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Impact of Various Seed Dormancy Breaking Methods on Viability and Early Growth of Soursop (*Annona muricata*) Seedlings

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ABSTRACT

Soursop (Annona muricata L.) is an economic fruit tree with a long history of traditional use. However, hard seed coat dormancy is a major setback, preventing rapid germination and seedling establishment. This study was conducted to break the hard seed coat dormancy of soursop (Annona muricata) seeds using different treatments. The experiment was conducted at the Department of Horticulture, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana. The experimental design was Randomized Complete Block Design with seven treatments replicated three times. The treatments were: no treatment (control), 500 mg/L Gibberellic Acid for 48 hours, 1000 mg/L GA3 for 48 hours, Hot water at 50°C for 5 minutes, Hot water at 50°C for 10 minutes, and cold water at 15°C for 24 hours. The study revealed that Soursop seeds treated at a concentration of 1000 mg/L of GA₃ for 48 hours broke the seed dormancy and promoted rapid germination at 25 days after planting. Soursop seeds treated with a concentration of 1000 mg/L of GA₃ for 48 hours also enhanced the vegetative growth of soursop. However, Soursop seeds treated with cold water for 48 hours produced plants with the lowest plant height, a low number of leaves and thinner stems. A linear regression showed that germination percentage significantly affected vigor index, such 70% of the variation in the vigor index was attributed to the germination percentage The study concluded that to obtain highest germination with improved vegetative growth of soursop, treatment with a concentration of 1000 mg/L of GA_3 for 48 hours is required.

Keywords: seed dormancy, soursop, seed viability, softening, radicle protrusion.

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Introduction

Soursop (Annona muricata L.) is a fruit tree with a long history of traditional use. It belongs to the Annonaceae family, which has more than 130 Genera and 2300 species (Patel and Patel, 2016; Errayes et al., 2020). According to Ilango et al. (2022), A. muricata is an evergreen plant that is mainly found in tropical and subtropical regions of the world. The tree produces the largest fruit in the Annona genus and has

been introduced to various tropical regions worldwide (Idowu *et al.*, 2022). It is also referred to as soursop, graviola, and guanabana (Nayak and Hegde, 2021). The name soursop is due to the sour and sweet flavour of its large fruit. Originating in India and Central America, soursop has drawn interest due to its commercial potential and possible health benefits (Hernández Fuentes *et al.*, 2021; Leal and Paull, 2023). The soursop, or Annona

muricata, is a distinctive and newly discovered tropical fruit species that holds great potential for application in the pharmaceutical industry, placing it in a promising position in the current fruit market (Yahaya, 2018; Amarasekera and Noor, 2024). According to Mutakin et al. (2022) the soursop plant is a fruit plant that contains bioactive substances such as tannins, phytosterols, flavonoids, saponins, and alkaloids. Annonaceous acetogenins, which are present in both the fruits and the leaves of soursop, are recognised to have medicinal qualities, including the ability to fight cancer (Prasad et al., 2019; Nambooze et al., 2024). According to Vincent et al. (2019) and Dalal and Medithi (2022), soursops contain Acetogenins, which are important in the treatment of several cancer types, and are strong inhibitors of the enzyme NADH oxidase (nicotinamide adenine dinucleotide phosphate-oxidase), which is found in the plasma membranes of cancer cells. Studies have also shown its antibacterial activity against oral infections, indicating promise in dental applications (Naha et al., 2021; Arruda et al., 2023). The fruit is eaten raw, blended into drinks, and transformed into a variety of culinary items (Afzaal et al., 2022). A sweet, white, creamy, juicy, soft, somewhat acidic pulp with great sensory qualities is present in the ripe soursop fruit (Dias and Jayasooriya, 2017; Almeida et al., 2024).

Despite its potential, urbanization and lack of awareness have led to a decline in soursop cultivation in some areas (Omere et al., 2023). Even with its great significance, soursop has a tough seed coat that delays and results in irregular germination seedling emergence (Sharma et al., 2022). According to Lamont and Pausas (2023), seed dormancy is an inherent feature of seeds that determines the environmental conditions under which they can germinate. This adverse condition poses a threat to the conservation and sustainable production of the crop. It is therefore important to research the best technique that will

facilitate both germination and production of healthy seedlings. Hard seed coat dormancy release involves the process of causing the seed coat to become permeable to gas or water by breaking, scraping, or modifying it mechanically (Ma et al., 2023; Kumar et al., 2023). Specific breaking dormancy techniques includes rubbing the seed between the jaws of a vice, filing or splitting the testa with a hammer, or rubbing it on sandpaper. For instance, the soaking of seeds in water to help soften the seed coat and make it more permeable. thereby facilitating germination, commonly practised. is Additionally, the use of growth hormones such as Gibberellic Acid (GA₃) to trigger rapid germination is reported in literature for plant species such as industrial hemp and Penstemon (de Mello, 2009; Du et al., 2022). Moreover, Mimi et al. (2023), reported of the significant role GA₃ played on sugar and lipid degradation during Annona x atemoya Mabb. seed germination. To this end, it became extremely imperative to research on techniques that could improve germination in soursop for easy adoption by nursery operators as well as updating the existing scientific literature. Therefore, the objective of this study was to determine the most effective method for breaking hard seed coat dormancy and promoting optimal germination and seedling establishment of soursop (Annona muricata).

Materials and Methods Study Area

The research was carried out on the experimental fields of the Department of Horticulture, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi. The location is situated within the semi-deciduous forest zone of Ghana and is located at latitude 5° 36' N and longitude 0° 10' E. The soil type of the experimental field is sandy loam. Studies show that the area experiences an average rainfall of 645 mm, and the mean minimum and maximum temperatures recorded are 22°C and 31°C,

respectively. Additionally, the average relative humidity ranged from 66% in the morning to 27% at noon.

Source of Experimental Materials

Soursop fruits were bought from the Bantama market, Kumasi, in the ripening stage, and the seeds were extracted manually. A tissue paper was used to clean the mucilage around the seeds, and they were allowed to dry at room temperature for 5 days.

Experimental Design and Procedure Experimental Design

The experimental design was Randomized Complete Block Design with seven treatments replicated three times.

The seeds were subjected to the following pre-sowing treatments at the laboratory of the Horticultural Department, KNUST.

T1- no treatment (control)

T2- 500 mg/L; GA₃ for 48 hours

T3- 1000 mg/L; GA₃ for 48 hours

T4- Hot water 50°C for 5 minutes

T5- Hot water 50°C for 10 minutes

T6- cold water (15°C) for 24 hours

T7- Cold water (15°C) for 48 hours

Experimental Procedure

T1 – The seeds were not subjected to any form of treatment.

T2-500 grams of gibberellic acid was dissolved in 1000 litres of distilled water to make a concentration of 0.5 moles. The seed were gently dropped into the solution for 48 hours at the Department of Horticulture lab, KNUST.

T3 – 1000 grams of gibberellic acid was dissolved in 1000 litres of distilled water to make a concentration of 1.0 moles. At the Department of Horticulture Lab, KNUST, 100 seeds were carefully put into the solution and left for 48 hours.

T4 – A thermometer was placed in a kettle containing water and boiled to 50°C. The water was then transferred into a beaker

after which 100 seeds were dropped into and kept for 5 minutes.

T5 – A thermometer was placed in a kettle of boiling water to reach a temperature of 50°C. After that, the water was poured into a beaker, into which the seeds were added and allowed to soak for 10 minutes.

T6 – The seeds were kept in a beaker containing normal cold water for 24 hours (a day).

T7 – The seeds were kept in a beaker containing normal cold water for 48 hours (2 days).

Nursery Establishment

Topsoil was obtained from the Department of Horticulture. The topsoil was sieved and later filled into 18cm × 26cm nursery bags.

Data Collection

The following data were taken during the growth period on the experimental field. The number of leaves, plant height, stem girth was recorded from the ninth week to the twenty first week after sowing and were in centimeters (cm).

Number of days to Emergence

Each seedling was observed and tallied to the day it first appears above the soil surface, the days were counted between sowing day to the emergence.

Seed Germination percentage

This was calculated as number of seeds germinated divided by the total number of seeds sown multiplied by 100.

Vigour Index

The shoot length (from the collar region to the tip of the plumule) and root length (from the collar region to the tip of the primary root) of 10 randomly sampled seedlings from each treatment were measured using a meter rule, and the mean results were reported in centimeters. Following the formula (Abdul-Baki and Anderson, 1973),

the vigour index was calculated and expressed as a whole number.

Vigour Index

- + Root Length)

Number of Leaves

The leaves per plant were taken from the ninth week to the twenty-first week after sowing. The leaves were counted, and the average was recorded.

Plant Height

The height of the plants was measured using meter rule from the soil to the topmost leaf, the figures were recorded, and the average or mean length was in centimeter (cm).

Stem Girth

This was measured and recorded using a Vernier calliper. The Vernier calliper was placed midway along the stem in the soil, and the figures were recorded to determine the average in centimetres (cm).

Data Analysis

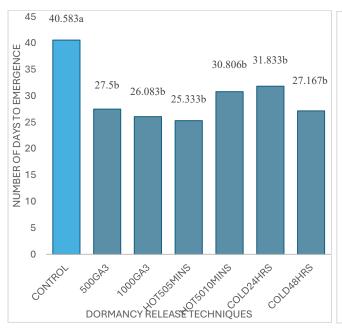


Figure 1: Effect of seed dormancy release techniques on number of days to emergence of soursop seeds. GA3: Gibberellic Acid, MINS: Minutes, HRS: Hours

The collected data were subjected to oneway analysis of variance (ANOVA) using Statistix Software version 10.0. Significant = Final germination percentage x (Shoot lengitherences between treatment means were declared at 5% and means were separated using Tukey's HSD. Figures and tables were used to present the results. A linear regression analysis was performed to establish a relationship between vigour index and germination percentage.

Results

Effect of Seed Dormancy Release Techniques on the Number of Days to **Emergence of Soursop Seeds**

Figure 1 shows the effect of the seed dormancy release technique on the number of days to emergence of soursop seeds. The results show that there was a significant difference among the treatment means, where seeds which were not subjected to any treatment (control) recorded the highest among the other treatments. Hot water at 50 degrees recorded the lowest, which means that it took the shortest days to germinate, and the control took the longest days to germinate.

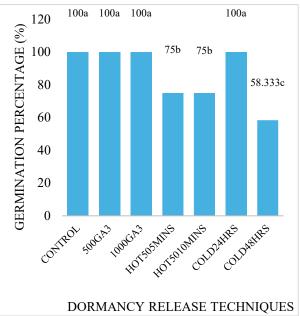


Figure 2: Effect of seed dormancy release techniques on germination percentage of soursop seeds after 41 days after planting. GA3: Gibberellic Acid, MINS: Minutes, **HRS:** Hours

effect of Seed Dormancy Release Techniques on Germination Percentage of Soursop Seeds Figure 2 above illustrates the effect of seed dormancy release technique on germination percentage of soursop seeds. The results show that there was significant difference among the treatment means where the seeds which were not submitted to any treatment (control), 500 mg/L of GA₃ for 48 hours, 1000 mg/L of GA₃ for 48 hours and cold water for 24 hours recorded the highest and cold water for 48 hours recorded the lowest. This means that the four which recorded the highest had all seeds germinated, and cold water for 48 hours had almost half of the seeds

Effect of Seed Dormancy Release Techniques on Vigour Index of Soursop Seeds

germinated.

Figure 3 shows the effect of the seed dormancy release technique on the vigour index of soursop seeds. The results show that there was a significant difference among the treatment means: 1000 mg/L of GA3 for 48 hours recorded the highest average of 3196.7, and cold water for 48 hours recorded the lowest average of 1398.3.

Effect of Seed Dormancy Release Techniques on Plant Height of Soursop Seeds

Table 1 reveals the effect of seed dormancy release technique on plant height of soursop seeds. The results showed that there were significant differences among the treatment means where 1000 mg/L of GA₃ for 48 hours recorded the tallest plants throughout the weeks, that is from week 9 to week 21

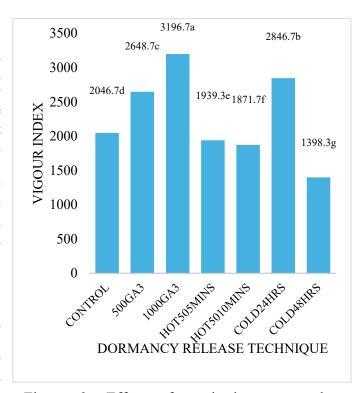


Figure 3: Effect of seed dormancy release techniques on vigour index of soursop seeds. GA3: Gibberellic Acid, MINS: Minutes, HRS: Hours

and cold water at 48 hours recorded the shortest plants from week 9 to week 21. Means with the same alphabets in a column are not significantly different from each other at 5% probability level. HSD: Honestly Significant Difference at 5% probability level, CV: Coefficient of variation, GA: Gibberellic acid, Hrs: Hours, Mins: Minutes, WK: Weeks.

Table 1: Effect of Seed Dormancy Release Techniques on Plant Height (CM) of Soursop Seeds

Treatments	WK 9	WK 11	WK13	WK 15	WK 17	WK 19	WK 21
Control	4.92a	7.47°	12.99°	17.87°	21.97^{bc}	24.64°	26.67°
500 GA ₃	6.72^{b}	10.27^{b}	14.72 ^b	20.04^{b}	22.24^{b}	27.94^{b}	31.67^{b}
1000 GA ₃	11.14 ^a	14.54 ^a	19.94ª	27.79 ^a	30.59^{a}	32.42a	37.67 ^a
Hot 50°C 5 Mins	6.14^{bc}	8.34°	13.47°	18.52°	20.84^{d}	23.14^{d}	26.17°
Hot 50°C 10 Mins	5.32^{cd}	7.54°	12.94°	16.72^{d}	21.42^{bcd}	23.34^{d}	25.79°

Cold 24Hrs	6.04 ^{bc}	10.27 ^b	13.42°	17.79°	21.19 ^{cd}	23.94 ^{cd}	25.68°
Cold 48Hrs	3.14^{e}	4.42^{d}	6.97^{d}	9.02^{e}	10.42e	12.77 ^e	15.17^{d}
HSD (5%)	1.01	1.01	1.01	1.01	1.01	1.01	1.01
CV (%)	4.65	3.22	2.14	1.58	1.36	1.20	1.07

Effect of Seed Dormancy Release Techniques on Number of Leaves of Soursop Seed

Table 2 indicates the effect of seed dormancy release technique on number of leaves of soursop seeds. The results showed that there were significant differences among the treatment means where 1000 mg/L of GA₃ for 48 hours recorded the highest number of leaves throughout the weeks, that is from week 9 to week 21 and cold water at 48hours recorded the lowest leaf numbers from week 9 to week 21.

Table 2: Effect of Seed Dormancy Release Techniques on Number of Leaves of Soursop Seed

Treatments	WK 9	WK 11	WK13	WK 15	WK 17	WK 19	WK 21
Control	1.92 ^b	5.50^{b}	7.42^{b}	12.42 ^b	16.17 ^b	17.92 ^b	19.67 ^b
500 GA ₃	2.42^{b}	4.42°	7.17^{b}	11.92 ^b	16.17^{b}	17.92^{b}	19.67 ^b
1000 GA_3	6.00^{a}	8.67 ^a	13.67 ^a	19.88a	23.50^{a}	27.00^{a}	27.00^{a}
Hot 50°C 5 Mins	2.42^{b}	3.92°	6.67^{b}	10.42 ^c	12.42^{d}	13.17°	13.42^{d}
Hot 50°C 10 Mins	1.92 ^b	4.17^{c}	6.67^{b}	9.42°	11.17 ^e	11.17^{d}	12.67^{d}
Cold 24Hrs	1.92^{b}	4.67^{bc}	7.42^{b}	11.92 ^b	13.92°	13.42°	16.67°
Cold 48Hrs	1.92 ^b	2.67^{d}	4.17^{c}	6.67^{d}	7.92^{f}	8.42 ^e	$8.92^{\rm e}$
HSD (5%)	0.99	0.99	1.07	1.01	1.15	0.99	0.99
CV (%)	10.31	5.84	4.00	2.45	2.26	1.82	1.69

Means with the same alphabets in a column are not significantly different from each other at 5% probability level. HSD: Honestly Significant Difference at 5% probability level, CV: Coefficient of variation, GA: Gibberellic acid, Hrs: Hours, Mins: Minutes, WK: Weeks.

Effect of Seed Dormancy Release Techniques on Stem Girth of Soursop Seeds Table 3 shows the effect of the seed dormancy release technique on the stem girth of soursop seeds. The results showed that there were significant differences among the treatment means where 1000 mg/L of GA₃ for 48 hours recorded the highest average throughout the weeks, that is from week 9 to week 21 and cold water at 48hours recorded the lowest average from week 9 to week 21.

Table 3: Effect of Seed Dormancy Release Techniques on Stem Girth of Soursop Seeds

Treatments	WK 9	WK 11	WK13	WK 15	WK 17	WK 19	WK 21
Control	1.67^{ab}	$2.17^{\rm abc}$	2.67^{a}	2.67^{ab}	3.92^{b}	4.17^{b}	4.17^{bc}
500 GA ₃	1.67^{ab}	2.67^{ab}	2.67^{a}	2.67^{ab}	4.17^{ab}	4.17^{b}	5.17^{ab}
1000 GA ₃	2.67^{a}	2.92^{a}	2.92^{a}	3.67^{a}	5.17 ^a	5.42 ^a	5.67^{a}
Hot 50°C 5 Mins	1.42^{b}	$1.92^{\rm abc}$	2.17^{ab}	2.42^{b}	$3.47^{\rm b}$	3.17^{bc}	$3.42^{\rm cd}$
Hot 50°C 10 Mins	1.67^{ab}	$1.92^{\rm abc}$	1.92^{ab}	2.17^{b}	3.47^{b}	3.17^{bc}	3.42^{cd}
Cold 24Hrs	1.67^{ab}	$1.92^{\rm abc}$	2.17^{ab}	2.67^{ab}	3.67^{b}	3.67^{b}	4.17^{bc}
Cold 48Hrs	1.17^{b}	1.17^{c}	1.42 ^b	$1.67^{\rm b}$	2.17^{c}	2.17^{c}	2.42 ^d
HSD (5%)	1.01	1.01	1.01	1.01	1.01	1.01	1.01

CV (%) 16.96 14.02 12.70 11.28 7.80 7.80 7.11

Means with the same alphabet in a column are not significantly different from each other at 5% probability level. HSD: Honestly Significant Difference at 5% probability level, CV: Coefficient of variation, GA: Gibberellic acid, Hrs: Hours, Mins: Minutes, WK: Weeks.

Linear Regression Analysis Between Seed Germination Percentage and Vigour Index

A linear regression showed that the germination percentage significantly affected the vigour index; 70% of the variation in the vigour index was attributed to the germination percentage (Figure 4).

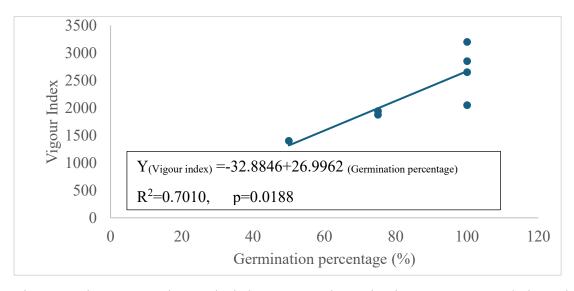


Figure 4: Linear regression analysis between seed germination percentage and vigour index

Discussion

Techniques of breaking seed dormancy significantly impact the germination and emergence of soursop (Annona muricata) seeds. The results from the field indicated that seeds under the control treatment had the longest germination time of 41 days. Joseph-Adekunle, (2014) reported that under sub-optimal conditions, soursop seeds take at least 2-3 months before germinating. It is possible that natural optimum conditions needed to trigger germination were available within the 41 days that led to the emergence of seeds in the control treatment. On the other hand, the soursop seeds that were subjected to hot water treatment emerged the earliest (25 days) among all the different treatments because hot water treatment can accelerate the physiological processes in seeds, such as the activation of germination enzymes or disruption of dormant mechanisms. Hot

water treatment aims to break seed dormancy and promote faster germination. Kumar (2024) reported that the hot water treatment softens the seed coat, allowing water to penetrate more easily. This action breaks down inhibitors that may prevent 2017). germination (Bradford, addressing dormancy, improving water intake, and enhancing nutrient absorption, pre-sowing treatments are essential for maximizing seed germination (Chowdhury al., 2024). By enhancing water absorption nutrients, pre-sowing and treatments promote robust seedling emergence, leading to more vigorous plants with faster early development (Jaliya et al., 2024).

Our results showed that there were no significant differences between the Control, 1000 mg/L of GA₃ for 48 hours, 500 mg/L GA₃ for 48 hours and cold water for 24

hours treatments. The application of GA₃ enhanced germination by helping in the manufacture of several enzymes, such as áamylase, which during the germination process breaks down starch into simple sugars (Singh and Maheswari (2007). Moreover, GA₃ helps to release Energy from these sugars which is needed for several metabolic and physiological processes connected to germination (Ali and Elozeiri, 2017; El-Maarouf-Bouteau, 2022). The enzymes that weaken the seed coat and permit the axis to break through are among the other enzymes that GA₃ activates (Zuo and Xu, 2020). According to Sánchez-Montesino et al (2019), GA₃ increases cell elongation, allowing the radicle to expand through the seed coat and endosperm that impede it. The cold-water treatment for 24 hours also recorded a high germination percentage because the water helped to soften the seed coat, which helped to hasten the germination process, which is in line with findings of Joseph-Adekunle (2014). The cold-water treatment for 48 hours recorded the lowest because the seed cells became extra turgid, which adversely affected germination, which is in line with findings of (Nawaz et al., 2013).

The highest seedling vigour observed in the treatment of seeds with 1000mg/L GA₃ for 48hours might be due to an increase in germination percentage and seedling height, which have contributed to higher vigour. The outcomes are in good agreement with the findings of Dinesh and Padmapriya (2022), who reported that guava seeds treated with GA3 had increased rates of germination as well as shoot, root, and seedling vigour index. The cold-water treatment for 48 hours recorded the lowest because the seed cells become extra turgid, which adversely affects the germination vigour (Sharaf-Eldin et al., 2022). This is because although soaking seeds in water and drying before sowing is the easiest way to achieve hydration, a major disadvantage is that it may result in uneven hydration and non-uniform germination. Soaking is not suitable for some plant species, as rapid hydration may cause leakage of essential nutrients out of the seed, resulting in seed damage, which also explains why the seeds treated with cold water for 48 hours produced the shortest plants, a low number of leaves and stem girth (Nawaz *et al.*,2013).

Varying response was noted for plant height in each treatment. Results indicated that there was a rapid increase in plant height; gibberellic acid (GA₃) is a wellknown plant hormone that plays a significant role in promoting growth by enhancing cell division and internode elongation. According to a study by Cosgrove (2024), GA₃ facilitates the elongation of plant cells by loosening the cell walls, thereby allowing cells to expand and contribute to the overall growth of the plant. In the case of soursop seedlings, GA₃ treatment promotes these cellular processes, leading to increased plant height.

In terms of a number of leaves, GA3 of 1000 mg/L was recorded as the highest, which proves that GA3 stimulates cell proliferation and elongation at the shoot apex; GA3 facilitates the development and elongation of leaf primordia. Gibberellins (GAs) are essential for nearly all aspects of plant growth and development, including cell elongation, leaf expansion, leaf senescence, seed germination, and the creation of leafy heads (Ritonga et al., 2023). There is a great deal of variation in leaf morphogenesis, flexibility, and leaf development differentiation, which results in a wide range of leaf senescence, size, shape, and angles. It has an impact on the shoot's apical meristem, which produces primordia and facilitates differentiation and growth of new leaves (Ritonga et al., 2023).

Our results showed the effects of GA₃ treatment on stem girth, such that the increased hormone levels led to changes in stem morphology, including alterations in stem girth. Shan *et al.* (2021) reported that

gibberellic acid (GA₃) is an essential plant hormones that significantly influence plant growth by promoting cell elongation and increasing cell numbers. These processes particularly important for elongation, where the internodes lengthen and stems become thinner, typically accompanied by higher levels of GA₃ in the main stems and leaves. While GA3 is wellknown for enhancing stem elongation, its impact on stem girth is also significant. In the case of soursop seedlings, GA₃ application results in thinner stems, highlighting the hormone's modulating not just the length but also the thickness of the stem. This thinning is due to GA3's ability to stimulate cambium activity, which is critical for secondary growth. The cambium, a cylindrical meristem consisting of pluripotent stem coordinates cell division differentiation to form secondary vascular tissues. It differentiates into secondary xylem on the inside and secondary phloem on the outside in a bidirectional manner (Zhang et al., 2024).

Germination Percentage (GP) and Seed Vigour Index (SVI) are both essential parameters in assessing seed quality and performance. In the current study, the linear regression analysis showed that germination percentage significantly affected vigour index, such 70% of the variation in the vigour index attributed to the germination percentage. The findings suggested that germination percentage can be a good indicator of field emergence and

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seedling performance for a variety of crop species, and it is strongly correlated with the seed vigour index. Hence, an increase in germination percentage considerable increase in seed vigour index. Kandpal et al. (2016) reported that seed vigour, is an essential indicator of seed quality and affects the rapidity and regularity of seed germination and the health of seedlings in the field. Further, seed vigour tests provide a more sensitive index of seed performance per se than the germination test and seeds of high germination have a high vigour index since they are directly related (Basu and Groot, 2023).

Conclusion

The results of this study suggest that Soursop seeds treated at a concentration of 1000mg/L of GA₃ for 48 hours had the greatest effect on breaking seed dormancy and promoted rapid germination. Soursop seeds treated with a concentration of 1000mg/L of GA₃ for 48 hours also enhanced the vegetative growth of soursop. Soursop seeds treated with cold water for 48 hours produced the shortest plants, the lowest number of leaves and the smallest stem girth. A linear regression showed that germination percentage significantly affected vigour index such 70% of the variation in the vigour index was attributed to by the germination percentage

Competing Interest

The authors declare no conflict of interest in the publication of this article.

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