

The Effect of Varying Concentrations of Gibberellic Acid (GA₃) Under Refrigeration on the Breaking of Dormancy and Seedling Growth of Apple (*Malus Domestica* Borkh.) Seeds in Jos Plateau State, Nigeria

¹Deshi, Kyenpiya Eunice, Ogbuka, Goodluck Ihuoma, Nanbol, Karya Kate and Satdom, Sylvia Makvereng

Department of Plant Science and Biotechnology, Faculty of Natural Sciences, University of Jos, Nigeria

ABSTRACT

A study was carried out at Ritdun Integrated Farm, Jos, Plateau State, Nigeria (Latitude 09 ° 85' N, Longitude 08 ° 86' E and altitude 1,319 m above sea level) from November, 2019 to February, 2020 to evaluate "the effect of varying concentrations of Gibberellic acid (GA₃) under refrigeration on the breaking of dormancy and seedling growth of apple (*Malus domestica* Borkh.) seeds in Jos Plateau, Nigeria". The experiment was laid out in a completely randomized design consisting of two varieties of Apple (green and red (Top red and Gold delicious respectively)) and three concentrations of GA₃ (0 ppm, 10 ppm, 15 ppm) giving a total of 6 treatment combinations which was replicated 3 times. The parameters assessed were mean number of days from treatment to breaking of dormancy, number of germinated seeds expressed as a percentage, number of leaves per seedling plant and plant height. The data collected was subjected to analysis of variance and the means were separated using least significant difference (LSD) at 0.05 probability level. The result obtained showed that variety did not have any significant ($p < 0.05$) effect on all the parameters assessed, however, GA₃ concentration had significant ($p < 0.05$) effect on the number of days to dormancy break. Seeds treated with 15 ppm GA₃ were the earliest to germinate in 23 days while seeds treated with 10 ppm GA₃ and the control took longer days to break dormancy (38.30 and 40.65 days respectively). Seeds treated with 15 ppm GA₃ had significantly ($p < 0.05$) higher mean establishment count (93.89%), plant height (12.88 cm), and number of leaves (13.66) at 6 weeks after planting. The interaction of variety and GA₃ concentration was not significant for all parameters assessed. The study indicates that the application of GA₃ (15 ppm) under refrigeration is an effective method to increase and accelerate the germination and growth of apple seedlings in Jos Plateau, Nigeria.

Keywords: Apple seeds, dormancy break, Gibberellic acid

Date of Submission: 26-12-2023

Date of Acceptance: 06-01-2024

I. INTRODUCTION

Apple (*Malus domestica* Borkh.) is a hardy, deciduous woody perennial tree of the rosaceae family that grows in all temperate zones (Nzaet *et al.*, 2021; Kumari *et al.*, 2023). Apples are one of the most widely cultivated tree fruits. China tops the list of the highest apple-producing countries in the world and plays an important role in international apple production (Wu and Pan, 2021). Kumari *et al.* (2023) observed that apart from higher production, several other factors make apples the most widely consumed fruit. These factors include easy market availability, cost affordability, long shelf life, variety of processed apple products such as jams, pies, canned apples, apple juice, smoothies etc. Apples are low in sodium, fat and cholesterol. They are also a very good source of vitamin C, fibre and antioxidants. The antioxidant activity of apples is mainly attributed to the phenolic compounds present in apples (Kumari *et al.*, 2023). It possessed rich content of polyphenols; flavanols and oligomeric flavonols (Kalinowska *et al.*, 2014). Multiple studies have reported that the polyphenol percentage in the overall phenolic and flavonoid content of apple peel extract is substantially higher than apple flesh (Nzaet *et al.*, 2021).

Mature apple seeds are dormant and do not germinate (Debska *et al.*, 2013). Dormancy is a physiological state when viable seeds do not germinate (Ciacka *et al.*, 2019). Dormancy is an innate feature of seeds that enables them to regulate timing of germination under favourable conditions for successful seedling establishment and growth (Finch-Savage and Leubner-Metzger, 2006). It can also be defined as the temporal cessation of growth of a plant meristem (Lewak, 2011). In apple seeds, all these blocks are removed as a result of cold treatment (stratification), but some of them are also affected by light and/or hormonal treatment. Seed

stratification, a commonly used technique, is used for dormancy removal and can be performed in moisture for an experimentally revealed time at warm or cold temperatures (Ciacka *et al.*, 2019).

Gibberellins are known as growth promoting hormones that are involved in several processes during the development of plants such as shoot growth, flower development, dormancy release and seed germination (Linkies and Leubner- Metzger, 2012). Gibberellic acid (GA₃) is known to be concerned in the regulation of plant responses to the external environment (Chakrabarti and Mukherji, 2003). Gibberellins eliminates the chilling requirements of peach and apple seeds and increased their germination (El-Barghathi and El-Bakkosh, 2005). Exogenously applied GA₃ overcomes seed dormancy in several species and promotes germination in some species that normally require cold stratification, light, or after-ripening (Kandari *et al.*, 2012). Gornik *et al.* (2018) found the most pronounced results in apple dormancy break were obtained after GA₃ treatment. Due to such application, the germination of apple variety 'Ligol' seeds increased by 100% in comparison to the control.

Poor seed germination is the major limiting factor of apple for production and cultivation. Apple fruit trees grow well in temperate climate zones where most commercial varieties satisfy their required chilling temperature, which is often expressed as less than 7°C (Tromp, 2005). More than 90% of the Nigerian apple fruit production comes from the colder regions with higher altitudes (Koornneef *et al.*, 2002). The Jos-Plateau has a semi temperate climate suitable for raising apple seedlings but there is scarcity of apple seedlings in Jos due to inability of apple seeds to germinate when extracted from the fruit and planted. This lack of germination is due to dormancy of the seed. One of the major constraints in the production of temperate fruit crops in tropical areas is the lack of effective accumulated chilling because warm winters result in prolonged dormancy leading to poor germination and blooming, strong apical dominance, unsynchronized growth patterns and, consequently, low yields (Huang *et al.*, 2010). Therefore, this study aimed at studying the effect of varying concentrations of gibberellic acid (GA₃) under refrigeration on the breaking of dormancy and seedling growth of apple (*Malus domestica* Borkh.) seeds in Jos Plateau State, Nigeria.

II. MATERIAL AND METHODS

The study was carried out between the months of November, 2019 to February, 2020 at Ritdun Integrated Farm Resources, Kangang road, off Miango Road, Dadin kowa, Jos, Plateau State, Nigeria (Latitude 09°85' N, Longitude 08°86' E and Altitude 1,319 metres above sea level) to evaluate "the effect of varying concentrations of Gibberellic acid (GA₃) under refrigeration on the breaking of dormancy and seedling growth of apple (*Malus domestica* Borkh.) seeds in Jos, Plateau State, Nigeria."

Two varieties of fresh apple fruits viz: red and green (Top red and Gold delicious respectively) were purchased from fruits store in Jos and sawdust was collected at Katako market in Jos. The gibberellic acid used in the experiment was imported from China.

The experiment was laid out in a completely randomized design consisting of two different varieties of Apple (Top red and Gold delicious) and three concentrations of GA₃ (0ppm, 10ppm, 15ppm) giving a total of 6 treatment combinations which was replicated 3 times and each replication was represented by twenty (20) seeds.

The sawdust collected was steam sterilized to eliminate soil-borne pathogens and ensure that there was no microorganism growth during the trial, it was allowed to cool and filled into plastic pots for planting of apple seeds. The seeds were collected by removing the seeds directly from the apple fruit using a knife.

To prepare the GA₃ solutions, 0.1g (100mg) of GA₃ was accurately weighed out using an analytical balance and added to a volumetric flask and 5mls of 0.5N NaOH solution was added to dissolve the Gibberellic acid powder. This was then brought to volume with distilled water, stirring the solution while adding the water to keep the material in solution. 100mg Gibberellic acid dissolved in 100 cm³ gives 1000ppm. Serial dilution was done to obtain the final concentrations needed. 10mls of the 1000ppm was pipetted and diluted in 100mls of distilled water to obtain 100ppm solution. 10mls and 15mls of the 100ppm solution was pipetted and diluted to 100mls distilled water each to obtain 10 ppm and 15 ppm solutions respectively. The Gibberellic acid solutions were prepared just prior to the immersion of the apple seed samples.

The apple seed samples were soaked in the prepared solution of gibberellin acid for 30 minutes after which they were removed and planted in the sterilized sawdust that was filled in to plastic pots. This was then placed in a refrigerator at 5°C for dormancy break and germination.

The parameters assessed include: number of days from treatment to breaking of dormancy, number of germinated seeds expressed as a percentage, number of leaves and plant height.

The data collected were subjected to Analysis of Variance (ANOVA) using SPSS and the means were separated using least significant difference (LSD) at 0.05 probability level.

III. RESULTS

Table 1 shows the effect of variety and GA₃ concentration on mean number of days to breaking of dormancy. Variety did not have any significant (P<0.05) effect on mean number of days to breaking of dormancy. However, GA₃ concentration has significant (P<0.05) effect on dormancy break. Seed treated with

15ppm of GA₃ was the earliest to break dormancy in 23 days. However, 10ppm and control took longer to break dormancy (40.65, 38.30 days) (Table 1). The interaction of variety and GA₃ concentration on mean number of days to dormancy break was not significant at 5% level of probability (Table 1).

Table 2 shows the effect of variety and GA₃ concentration on mean establishment count at week 3, 5 and 7 weeks after transplanting. Variety did not have any significant (P<0.05) effect on mean establishment count at all the sampling dates (3, 5 and 7 weeks after transplanting) (Table 2). GA₃ concentration had significant (P<0.05) effect on the establishment count. Seed treated with 15ppm of GA₃ had significantly higher mean establishment count at 3 and 5 weeks after transplanting (83.89 and 93.89% respectively) than 10ppm GA₃ and the control. However, at 7 weeks after transplanting 15ppm and 10ppm of GA₃ concentration resulted in a similar and significantly higher mean establishment count (93.89 and 93.88% respectively) than the control (67.225) (Table 2). The interaction of variety and GA₃ concentration on mean establishment count was not significant at all sampling dates (Table 2).

The effects of variety and GA₃ concentration on mean plant height is presented in Table 3. Variety did not have significant (P<0.05) effect on mean plant height at all the sampling dates. However, GA₃ concentration had significant (P<0.05) effect on mean plant height at 2 weeks after transplanting only, it was not significant at 1,3,4,5 and 6 weeks after transplanting (Table 3). The interaction of variety and GA₃ concentration on mean plant height was not significant at all the sampling dates at 5% level of probability (Table 3).

The effects of variety and GA₃ concentration on mean number of leaves per plant is represented in Table 4. The number of leaves per plant increased with time from one to six weeks after transplanting in both varieties and at all levels of GA₃ concentration. Variety did not have any significant (P<0.05) effect on mean number of leaves produced per plant at all the sampling dates. Generally, seed from green apple produced a slightly higher mean number of leaves than those from the red apples. Mean number of leaves produced per plant was not significantly (P<0.05) affected by GA₃ concentration. However, seeds treated with 15ppm of GA₃ resulted in highest mean number of leaves at all the sampling dates (Table 4). There was no significant (P<0.05) interaction of variety and GA₃ concentration on the mean number of leaves produced per plant at all the sampling dates (Table 4).

IV. DISCUSSION

Varieties did not have any significant effect on mean number of days to dormancy break, both varieties responded in similar manner. Pauwels *et al.* (1998) found a clear influence of cultivar in the time of seed germination with late ripening cultivars showing earlier germination than early ripening ones. According to Sharma *et al.* (2006) the embryonic dormancy in apples is a set of block imposed upon a process cardinal for growth, and these blocks are found in all apple seeds regardless of the type of variety or species of apple.

Treatment with Gibberellic acid (GA₃) had significant effect on dormancy break. Seeds treated with 15ppm GA₃ were the earliest to break dormancy in 23 days. Gornik *et al.* (2018) observed that the application of SA, GA₃, BAP and JA during seed stratification stimulated the seeds' germination rate as well as the growth of seedlings. They obtained the most pronounced results after stratification in GA₃ alone or in a mixture containing SA, GA₃, BAP and JA. Ailero (2004) reported that internal GA₃ percentage is at a high level, but the proportion of ABA is at lower level in dormant seeds. While GA₃ in the structure increases the enzymatic activities, it slows the ABA activity. Gibberellins have been shown to increase germination in several species (Karam and Al-Salem, 2001).

GA₃ concentration had significant (p<0.05) effect on plant height. Treatment of seeds with GA₃ at 15 ppm for 30 minutes before sowing was found to increase height of seedlings to 12.88cm. Rayees *et al.* (2014) reported apple seeds treated with GA₃ at 500 ppm for 40 hours before sowing with an increased seedlings length of 8.94 cm. Additional GA₃ activated α -amylase which digested the available carbohydrate into simpler sugar, so that energy and nutrition were easily available to faster growing seedlings.

Variety had no significant (P<0.05) effect on mean number of leaves per plant. The varieties both responded in a similar manner. This agreed with the previous work of (Welsh and McClelland, 1990). However, treatment with GA₃ promotes seed germination and number of leaves at 15ppm as shown in Table 4. This was found to be consistent with the findings of Chakrabarti and Mukherji (2002). GA₃ could overcome the adverse effect in the seed physiological activity of apple seedlings growth.

V. CONCLUSION

It can be concluded that the application of Gibberellic acid (GA₃) enhance dormancy break, germination and growth of apple seeds. GA₃ application at 15ppm was the most successful in breaking of dormancy and seedling growth of apple. GA₃ application is an effective method to increase and accelerate the germination of seeds and growth of seedlings.

It is also recommended that other higher concentrations of GA₃ could be researched into.

REFERENCES

- [1]. Ailero, B.L. (2004). Effects of Sulphuric Acid Mechanical Scarification and Wet Heat Treatments on Germination of Seeds. *Journal of Biotechnology*, 3(3):179-181.
- [2]. Chakrabarti, N. and Mukherji, S. (2002). "Effect of Phytohormone Pretreatment on Metabolic Changes in Vigna Radiate Under Salt Stress," *Journal of Environmental Biology*, 23: Pp. 295-300
- [3]. Chakrabarti, N. and Mukherji, S. (2003). "Effect of Phytohormone Pretreatment on Nitrogen Metabolism in Vigna Radiate Under Salt Stress," *Biology Planta*, 46: 63-66.
- [4]. Ciacka, K., Krasuska, U., Otulak-Kozielec, K., and Gniazdowska, A. (2018). Dormancy Removal by Cold Stratification Increases Glutathione and S-Nitrosoglutathione Content in Apple Seeds. *Plant Physiology and Biochemistry*, 138: 112-120.
- [5]. Dębska, K., Krasuska, U., Budnicka, K., Bogatek, R., and Gniazdowska, A. (2013). Dormancy Removal of Apple Seeds by Cold Stratification is Associated with Fluctuation in H₂O₂, No Production and Protein Carbonylation Level. *Journal of Plant Physiology* 170:480-488.
- [6]. El-Barghathi, M.F. and El-Bakkosh, A. (2005). Effect of Some Mechanical and Chemical Pre-Treatments on Seed Germination and Seedling Growth of Quercus Coccifera (Kermes Oaks). Jerash Private University.
- [7]. Finch-Savage, W.E., and Leubner-Metzger, G. (2006). Seed Dormancy and the Control of Germination. *The New Phytologist* 171:501-523.
- [8]. Górnik, K., Grzesik, M., Janas, R., Żurawicz, E., Chojnowska, E., Górska, R. (2018). The Effect of Apple Seed Stratification with Growth Regulators on Breaking the Dormancy of Seeds, the Growth of Seedlings and Chlorophyll Fluorescence. *Journal of Horticultural Research* 26(1): 37-44.
- [9]. Huang, X., Schmitt, J., Dorn, L., Griffiths, C., Effgen, S., Takao, S., Koornneef, M., and Donohue, K. (2010). The Earliest Stages of Adaptation in an Experimental Plant Population: Strong Selection on QTLs for Seed Dormancy. *Molecular Ecology* 19:1335-1351.
- [10]. Hyson, D.A. (2011). A Comprehensive Review of Apples and Apple Components and Their Relationship on Human Health. *Journal of Nutrition and Biochemistry*. 2:408-420.
- [11]. Kalinowska, M., Bielawski, A., Lewandowska-Siwkiewicz, H., Priebe, W. and Lewandowski, W., (2014). Apple: Content of Phenolic Compound Vs. Variety, Part of Apple and Cultivation Model Extraction of Phenolic Compound Biological Properties. *Plant Cell Environment*, 84: 169-188.
- [12]. Kandari, L.S., Rao, K.S., Payal, K.C., Maikhuri, R.K., Chandra, A. and Vanstaden, J.V. (2012). Conservation of Aromatic Medicinal Plant Rheum Emodi Wall Ex Messis Through Improved Seed Germination. *Seed Science and Technology*, 40: 95-101.
- [13]. Karam, N. S. and Al-Salem, M. M. (2001). "Breaking Dormancy in Arbutus Andrachne L. Seeds by Stratification and Gibberellic Acid," *Seed Science and Technology*, 29: Pp. 51-56, 2001.
- [14]. Koornneef, M., Bentsink, L., and Hilhorst, H. (2002). Seed Dormancy and Germination. *Current Opinion in Plant Biology*, 5:33-36.

Table 1: Effects of variety and GA₃ Concentration on the mean number of days to dormancy break.

Treatment	Number of days to seed germination
Variety	
Green	30.44a
Red	37.56a
LS	NS
LSD _{0.05}	7.98
GA₃ concentration	
0	38.30a
10	40.65a
15	23.00b
LS	*
LSD _{0.05}	9.73
Interaction	
(Variety x GA ₃ Concentration)	NS

NS= Not Significant

*=Significant.

Table 2: Effects of Variety and GA₃ Concentration on mean Establishment Count at 3, 5 and 7 weeks after planting.

Treatment	Establishment count(%)		
	Weeks after planting		
	3	5	7
Variety			
Green	56.67a	64.44a	76.66a
Red	53.70a	68.52a	74.07a
LS	NS	NS	NS
LSD _{0.05}	16.83	20.13	18.95
GA₃ concentration			
0	36.11b	49.99b	67.22b
10	45.55b	55.56b	93.88a
15	83.89a	93.89a	93.89a
LS	*	*	*
LSD _{0.05}	20.61	24.72	23.20
Interaction			

(Variety x GA ₃ Concentration)	NS	NS	NS
---	----	----	----

NS= Not Significant

*=Significant.

Table 3: Effects of variety and GA₃ Concentration on mean plant height at 1, 2, 3,4, 5 and 6 weeks after transplanting.

Plant height (cm)						
Treatment	1	2	3	4	5	6
Variety						
Green	7.90a	7.19a	7.89a	9.64a	11.95a	12.19
Red	6.96a	6.57a	7.77a	10.22a	11.41a	12.61a
LS	NS	NS	NS	NS	NS	NS
LSD _{0.05}	1.73	1.31	1.88	1.83	1.87	1.99
GA₃ Concentration						
0	6.30a	6.88b	7.21a	9.51a	11.03a	11.97a
10	6.96a	7.46a	7.84a	9.94a	11.33a	12.37a
15	6.90a	8.37b	8.44a	10.35a	12.70a	12.88a
LS	NS	*	NS	NS	NS	NS
LSD _{0.05}	2.12	1.60	2.29	2.23	2.29	2.43
Interaction						
V x GA ₃	NS	NS	NS	NS	NS	NS

NS= not significant

*= significant

Table 4: Effects of variety and GA₃ concentration on the man number of leaves at 1, 2, 3, 4, 5, and 6 weeks after transplanting

Number of Leaves						
Treatment	1	2	3	4	5	6
Variety						
Green	7.72a	8.54a	9.99a	11.33a	11.63a	12.86a
Red	7.05a	7.58a	9.81a	11.25a	11.47a	12.49a
LS	NS	NS	NS	NS	NS	NS
LSD _{0.05}	1.33	1.43	1.86	1.65	1.18	2.43
GA₃ Concentration						
0	7.22a	7.73a	9.73a	11.08a	11.13a	12.44a
10	7.37a	7.70a	9.22a	10.66a	10.89a	12.59a
15	7.85a	8.75a	10.66a	12.14a	12.65a	12.66
LS	NS	NS	NS	NS	NS	NS
LSD _{0.05}	1.63	1.82	2.28	2.87	1.44	2.97
Interaction						
V x GA ₃	NS	NS	NS	NS	NS	NS

NS= not significant *= significant