APST

Asia-Pacific Journal of Science and Technology

https://www.tci-thaijo.org/index.php/APST/index

Published by Research Department,
Khon Kaen University, Thailand

Evaluation of the morphological and physiological changes of Saluyot (*Corchorus olitorius*) during seed maturation

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Received 29 January 2024
Revised 2 April 2024
Accepted 28 May 2024

Abstract

This paper explores the morphological and physiological changes in Saluyot seeds as they reach physiological maturity. The experiment encompassed the period from anthesis to physiological maturity, with observations conducted at three-day intervals. Monitored parameters included seed pod and seed color, size, seed moisture content, seed dry weight, and seed germination rate. The results indicate a gradual increase in seed size from 21 days after anthesis (AA) to 39 days AA, followed by a decline at 42 days AA, with average seed sizes ranging from 1.18 mm to 1.46 mm and 1.42 mm, respectively. Additionally, the color of the seeds and their pods gradually changes from green to the formation of black spots, signifying chlorophyll degradation. The moisture content (MC) of the seeds also decreases progressively from 9 days to 24 days AA, with a rapid loss recorded from 27 days to 42 days AA, averaging between 86.78% and 78.21%, and 40.54% to 16.64%, respectively. Regarding seed dry weight, the maximum weight was observed between 39- and 42-days AA, at 0.71 mg and 0.70 mg, respectively, indicating the physiological maturity of seeds within this period. Moreover, Saluyot seeds displayed a low germination rate, with a maximum of 40% observed on 39 days AA. This low germination rate suggests the onset of dormancy in Saluyot seeds post-harvest. In summary, the physiological maturity of seeds, as indicated by maximum seed dry weight and germination rate, occurs at 39 days AA.

Keywords: Saluyot, saluyot seeds, physiological maturity, moisture content, seed dry weight, seed maturity, seed science.

1. Introduction

A seed is an embryo, an immature diploid sporophyte that develops from the zygote and is covered in a seed coat and nutritive tissue [1]. The process of seed development begins with the onset of flowering, leading to the creation of floral structures and successful pollination [2]. According to Delouch [3], the progression of seed development and maturation involves a sequence of morphological, physical, physiological, and biochemical transformations that take place from the fertilization of the ovule until the point at which seeds achieve physiological independence from the parent plant. The transformation from a fertilized ovule to a dormant seed involves crucial changes that are essential not only for the development of the seed but also for determining its quality and viability. Physiological maturity, a critical stage in seed development, underscores the intricate relationship between these changes and their influence on seed quality, storage potential, and germination success.

Numerous indicators could forecast the correct stage of physiological maturity such as seed moisture content, seed size, seed dry weight, the germination potential of the seed, and the seed vigor [2]. As the seed reaches its physiological maturity, its moisture content also gradually declines and intensifies after physiological maturity [4], seed dry matter increases and becomes maximum during physiological maturity [2]. Furthermore, physiological maturity can also be characterized when seeds reach the maximum dry weight, germination, and vigor.

Physiological maturity and seed quality are closely related; seed quality is an essential component of sustainable agriculture. Optimal vigor and storability are possessed by seeds during physiological maturity. They can better handle the difficulties of handling, storing, and working in harsh environments because they are more stable to environmental pressures. Seeds harvested before reaching physiological maturity may lose some of their vitality and germination capacity. On the other hand, collecting seeds beyond their physiological development might result in a reduced capacity for storage and possible difficulties in breaking dormancy.

Saluyot, scientifically known as *Corchorus olitorius*, is a leafy green vegetable renowned for its nutritional value and culinary versatility [5]. As a leafy vegetable, it serves as an excellent source of proteins, dietary fiber, carotene, and vitamins (A, C, E), as well as mineral nutrients such as potassium, calcium, phosphorus, and iron [6]. Saluyot leaves contain plentiful antioxidant compounds with diverse biological effects, including diuretic, analgesic, antipyretic, and antimicrobial properties, as well as antitumor and phenolic antioxidative attributes, making them suitable for biomedical applications [7]. In recent years, studies have also explored the potential of Saluyot powder-based composites for sustainable applications [8, 9], as well as the potential of Saluyot-based hydrocolloids [10].

The applications of Saluyot are expected to increase in recent years due to the need for alternative sources of bioactive compounds for food and medical applications. However, despite its importance, to the best of our knowledge, there has been no dedicated study on the physiological maturity of Saluyot seeds, which is crucial for successful production and cultivation. To address this gap, the current work aims to evaluate the physiological maturity of Saluyot seeds after anthesis. This study will monitor changes in seed pod and seed color, seed size, dry weight, moisture content, and germination rate until physiological maturity is observed.

2. Materials and methods

Due to time constraints, instead of cultivating our own Saluyot plants from seeds to full maturity, a flowering and ready-to-flower Saluyot plant from Institute of Crop Science (ICrops), University of the Philippines Los Baños (UPLB) were used in this exercise. More than 100 flower buds were carefully tagged to ensure that samples collected throughout the experiment were of the same maturity levels. The tagged flower buds were then observed daily until anthesis occurred, which was noted on the third day after tagging. At three-day intervals post-anthesis (AA), a sufficient number of tagged samples were collected to monitor changes in fruit appearance, seed size, moisture content, dry weight, germination rate, and embryo development, observed using a microscope. This experiment was conducted at SeedTech Laboratory, ICrops, UPLB. Data gathering took place from October to December 2023, with daily mean temperatures ranging from 26.15°C to 25.41°C.

2.1 Seed size characterization

Saluyot seeds are relatively small in nature and are difficult to extract from 21 d before anthesis. In this exercise, the seed size was monitored in three replications from 21 to 42 d AA. The size of the seed was measured at the side part using a digital caliper as illustrated in Figure 1.

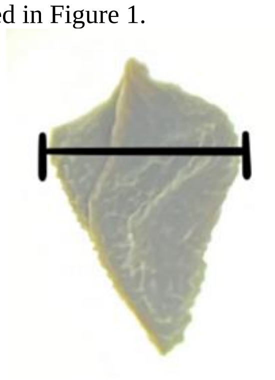

Figure 1 The part of the seed where size was taken from 21-42 d AA.

2.2. Seed germination test

The seed germination test was performed every 3 d AA. 30 seeds were extracted from Saluyot pods of the same maturity levels and sown in the petri dish lined with moistened filter paper. The samples were then monitored periodically to observe the emergence of the radicles. Seeds were considered germinated when radicles were visibly protruding, reaching an approximate length of 2 mm [11, 12]. The germination rate was calculated using Equation (1).

$$GR = \frac{\text{\# germinated seeds}}{\text{total \# of seeds sown}}(100\%) \quad (1)$$

Where GR is the germination rate (%).

2.3. Seed moisture content and dry weight determination

Seeds of the same maturity were extracted from the pod or fruit of Saluyot every 3 d AA. 2-15 fruits were used until sufficient weight of seeds were collected. Saluyot seeds are very slimy and tiny, hence; instead of counting the number of seeds utilized for seed dry weight determination, a separate batch of Saluyot fruits was collected, and a number of seeds was counted. The initial weight (wet weight) of the extracted seeds was obtained and the weighed seeds were then oven-dried at 102-105°C for 17 hrs. After drying, the samples were temporarily held inside a desiccator to prevent the dried samples from absorbing moisture from the surroundings before weighing the final moisture content (dry weight) using an analytical balance (U.S. Solid 200 x 0.0001g Analytical Balance) at the Physiology Laboratory, ICrops UPLB. The seed moisture content, on a wet basis, was calculated using Equation 2, while seed dry weight was calculated using Equation 3.

$$MC_{wb} = \left(\frac{w_i - w_f}{w_i}\right) \quad (2)$$

$$DW = \frac{w_f}{\text{\# pods } (\#\frac{\text{seeds}}{\text{pod}})} \quad (3)$$

Where MC_{wb} is moisture content wet basis (%), w_i is initial weight (mg), and w_f is seed weight after oven drying (mg). DW is expressed in mg/seed.

2.4. Data Analysis

The collected data were analyzed and graphically generated using Microsoft Excel 365.

3. Results and discussions

To clarify the successive morphological and physiological changes connected to the transition from flower buds to flowers and then to fruits, changes in the shape and color of Saluyot flower buds were observed as they grew into ripe fruits, as shown in Figures 2 and 3. Notable variations in fruit size were observed during this phase, showing an expansion trend. Moreover, the progressive change in fruit color from green to green with black spots at 21 d AA was connected to the breakdown of chlorophyll, indicating physiological changes that occurred under the surface throughout the maturity of Saluyot fruits.

Figure 2 Transformation of Saluyot flower buds into matured fruits captured every three-day interval.

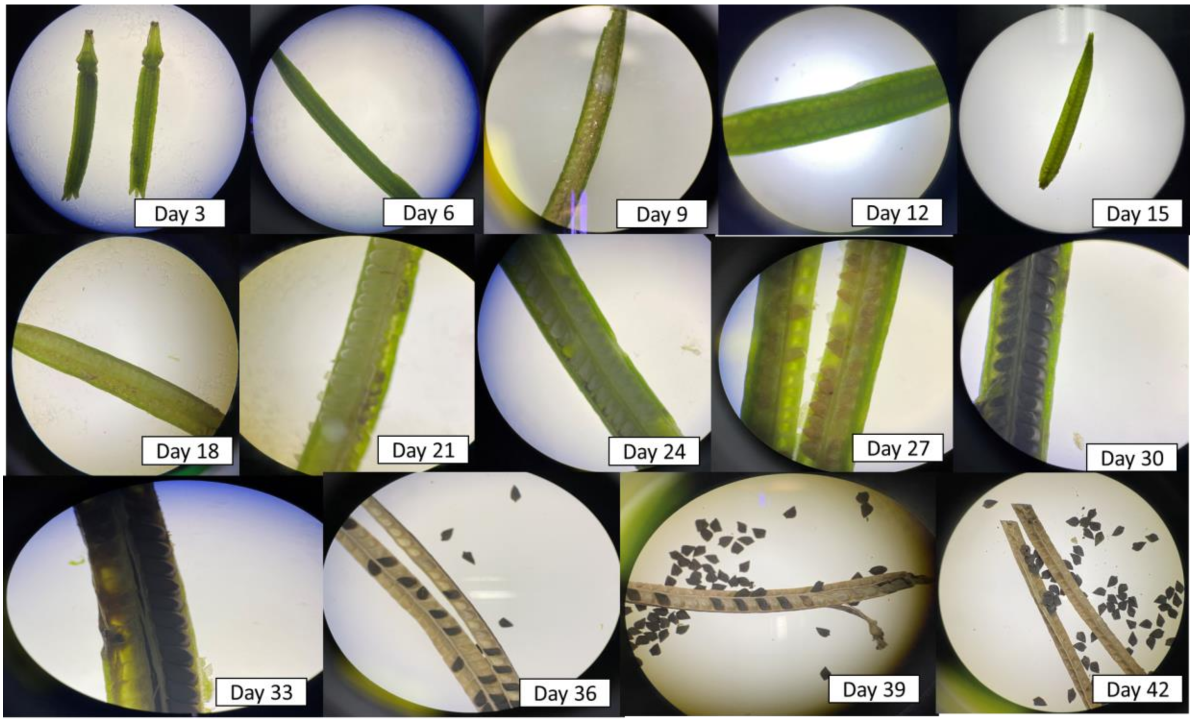

Figure 3 Transformation of Saluyot fruit from day 3 AA to day 42 AA under microscopic view.

In Figure 3, the fruits were sectioned and examined under a microscope to visualize the seeds within. Concurrent with the alteration in the fruit peel color, the seed color transitioned from a lighter to a darker shade. This color shift became particularly pronounced on 24 d AA. These changes in Saluyot pod and seed color from green to dark color are due to the degradation of chlorophyll which is common in most of the seeds [2]. For these reasons, seed color has also been considered as a marker for assessing seed physiological maturity [13].

3.1 Changes in seed size

The changes in the seed size of Saluyot were also monitored as physiological maturity proceeds. As shown in Figure 4 are the changes in seed size from days 18 to 42 d AA. Although changes in seed size in upward trend were noticed from 18 d AA (1.18 mm) to the final days of reading (1.42 mm), the overall changes appear to be relatively flat. The early stages of embryogenesis are marked by rapid cellular division and expansion, which causes a progressive rise in seed size [2]. The development and expansion caused by the massive accumulation of storage components (proteins, lipids, and/or carbohydrates) and the water intake by cotyledon or endosperm cells are the factors that contribute to this increase in seed size [14].

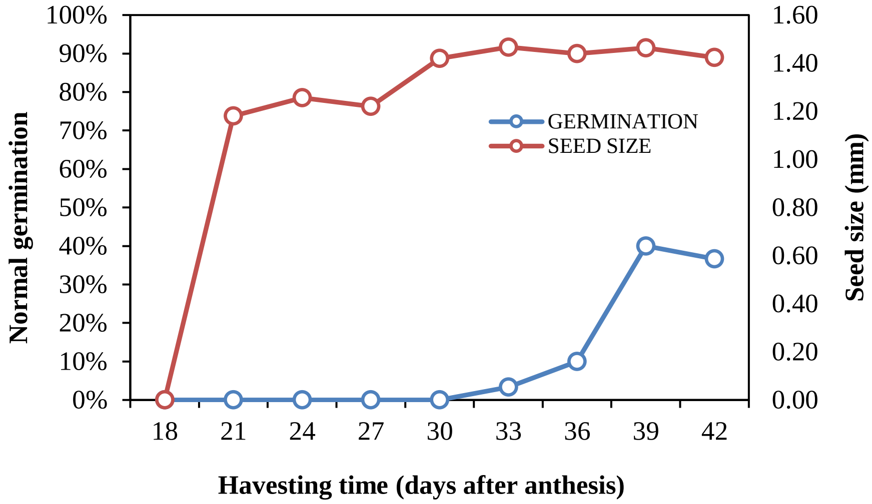

Figure 4 Changes in seed size (mm, orange), and normal germination rate (percent, blue).

3.2 Changes in seed germination rate

The germination rate of extracted seeds from freshly harvested Saluyot pods were monitored until physiological maturity was observed. In Figure 4, the trend in germination rate of the seeds is drawn. Germination only occurs at 33, 36, 39, and 42 d AA with germination rates of 3.33%, 10.00%, 40.00%, and 36.67%, respectively. Although the seeds were able to germinate in a moist petri dish, it can be said that the germination rate of the seeds is very low with no germinations observed from days 30 before anthesis.

The observed low germination of Saluyot seeds is explained by the fact that Saluyot seeds this time undergo dormancy. While seed dormancy serves vital functions in ensuring species survival by avoiding unfavorable conditions [15], it may not always be advantageous for agricultural purposes. Kak et al. [16] emphasized that *corchorus* spp. normally exhibit 100% seed dormancy due to the impermeability of their seed coat. The nonsynchronous germination observed in this scenario is also explained by the erratic release of seed dormancy [17]. Since in this experiment, the germination rate of Saluyot seed was observed right after the extraction of seed and no special dormancy breaking technique was employed, the 40% germination rate observed at 39 d AA was considered acceptable.

In recent times, multiple authors have documented successful scarification of *Corchorus olitorius* seeds using concentrated sulfuric acid [16, 18, 19]. Maina et al. [20] also explored the different techniques for breaking the dormancy of Saluyot, in which they suggested that the most effective treatment was mechanical scarification, leaching and soaking in hot water.

3.3. Changes in seed dry weight and moisture content

In this experiment, changes in seed dry weight (DW) and moisture content (MC) have been monitored every three days intervals after anthesis. The graphical representation of the findings is illustrated in Figure 5. Regarding seed dry weight, the trend indicates a continuous increase from day 9 after anthesis because of accumulation of food reserves and water uptake [2]. The peak seed dry weight was recorded at 39, 42, and 36 d AA, with corresponding values of 0.71 mg/seed, 0.70 mg/seed, and 0.69 mg/seed, respectively. As stated by Egli [21], the process of growing and developing seeds usually consists of three partially overlapping phases: the lag phase, which begins at fertilization and is characterized by cell proliferation and minimal dry weight gain (phase I); the seed filling phase, which is a linear phase of large dry weight gain linked to cell enlargement and storage compound accumulation (phase II); and the final phase, which is associated with reduced dry weight gain and is linked to desiccation and dormancy (phase III). In this case, minimal increase in seed dry weight was observed in days 6-18 AA (phase I), large dry weight gain was noticed in 21-39 d AA (phase II), and a slight decline at 42 d AA (phase III). Physiological maturity of seeds is usually associated with maximum dry weight where maximum accumulation of seed dry matter occur [20, 22], which in this case, the maximum dry weight was observed at 39 d AA.

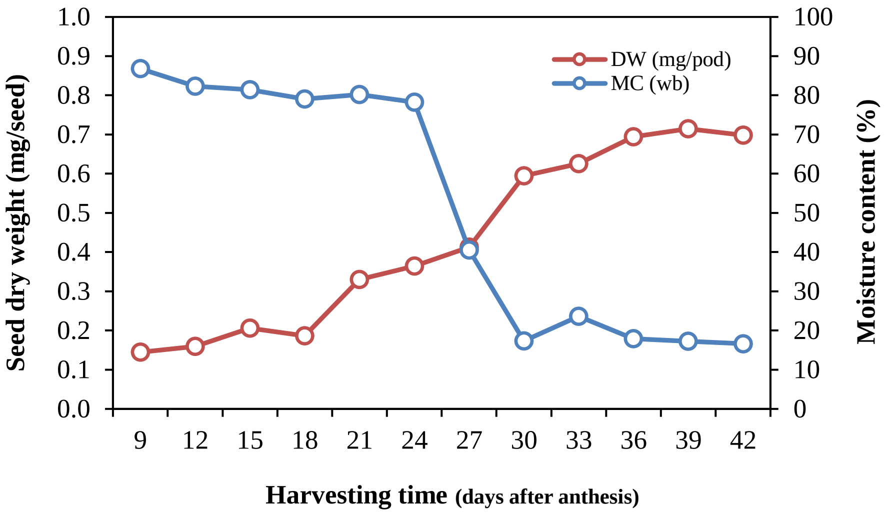

Figure 5 Changes in seed dry weight (mg/pod, orange) and moisture content (% wet basis, blue). The average number of seeds per pod is 159 seeds.

The moisture content of the seeds was also found in decreasing manner (Figure 5). The graph reveals a gradual decline in seed moisture content from day 9 (86.78%) to day 24 (78.21%) after anthesis, followed by a rapid reduction in moisture levels observed between days 27 (40.54%) and 42 (16.64%). Although the moisture content drops during the maturation process, it stays relatively high for most of that time (9-24 d AA) because water is the medium through which nutrients are transferred from the parent plant to the developing seeds. Furthermore, at high water contents, enzymes including those required to produce the storage compounds may become more active. Dehydration was found to occur slowly in the early stages and to pick up speed once the seed reaches mass maturity [2].

4. Conclusion

This study has highlighted the changes in physiological properties of Saluyot seeds from anthesis to physiological maturity. Changes in seed pod and seed color were observed from 24 d AA and these changes are associated with the degradation of the chlorophyll in seed pod and Saluyot seed. Other than these properties, a slight increase in seed size, an increase in seed dry weight, and a decline in seed moisture content was observed which is due to accumulation of food reserved and water uptake during seed development declines as development proceeds. In terms of seed germination, a low germination rate was observed from 33 to 42 d AA, with no seeds germinating from 30 days before anthesis, indicating that seeds undergo dormancy during this period. In brief, the physiological maturity of Saluyot seeds occur at 39 d AA with maximum dry matter (food reserve) and maximum germination rate was observed of 113.64 mg/pod, and 40.00%, respectively.

5. Acknowledgements

The author extends heartfelt gratitude to Ma'am Ruth Palmes for her exceptional dedication and effort, particularly in overseeing the crucial task of oven-drying the samples. Special thanks are also extended to SeedTech Laboratory for their generous provision of facilities and equipment. MCA would also like to acknowledge the Department of Science and Technology – Engineering Research and Development for Technology (DOST-ERDT) for the scholarship support. ADB Jr. would like to thank the German Academic Exchange Service and Southeast Asian Regional Center for Graduate study and Research in Agriculture for the support.

6. Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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