum germination decreased in storage time of 6 to 10 days, it can be said that the seeds do not germinate on such treatment.

Germination of seeds are packed in plastic bags divakum stored for 4 days by 60%, when compared with vacuum packaging and plastic bags without "waring", higher germination is 71.67% and 78.33%. The lowest percentage of germination in seeds are packed in plastic bags without vacuum is 63.33% while the "waring" by 65%, and higher than the lowest percentage of germination in seeds that are packed with plastic bags divakum is 60%. Seeds are packed in plastic bags divakum germination started to decline from the old 2 to 4 days of storage, whereas seeds stored in plastic bags without vacuum germination began a long decline from 2 to 6 days of storage. While the seeds are packed with "waring" germination began to decline when stored for 2 to 10 days, the decline is quite low germination occurs when seeds are stored for 10 days. The percentage of seed germination of sugarcane G2 can be seen in Figure 5.

2.2 The length of shoots

Observations indicate that the treatment a variety of ways of packaging and storage time affects the parameter length of shoots at each age of observation. At the age of 14 HST observations of the highest shoot length in seed cane which is packed with "waring" that is stored for 6 days is 11.65 cm, cane seeds are packed in plastic bags that are not stored divakum had the highest shoot length is 29.57 cm at the age of 28 days of observation. Meanwhile, the seeds are packed in "waring" that is stored for 2 days at the age of 42 HST observations have the highest shoot length is 67.15 cm. Observations at various ages observations show that the average length of shoots varied.

Seeds are packed with sugar cane using a plastic bag divakum showed the best growth of shoots is not stored in the seed. Seeds are packed in plastic bags without vacuum growth is the best bud seeds stored for 6 days. While the seeds are packed with "waring", the seeds stored for 2 days had the highest shoot length. Overall it can be seen that the growth buds on seed that is packaged by using packaging "waring" has a good growth. Effect of different ways of packaging and storage time at different ages of observations can be seen in Figure 6.

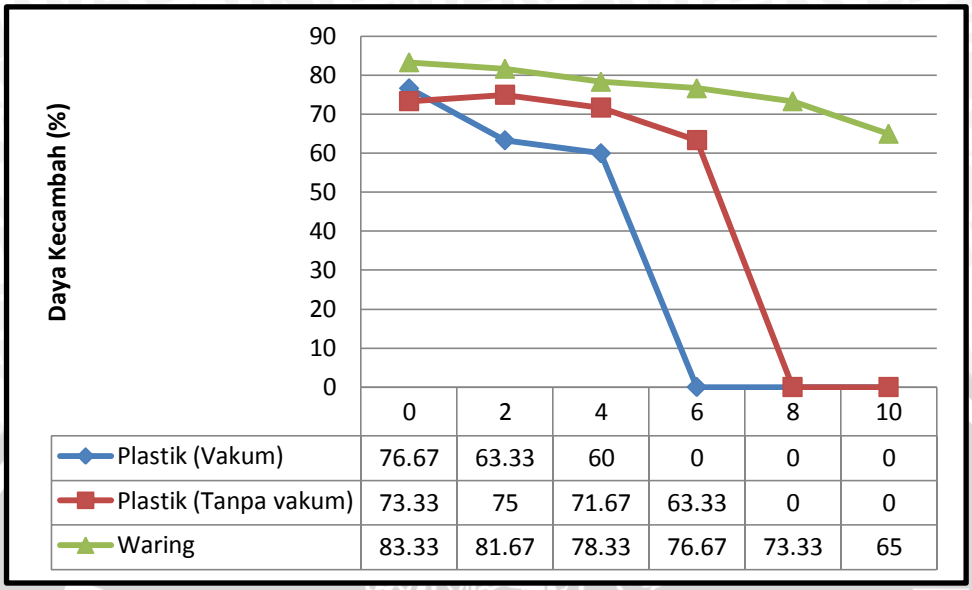
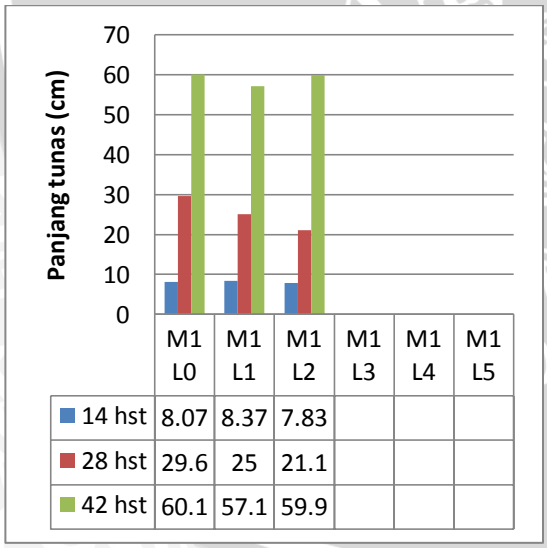
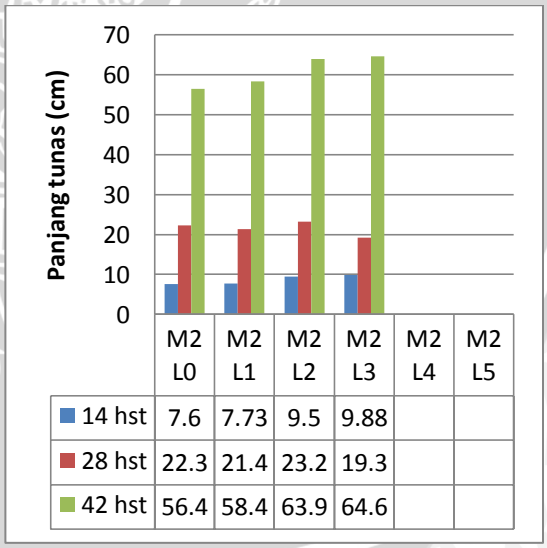


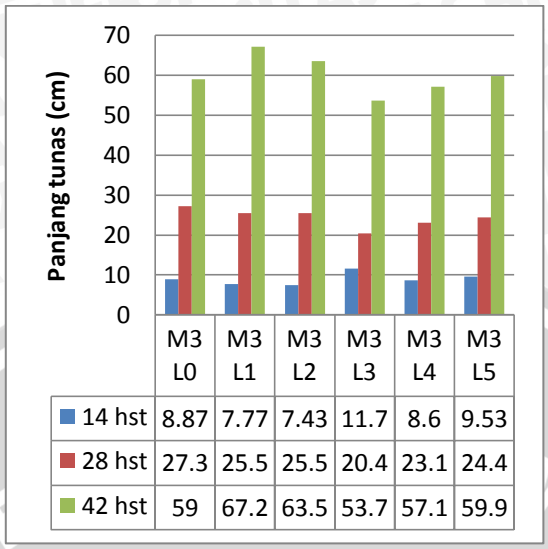
Figure 5. Graph G2 sugarcane seed germination



(a)



(b)



(c)

Figure 6. Histogram of the length of the seed cane shoots G2 at different ages of observations that are packed with (a) plastic divakum (b) plastic with no vacuum and (c) "waring".

3. Vegetative growth Height

3.1 Trunk

Different ways of packaging and storage time affect seed cane stem height at each age of observation G2. Stem height observations made when plants are 10 to 16 MST. High seed rods in general has increased quite high at the age of 12 MST observation. Observation of the age of 16 MST seed cane are packed in plastic bags divakum have the highest stem growth in seeds stored for 2 days ie stem height reached 164.67 cm. At the age of 10 MST cane seeds are packed with plastic bags without vacuum and stored for 4 days had a mean stem height of the highest compared with other treatment storage time is 95.8 cm.

Observations on the age of 12 and 14 MST on sugarcane seeds stored for 2 days had a mean stem height of the highest. Not saved seed has the best stem growth at the end of observation at 16 MST is 170.33. Packaging by using the "waring" at age 10 to 16 MST did not show a very real difference. Observations stem height at the age of 16 MST showed that the growth of sugarcane seeds are packed with "waring" that is stored for 10 days to have a good stem height growth compared with other treatments that stem height reached 179.67 cm.

3.2 Diameter

The results obtained are at the age of the 10 MST seed cane rod diameter ranging from 1.3 to 1.5 cm, while that at the age of 12 MST trunk diameter reaches 2.1 to 2.9 cm. Stem diameter ranged from 2.4 to 3.1 cm at the age of 14 HST observations and at the age of 16 MST has reached a trunk diameter of 3.1 to 3.4 cm. At the age of the 10 highest MST diameter rod that is in the seed cane is packed with "waring" that is not stored and are packed in plastic bags without vacuum and stored for 2 days which is 1.53 cm. Cane seeds are packed with "waring" that is stored for 4 days had the highest stem diameter is 2.93 cm at the age of 12 MST observation. Meanwhile, the seeds are packed in plastic bags divakum stored for 4 days at the age of 14 and 16 observations of the MST has a diameter at least as high as 3.17 cm and 3.37 cm. G2 seed cane rod diameter can be seen in Figure 8. Observations on the packing diameter rod with

a plastic bag in each respective divakum age observations showed no significant difference. Packaging seeds in plastic bags that are not divakum at the age of the 10 MST with storage time 2 and 4 days showed a real difference, it is also seen in the observations of the age of 14 and 16 MST. While the seeds are packed with sugar cane using the "waring" at age 10 to 14 MST showed no real difference, but at the age of 16 MST apparent difference in the seeds stored for 8 and 10 days.

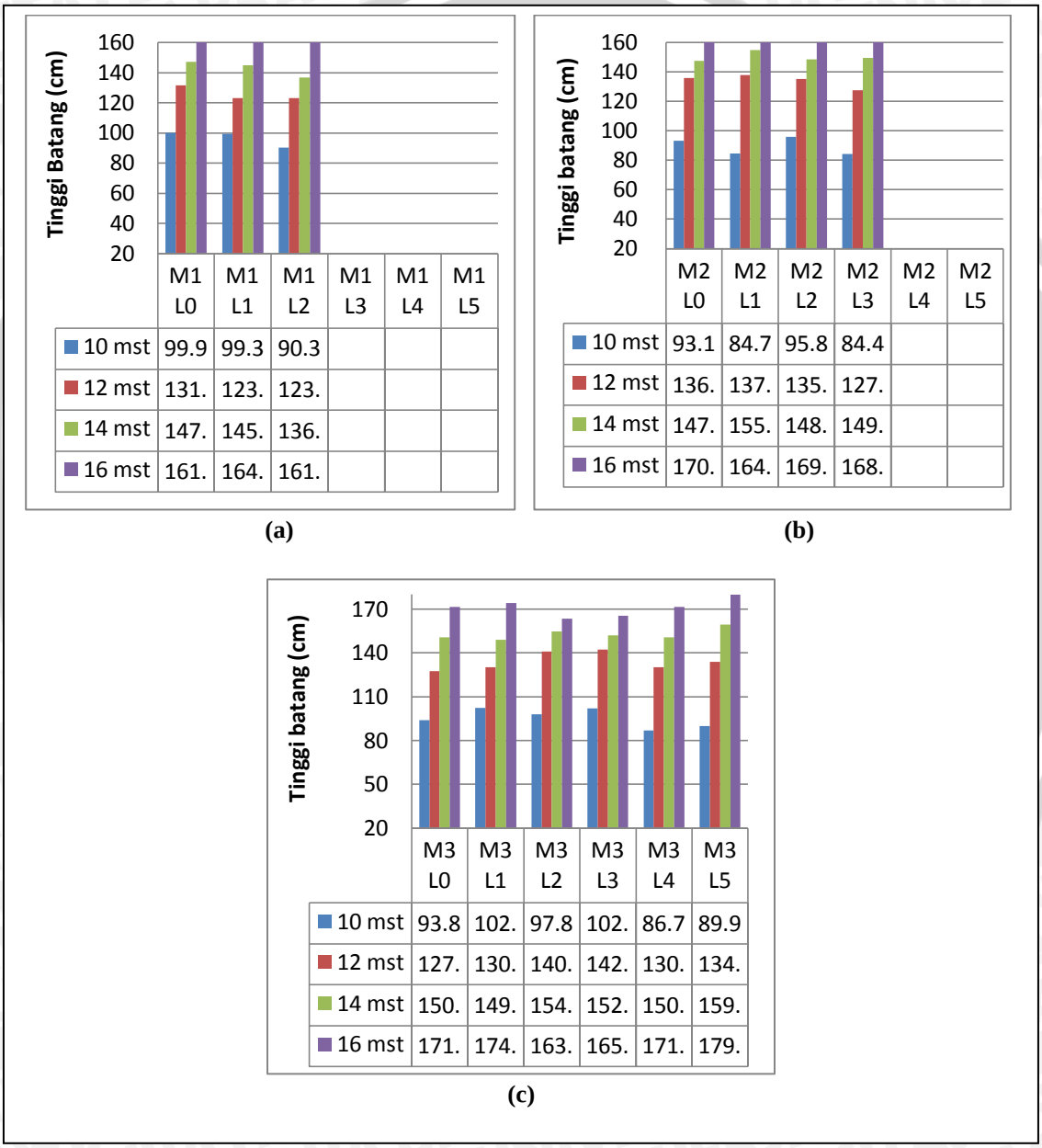


Figure 7. Histogram of seed cane G2 stem height at different ages of observations that are packed with (a) plastic divakum (b) plastic with no vacuum and (c) "waring"

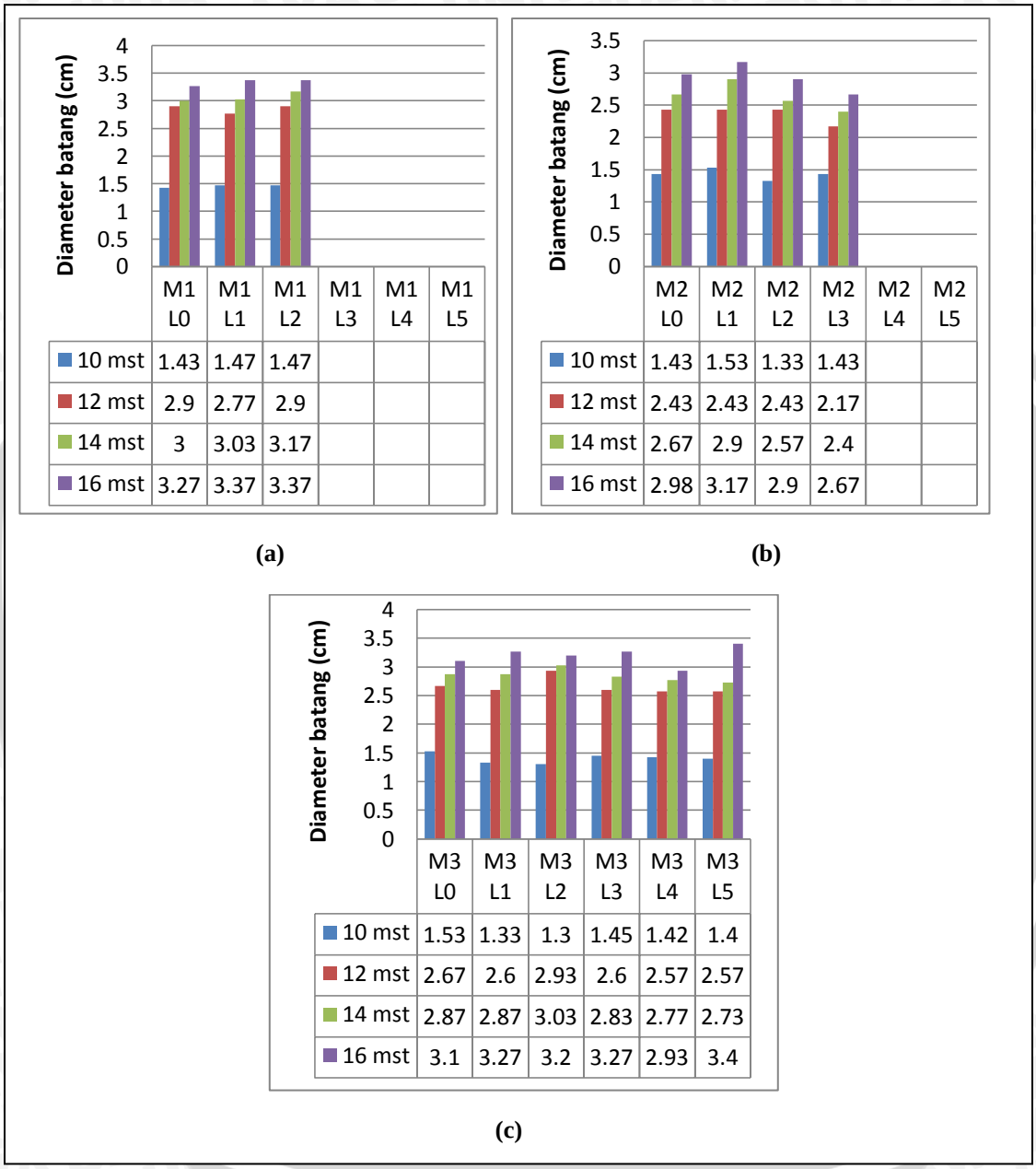


Figure 8. Histogram of the diameter of the seed cane rod G2 at different ages of observations that are packed with (a) plastic divakum (b) plastic with no vacuum and (c) "waring".

3.3 The number of tillers

Different ways of packaging and storage time affects the amount of seed cane seedlings G2. Observations made on the number of puppies aged 8 to 16 MST. At the age of 8 to 12 observations MST increased number of tillers in general, but when the seed was 14 MST number of tillers decreased until the age of 16 MST. The number of seedlings of seed cane with a variety of ways of packaging and storage time can be seen in Figure 9. Seeds are packed in plastic bags divakum indicates that the highest number of tillers present in seed that is not stored. Seeds are packed in plastic bags without vacuum stored for 2 days had a mean number of tillers of the most high. While the seeds are packed with "waring" saved 4 days the number of tillers is higher than the other treatment storage time. The number of pups at the age of 8 MST ranged from 3 to 5, where the highest number of tillers present in sugar cane seeds are packed in plastic bags that are not stored divakum and "waring" that is stored for 8 days. Packing in plastic bags stored divakum longer declining number of tillers. The situation is different from the seed that is packaged with a plastic bag without vacuum, where the number of tillers decreased in 2 days storage time, but when stored for 4 days and number of tillers is higher than the storage for 2 days. While the seeds are packed with "waring" the longer the higher the recorded number of tillers, but decreased at 10 days storage time.

Observations on the age of 10 MST, the number of puppies reach 6 to 7, where the seeds are packed with "waring" that is stored for 8 and 10 days had a mean number of tillers of the highest compared with other treatments. When the seed was 12 MST in general increases the number of tillers, but the seeds are packed "waring" with storage time for 8 and 10 days decreased the number of tillers than when the seed was 10 MST.

3.4 Number of leaves

The number of leaves at the age of 8 MST observation, sugarcane seeds are packed in plastic bags without vacuum and stored for 4 days had a mean number of leaves of the highest of 28.33, while the seeds are packed divakum plastic bags and stored for 4 days at the age of 10 observations MST has the highest average number of leaves is 48.67. Meanwhile, at the age of 12 observations of the MST, the seed cane which has a mean number of leaves was the highest that is packed with "waring" and stored for 10 days is 61.67.

In general, increasing the number of leaves occur at the age of the 10 MST. After the age of 12 MST seed leaf number did not experience a high increase in the number of leaves. Seeds are packed in plastic bags divakum averages the highest number of leaves contained in the seeds that are not stored. The longer the seed is stored then the average number of leaves decreased. Seeds are packed in plastic bags without vacuum present in seeds stored for 4 days. The mean number of leaves increased until the seeds are stored for 6 days, but decreased in seeds stored for 8 days. While the seeds are packed with "waring", the highest average number of leaves found in the seeds stored for 10 days with a mean number of leaves at 61.67.

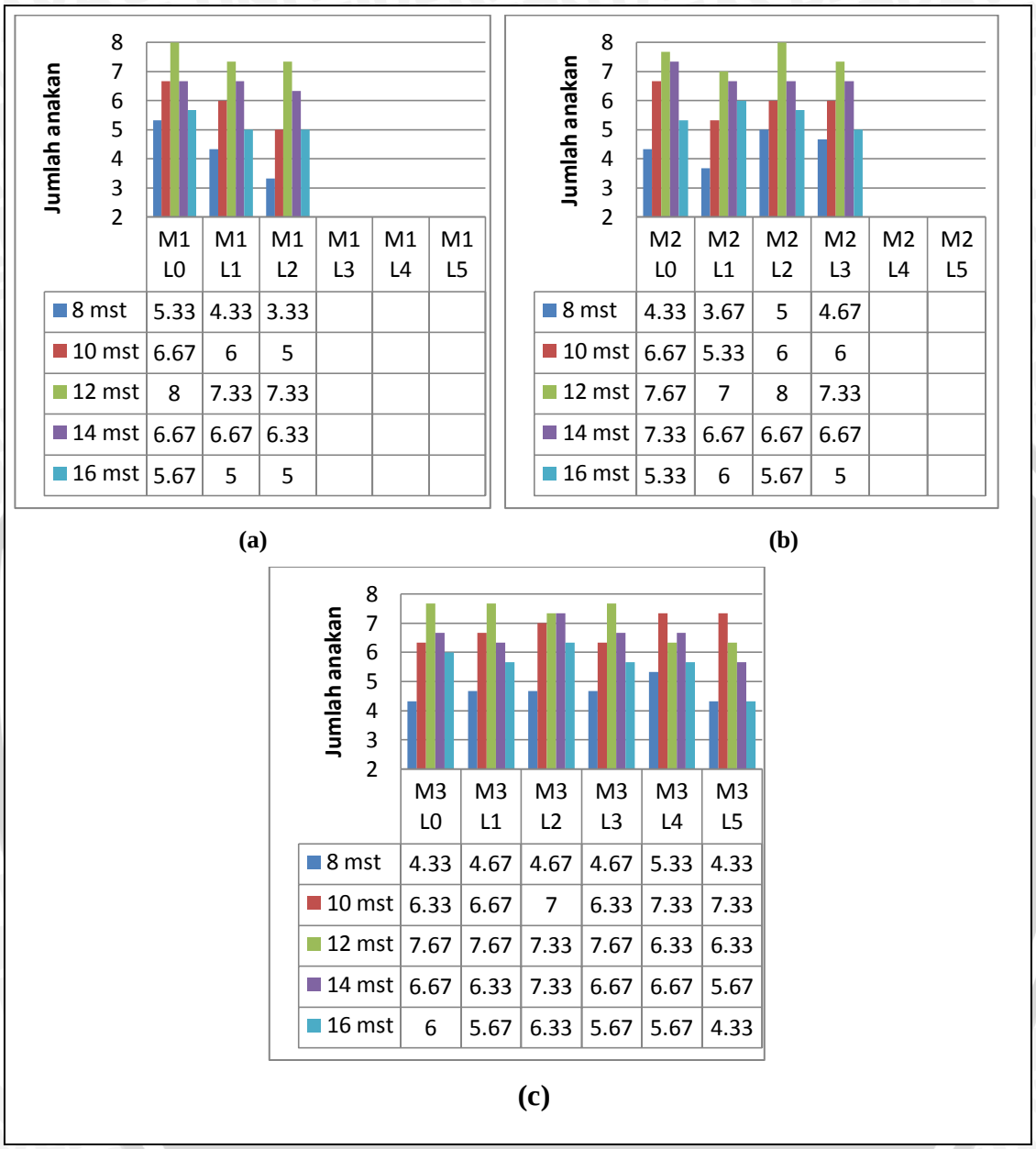


Figure 9. Histogram of the number of seed cane seedlings at various ages observations G2 is packed with (a) plastic divakum (b) plastic with no vacuum and (c) "waring".

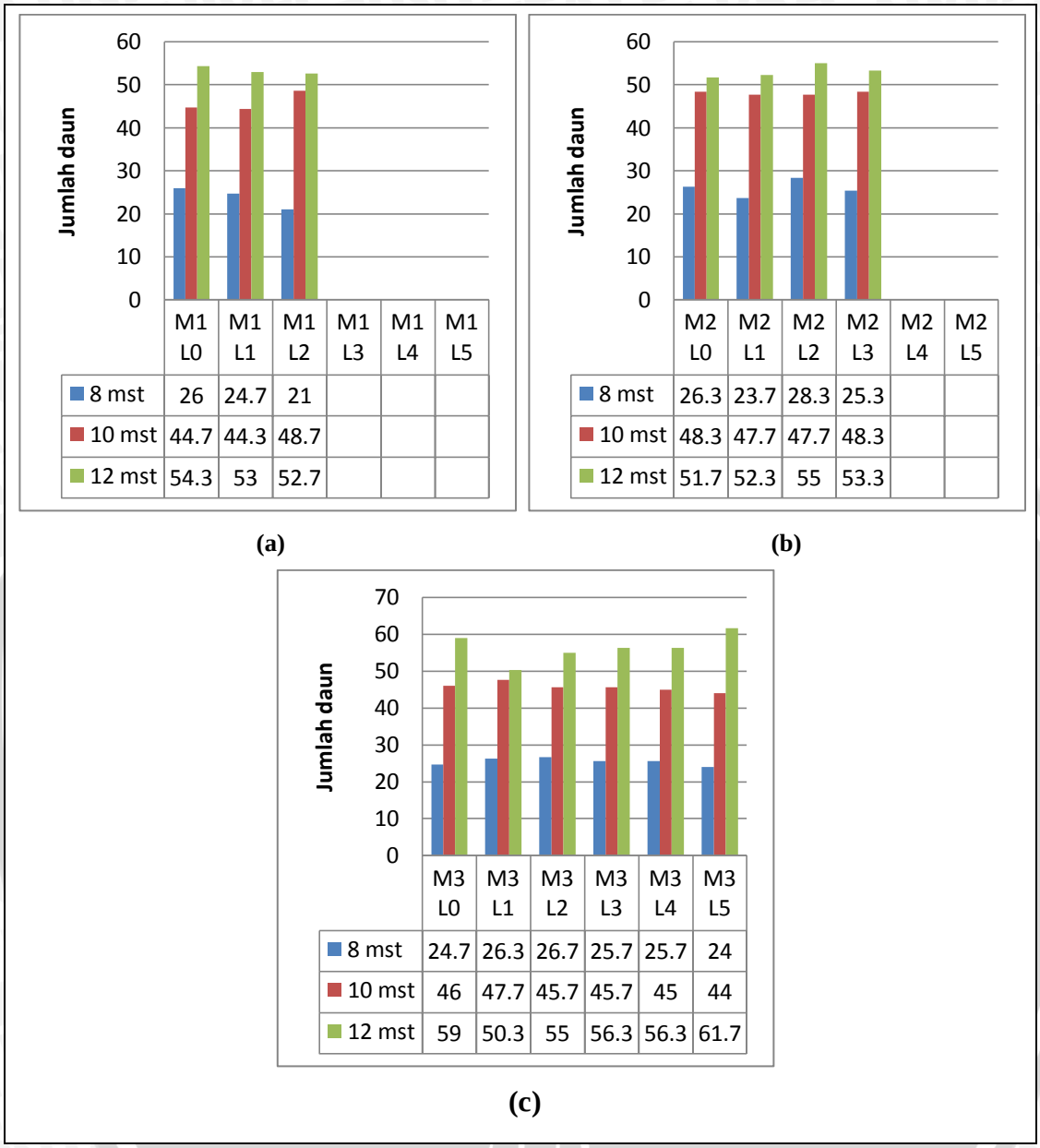


Figure 10. Histogram of the number of leaves of sugarcane seed G2 at different ages of observations that are packed with (a) plastic divakum (b) plastic with no vacuum and (c) "waring".

3.5 Number of segments

Observations made by counting the number of segments ranging from sugar cane land surface to the point of growing sugar cane. Observations on the age of 14 sugarcane MST segment number average number of 7 to 9. The mean number of segments the highest of the packaging with "waring" that is stored for 10 days. At the age of 16 seeds reached the MST, the number of segments account for 13 to 14, wherein the average number of segments is the highest seed cane are packed in plastic bags without vacuum divakum and

stored for 2 days. Observations on 18 MST showed that the number of segments reaches 15 to 16, where the highest number of segments contained in the seeds are packed with plastic bags without vacuum are not saved and stored for 2 days.

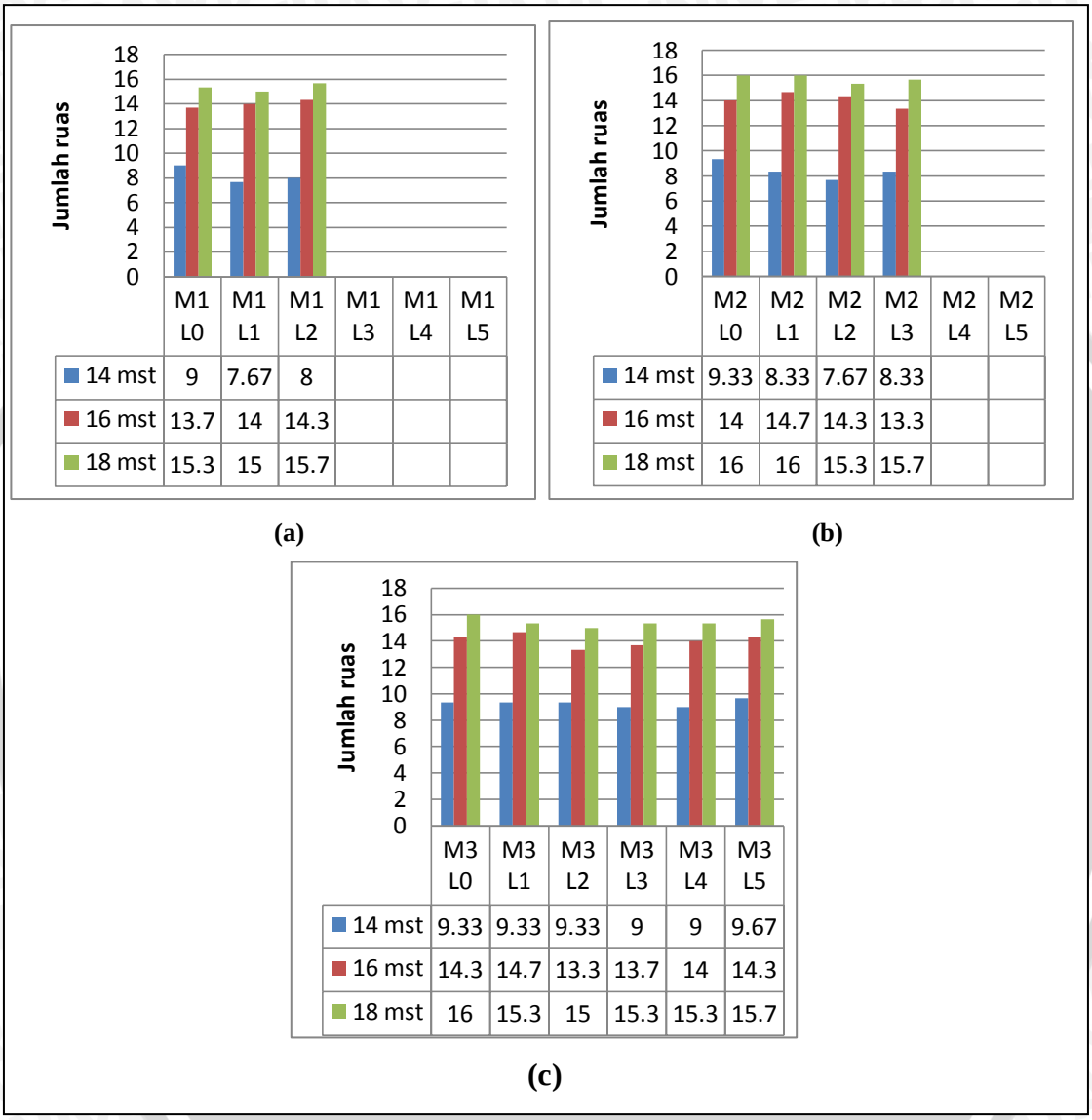


Figure 11. Histogram of the number of seed cane segment G2 at different ages of observations that are packed with (a) plastic divakum (b) plastic with no vacuum and (c) "waring".

DISCUSSION

Effect of different ways of packaging and storage time can be seen at the time of seed cane has been packed and then stored. Parameters that can be observed at the time depreciation is stored seed weight of seed cane G2, the percentage of seed cane is moldy during storage, the percentage of seed cane normal eye, the percentage of roots that grow and change in color that occurs on the surface of the seed cane pieces G2.

The results obtained that the highest seed weight shrinkage occurs in the seeds are packed with "waring" that is stored for 10 days. It may happen because of the packaging "waring" is porous so that the evaporation of liquid occurs in seeds and seed moisture content resulted in a shrinking. The longer the seed is stored it will decrease the water content of seeds so that the depreciation will be the higher seed weight. Packaging materials is critical to the security store. Packaging materials that are porous can cause the exchange of air from outside to inside or vice versa is very large, consequently the water content of seeds in the materials will decline more rapidly, as described by Harnowo and Utomo (1990). Moldy seed that occurs predominantly in the seeds are packed with "waring". Berjamurnya seed cane can occur because of the packaging "waring" air can enter through the hole on the packaging. In the air there is a bacterial spores, fungi, and viruses that are spread media for microorganisms. With the air entering the package "waring" then lead the growth of fungi on seed cane G2. The results show the percentage of roots that grow predominantly found in the seeds are packed with "waring". It may happen because of the packaging "waring" O2 in through the hole on the packaging. Which serves as the respiration of oxygen in the process of respiration occurs reshuffle sucrose into glucose. Glucose is converted in the process of respiration to energy (ATP) and amino acid compounds which function to form new cells so that the roots of the seedlings to grow sugar cane.

Discoloration of the cut surface of the seeds occurs during storage in which the packaging using the "waring" average color of the seed surface in maroon. Discoloration may occur due to sugar cane contain phenolic compounds with O2 to form an oxidized quinone compound, as described by Bariyus (2008). Color changes to red brown seed pieces are influenced by the presence of oxidase enzymes polypenol and oxygen into the package "waring" where polypenol oxidase enzyme activity, which with the help of oxygen will change the group monophenol into o-hydroxy phenol, which then changed to o-quinones . O-quinone groups thus form a brown color on the piece of cane. Enzymatic browning can occur because of an injured plant tissue, such as cuts and other treatments that can cause damage to plant tissue. Often lead to tissue damage in contact with the enzyme substrate. The enzyme responsible for enzymatic browning reaction is called fenolase oxidase, fenoloksidase, tyrosinase, polifenolase, or katekolase, as described by Cheng & Crisosto (2005).

On the germination parameters showed that the seeds are packed with sugar cane G2 uses "waring" have a higher germination than the seeds are packed with plastic bags either divakum or without vacuum. This is due to that in the plastic, especially the divakum O2 availability is limited, so that respiration rate is low while the seed cane require O2 for breathing (respiration). In the process of respiration takes sugar as a respiratory substrate, which is produced by hydrolysis of starch. During the renovation process of starch which is assisted by the availability of oxygen, will produce compounds such as glucose simpler during storage, as described by Muchtadi (1992). Food reserves in the form of glucose is converted in the process of respiration to energy (ATP) and to amino acid compounds which function to form new cells,

which then differentiate into tissue - tissue of sugarcane sprouts, it is consistent with that described by Dillewijn (1952).

Packaging affects seed germination of the seed cane. On cane seeds are packed with plastic bags that divakum, O2 is removed so the cane can not be berespirasi seed. Seeds are packed in plastic without using vacuum, O 2 can still remember packing into the packing is not too tight. While the seeds are packed with "waring" that is porous O2 in through holes in the packaging so that the seed cane can berespirasi. Seed cane requires O2 to breathe due to the respiration of the glucose is necessary for the growth of roots and buds. On the observation has been explained that the roots of sugar cane grown on the seed contained in the seeds are packed with "waring". Seed cane that has roots and buds that had grown sugarcane indicates that the seed has good germination and growth.

The results obtained by the observation that the seeds are packed with "waring" has a good growth on the parameters of stem height, number of segments, number of leaves and stem diameter. This suggests that the type of packaging affect the growth of seed cane. In the seeds are packed with "waring" O2 is very important to the process of respiration respiration in which the compound-forming dihasilnya new cells that affect germination. Seeds are packed with "waring" stored at the roots and buds have grown while the seeds are packed with plastic bags of roots and buds do not grow. From these results it can be seen that the seeds are packed with "waring" compared with the first germinated seeds are packed in plastic bags either vacuum or without vacuum. At the time of planting, the seeds have germinated when stored in advance will experience faster growth than sugar cane seeds are packed in plastic bags which take time to germinate when planted in advance. Thus affecting the growth of stem height, leaf number and stem diameter. The results showed the number of pups at week 8 to week 12 have increased, but at week 14 to the last observation at week 16 decreased the number of tillers. This occurred in connection with the ongoing competition between individuals within a population. In the study of seed that is packaged with "waring" long and 4 days of storage has a mean number of tillers of the most high. The average number of chicks reach 5-7 puppies. In the seeds are packed with "waring" higher than the number of progeny seeds are packed with plastic. In the vegetative period, sugarcane requires adequate water, nitrogen, phosphate, CO2, and sunlight, so that when the chicks grow up not having the disorder, the condition of sugar cane must be optimal, which is supported by the availability of the materials mentioned above, as described by Kuntohartono (1999).

The results showed that the variable number of segments, the average number of segments in the sugar cane crop at 14 weeks had a number of sections 13-14 segment, whereas at the age of 4 months (week 16) approximately 15-16 segments. At the last observation that the data obtained in plants that use plastic packaging method without vacuum and "waring" that is not stored has a mean number of segments that are not significantly different and the highest among other

KESIMPULAN

1. Seed packaging uses "waring" gives good results compared with the germination of seed that is packaged using plastic bags. On packaging "waring" higher O2 availability, O2 where the seed cane required for respiration in order to germinate the seeds properly.

2. Seeds are packed with sugar cane using the "waring" gives better growth than seeds that are packaged using plastic bags.

ADVICE

Concept needs to be studied in a controlled atmosphere packaging G2 seed cane. With the method of controlled atmosphere, the concentration ratio of O2 gas, CO2, and N2 in a plastic bag can be set so that the seed cane can berespirasi G2 and seeds can be stored for longer.

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