Viability Test and Breeding Growth on Poly-embryos Seeds

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Abstract. Seeds are mature ovules. One or more ovaries form in an ovary in a legume, but never more than one seed is formed in an ovary in a monocot. Each ripe seed always consists of at least two parts, namely: (1) Embryo, (2) Seed coat (Seed coat or testa). Embryo formed or derived from fertilized eggs (zygote) by undergoing cell division in the embryo. The seed coat is formed from the integument (one or more) of the ovules. In legumes generally, there are two layers of the seed coat. Every very young and growing seed always consists of three parts, namely: (1) Embryo, (2) seed coat, (3) Endosperm. Endosperm is a storage food storage network that is absorbed by the embryo before or during seed germination and is always present in very young seeds. Poly-embryos is the presence of more than one embryo in one seed, but these embryos do not always mature or mature, remain undeveloped or degenerate. The purpose of this research is to study the germination of one seed that has more than one embryo and to determine the growth of seedlings from poly-embryonic seeds. The results showed that the highest plant growth (plant height, number of leaves, fresh weight and dry weight of orange seeds) was found in orange seeds that had 1 embryo compared to orange seeds which had 2 and 3 embryos. Seed germination and growth are strongly influenced by the number of food reserves stored in seeds.

Keywords: Citrus Beans, Viability, Poly-embryos

1. Introduction

Seeds are mature ovules. One or more of the ovaries formed in the legume, but never more than one seed formed in the ovaries of monocots. Each ripe seed always consists of at least two parts, namely: (1) Embryo, (2) Seed coat (Seed coat or testa). The embryo is formed or derived from fertilized eggs (zygote) by undergoing cell division in the embryo sac. The seed coat is formed from the integument (one or more) of the ovules. In legumes generally, there are two layers of the seed coat. The inner layer is thin and soft, while the outer layer is thick and hard as a protective layer against temperature, disease, and mechanical touch. Every very young and growing seed always consists of three parts: (1) Embryo, (2) seed coat, (3) Endosperm. Endosperm is a storage food storage network that is absorbed by the embryo before or during seed germination and is always present in very young seeds [1]. Relationships in the reproductive structure from the flower phase to the fruit or ripe seeds are: ovule becomes seeds, ovaries become fruit, ovaries wall becomes pericarp (2n), nucellus becomes perisperm (2n), integument becomes testa or seed coat, 2 polar nuclei + sperm the nucleus becomes endosperm (3n), egg nucleus + sperm nucleus becomes zygote-embryo (2n), embryo sac will disappear, micropyle becomes micropyle, funiculus becomes hilum, funiculus + integument becomes raphae. In legumes (beans), seeds have 2 cotyledons without endosperm. The seed coat of the legume
is generally easily released from the seeds after soaking in hot water so that the whole seed or embryo is visible. This embryo consists of parts: (1) two cotyledons or seed leaves, (2) usually two small leaves around the growing point, (3) hypocotyl, (4) radicle. Food reserves contained in seeds can be in the form of (a) carbohydrates, especially in the form of flour, hemicellulose, sugar, (b) fat or oil and (c) protein [2].

Poly-embryonics is the presence of more than one embryo in one seed, but these embryos do not always mature or mature, remain undeveloped or degenerated. Poly-embryonics are formed because:
1. Cleavage during pro-embryo
   a. Zygotes divide irregularly to form groups of cells that grow simultaneously and form several embryos
   b. Pro-embryos form small shoots that can function as embryos
   c. The embryos that form the filaments become branched and each grows into an embryo
2. Derived from cells other than eggs (synergid)
3. There are more than 1 embryo sac in 1 ovule
4. Derived from sporophyte cells in 1 ovule (adventive embryo)

These poly-embryonic orange seeds, when germinated, can produce two kinds of seedlings, namely generative seedlings that come from meeting male and female cells, also called zygotes, while vegetative seedlings originate from embryos that are formed from a cell or a group of cells in the nucellus or integument. These vegetative seedlings are also called nucellar seedlings. The number of generative seedlings is only one, while nucellar/vegetative seedlings can amount to more than one seedling [3]. The percentage of sprouts of siam oranges from Kampar aged 1 month has a percentage of 43, 75%. Percentages can increase within 2 months because the seeds being sown have different vigor so that the growth of seeds is uneven [4].

The purpose of this research is to study the germination of one seed that has more than one embryo and to determine the growth of seedlings from poly-embryonic seeds.

2. Literature Review

Seed as plant material in maintaining its species is by trying to grow and develop. But in these seeds often found more than one embryo so that in one seed can grow more than one plant depending on the number of embryos contained in these seeds. The incident was first discovered by Antoni van Leeuwenbock in 1719 which occurred in orange seeds.

According to Nugroho et al (2006), poly-embryony is the presence of more than one embryo in one seed [5]. Poly-embryo occurs mostly in angiosperms, possibly due to several reasons, among others: (1) cleavage poly-embryony, (2) embryo originates from leaf cells in the bladder of institutions other than fertilized eggs, (3) formed institutions that are numerous in one ovule and (4) the activity of sporophytic cells (soma cells) in the ovule.

The germination of siam oranges from Kampar produces 1 to 3 seedlings in one seed planted for 1 month. The seedlings are derived from mono embryo type seeds that produce one seedling, di-embryo type seeds that produce him seedlings and triembryo type seeds that produce three seedlings (Widianti et al, 2013).
The yield of polyembryony seedlings is influenced by the shape and size of the seeds, where Siamese and Kasturi cultivars have ovoid and long ovate seeds, while Nipis cultivars have ovoid, long ovate and flattened seeds.

In the component of an embryo, orange seeds consist of plumula, radicals, and pieces of seeds. Plumula is a candidate for stems and leaves, radicals are candidates for roots, while the seed pieces are usually large and thick functioning as food storage organs (Frost and Soost, 1968 Cit. Ulin, 1983). Ashari et al. (1988) suggested that the process of seed formation in oranges in two ways, namely the merging between eggs and sperm produces generative embryos, while vegetative embryos develop due to the division of nucleus tissue adjacent to the embryo sac [6].

The percentage of the number of polyembryony and the number of seed embryos that can germinate on citrus plants is influenced by nutrient status and physiological activity of the plant, as well as the branches where the fruit is formed, the age of the plant and the period of fruiting also influence the number of seed embryos per fruit [7].

Citrus seed and Lanshe fruit including recalcitrant seeds that do not have a dormant period. According to Castle (1981), orange seeds are recalcitrant seeds, which means they easily lose viability in certain (low) water contents. Because it must remain in a fresh state. However, even though it is germinated fresh, the speed of germination is still relatively slow and non-uniform [8].

3. Methods

3.1 Materials and tools
The research experiment was conducted in October 2016 at the General Laboratory of the Faculty of Agriculture, Teuku Umar University. The ingredients used are orange seeds, manure, filter paper, and cotton. The tools used include germination tanks, rulers, ovens and analytical scales.

3.2 Experiment Method
The experiment used a non-factorial design which was arranged in a completely randomized design with 3 replications. Data were analyzed using an LSD level of 5%. The factor tested was the separation of embryos consisting of 3 levels, namely:
   a. Nominated seedlings 1
   b. Nominated seedlings 2
   c. Nominated seedlings 3

3.3 Research Implementation
a. Select fresh and ripe oranges, separate the seeds from the fruit, then wash them thoroughly and dry in the sun until the orange seeds are no longer sticky when held.

b. Prepared like germination, covered with cotton and then moistened with water. Seeds are arranged on cotton that has been prepared, imbibition fruit seeds a few days until the seeds swell. These swollen seeds make it easy to see the number of embryos and seeds that have both complete and separate embryos.

c. Selected seeds that have seedlings 1, 2 and 3. Nusantara seeds are planted according to the embryo amount of 10 seeds each.

3.4 Observation
3.4.1 Germination
The percentage of poly-embryonic seeds germinated from each treatment was calculated, using the formula:

\[
\% \text{ sprouts} = \frac{\text{number of sprouts produced}}{\text{number of seeds planted}} \times 100\%
\]

\[
\% \text{ poly-embryos} = \frac{\text{Number of poly-embryos seedlings}}{\text{Number of seeds germinated}} \times 100\%
\]

3.4.2 Seedling growth
After 1 month 5 samples of seedlings were taken randomly to observe their growth in the parameters:
- Seed height
- Leaf area
- Number of leaves
- Fresh weight of seeds
- Seed dry weight

4. Results and Discussion

4.1 Results

4.1.1 Germination
The percentage of germination of citrus seeds has a percentage of 70.00%, while the percentage of poly-embryonic seeds is only around 71% of the germinated seeds. Kultunow in Widianti et al 2005, stated that in the formation of poly-embryonic seeds in Citrus plants many non-zygotic nucellar embryos were initiated directly from their parent cells, where the nucellar cells surround embryonic sacs containing developing zygotic embryos. During embryonic sac development, these embryonic cells enter the endosperm tissue and then develop into embryos in zygotic embryos. These embryos of the archipelago continue to develop and produce several seedlings with the same genotype as female elders

4.1.2 Plant Height
The results of the F test in the analysis of variance (even-numbered attachments) showed that polyembryony seeds had no significant effect on plant height at 7 days after planting (HST), but had a significant effect at 14, 21 and 28 days after planting. The average height of citrus seedlings in some embryos can be seen in Table 1.

| Treatment                            | Plant Height (cm) |
|--------------------------------------|-------------------|
|                                      | 7 HST | 14 HST | 21 HST | 28 HST |
| B1 (1 Nominated seedlings)           | 3.63  | 5.51 a | 6.21 b | 6.66 b |
| B2 (2 Nominated seedlings)           | 3.38  | 4.23 a | 4.86 a | 5.31 a |
| B3 (3 Nominated seedlings)           | 3.50  | 4.37 a | 4.92 a | 5.49 a |
| BNT                                  | -     | 0.59   | 0.60   | 0.46   |

Note: The mean in the same column and followed by the same letter are not significantly different according to LSD 5%

Table 1 shows that orange polyembryonic seeds had no significant effect on plant height at 7 HST. This is presumably due to the initial growth of plants starting with the height characteristics of the plant itself and then followed by other growth along with increasing plant age so that the results of the analysis of variants showed the same trend [9]. At the age of 14, 21 and 28 days after planting the highest plants were found in orange seeds that had 1 embryo compared to orange seeds that had 2 and
3 seedlings. This is because 1 orange embryo seed (nucellar seed) does not have competition between other embryos to grow so that with existing food reserves can encourage better seed growth. While the orange seeds that have 2 and 3 embryos (seedlings nucellar) there is competition between other embryos. Existing embryos in orange seeds will become new plants so that food reserves in the seeds are not sufficient to grow and develop for the orange seeds. Seed germination and growth are strongly influenced by the number of food reserves stored in seeds (Magagula and Ossom 2011 in Hasnah M, 2013).

4.1.3 Number of Leaves
Test results F on the analysis of variance (even-numbered attachments) showed that the orange seed polyembryonic seed significantly affected the number of leaves of plants aged 7, 14, 21, and 28 days after planting.

| Treatment               | Number of Leaves |
|-------------------------|------------------|
| Symbol                  | 7 HST 14 HST 21 HST 28 HST |
| B1 (1 Nominated seedlings) | 2.00 a 4.11 a 4.89 a 5.44 a |
| B2 (2 Nominated seedlings) | 3.78 b 5.33 b 5.89 b 6.22 b |
| B3 (3 Nominated seedlings) | 5.78 c 6.44 c 6.67 c 7.22 c |
| BNT                     | 0.40 0.50 1.14 0.91 |

Note: The mean in the same column and followed by the same letter are not significantly different according to LSD 5%

Table 2 shows that orange polyembryonic seeds significantly affect the number of leaves aged 7, 14, 21 and 28 days after planting. This is due to the character of the orange seed itself where the orange seeds that have 1 embryo only have 2 leaves, while seeds that have 2 embryos have sprouted with 4 leaves and also shown on seeds that have 3 embryos have 6 leaves on the seed sprouts the orange.

4.1.4 Fresh Weight and Dry Weight Citrus seed (gr)
Results test F on the analysis of variance (even-numbered attachments) showed that the orange seed polyembryony seed significantly affected the fresh weight and dry weight of orange seeds. The average fresh weight and dry weight of some embryos can be seen in Table 3.

| Treatment               | Fresh weight | Dry weight |
|-------------------------|--------------|------------|
| B1 (1 Nominated seedlings) | 1.27 a       | 0.42 a     |
| B2 (2 Nominated seedlings) | 1.35 a       | 0.52 a     |
| B3 (3 Nominated seedlings) | 1.54 b       | 0.63 b     |
| BNT                     | 0.10         | 0.15       |

Note: The mean in the same column and followed by the same letter are not significantly different according to LSD 5%

Table 3 shows that the best fresh weight and dry weight are found in orange seeds that have 3 embryos. This is because embryo 3 seeds have more plant organs (number of leaves) so that the fresh weight and dry weight are also heavier than those of embryos 1 and 2.

4.2 Discussion
The percentage of polyembryony seeds can increase with increasing seeding time because the seeds sown have different strengths so that the seed growth is uneven. The percentage of the number of polyembryonic and the number of seed embryos that can germinate in citrus plants is influenced by the nutritional status and physiological activity of the plant, as well as the branches where the fruit is formed, the age of the plant and fruit period also affect the number of seed embryos per fruit (Maheswari and Swamy, 1957). In addition, polyembrioni seed orange plants are also influenced by
the shape of the seeds themselves which are in the form of round and ovoid seeds can produce two to three seeds in one seed (Awuy, 1993 in Widianti et al, 2005).

For the growth of the whole one embryo citrus seedlings showed better growth compared to two whole and three whole embryos. This is because food reserves are not divided by the number of embryos contained in the seeds so that seeds that have a smaller number of embryos will grow faster than those who have more embryos. In the germination process needed food reserves that are ready to be decomposed for metabolic activities, so that seeds which have more food reserves will germinate faster if placed in optimum conditions. This is because the supply of food reserves is sufficient to support plant growth.

According to Magagula and Ossom. 2011, germination and growth of seedlings are strongly influenced by the number of food reserves stored in seeds. Seed mean shows the amount of food reserves, protein, mitochondrial activity, speed/ability of respiration / ATP production and growth potential (Rahmawati & Saenong, 2010). The content of food reserves will affect the weight of a seed, it will affect the amount of production and speed of seed growth. Because the seeds are heavy with a large content of food reserves will produce greater energy during the germination process [10]. Thus it will affect the number of sprouts that come out and the weight of the plant.

According to Roach and Wulff (1987) in Hasnah, M (2013), the mother tissue around the embryo such as differences in the skin layer can affect germination.

5. Conclusion

The best polyembryony orange seeds are found in orange seeds that have 1 seedling strand compared to 2 seedlings and 3 seedlings. It is hoped that further research will be carried out on the growth of other polyembryonic plants over a longer period.

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