

Application of hormones and mechanical treatment on breaking the dormancy of G₀ “Granola” (*Solanum tuberosum*) minitubers in Northern Mindanao, Philippines

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Abstract. Macasambat RLP, Tan RJ, Margate RE. 2023. Application of hormones and mechanical treatment on breaking the dormancy of G₀ “Granola” (*Solanum tuberosum*) minitubers in Northern Mindanao, Philippines. *Asian J Agric* 7: 8-14. The study was conducted to identify the best treatment combination for dormancy breaking and sprouting quality for “Granola” minitubers. A comparison was made by wounding and dipping the one-week-old minitubers in different levels of GA₃ and BAP (0, 50, and 100 mg/L). Results showed that either 50 or 100 mg/L of GA₃ solely influenced the average number of sprouts in minitubers while wounding minitubers highly influenced tuber weight loss by increasing the percent loss in minitubers. In combination with factors such as wounding and 50 mg/L of GA₃, it positively influenced the average sprout length, fresh mass of sprouts, and dry mass of sprouts. While the dormancy period and days to dormancy break time (50% minitubers achieved > 2 mm sprout length) were reduced for more than 45 and 50 days, respectively, when factors were combined, such as wounding the minitubers and dipping in the treatment solution having 50 mg/L GA₃ and 50 mg/L BAP. The levels of GA₃ and BAP (50 and 100 mg/L) showed no significant differences in achieving early dormancy breaking and high sprouting quality in G₀ minitubers. Overall, for most of the results in this study, wounding combined with the application of 50 mg/L GA₃ was commendable for dormancy breaking and attaining high sprouting quality of “Granola” minitubers.

Keywords: Dormancy breaking, G₀, Granola, minitubers, *Solanum tuberosum*

INTRODUCTION

Potato (*Solanum tuberosum* L.) is one of the world's most important crops next to wheat and rice in terms of demand and consumption (CIP 2020; Maharijaya et al. 2021). It is grown in almost 155 countries worldwide, and billions of people consume it because of its versatility and nutritional values, such as high in phosphorus and vitamins B1, B2, and C (Gonzales et al. 2016), which are the main reasons why countries consider potato as one of the staple crops. Countries such as China and India are the biggest producer of potatoes worldwide (Statista 2019). On the other hand, in the Philippines, potato production has a fluctuating trend (PSA 2018; 2020). In recent data, from July to September 2020, Cordillera Administrative Region (CAR) was registered as the country's largest potato producer, followed by Davao Region and Northern Mindanao, Philippines (PSA 2020). Even with high production in some regions in the country, it is not enough to supply the increasing demand for potatoes not just in the Philippines but also in other countries since it is inevitable that production may decrease due to techniques that the Philippine potato industry has not yet adapted; specifically, dormancy breaking of tubers.

Potato tubers naturally undergo deep dormancy after reaching physiological maturity (Mani et al. 2017). Depending on the variety, dormancy can last up to two to five months without chemical or physical treatments before

sprouting (Wrobel et al. 2017). Countries under temperate climatic conditions often consider long dormancy advantageous. However, shorter dormancy is favored in subtropical countries, like the Philippines, where two planting cycles can be achieved per year (Struik and Lommen 1999). According to Wrobel et al. (2008), shortening or breaking the dormancy is a prerequisite, especially during the seed multiplication program and for rapid postharvest quality testing of tubers. Faster distribution of active tubers all year round is one of the keys to eliminating fluctuating production in the country.

During a visit to Northern Mindanao Agricultural Crops and Livestock Research Complex (NMACLRC) at Dalwangan, Malaybalay, Bukidnon, Philippines, which specializes in producing disease-free tubers, one of their concerns is the long dormancy phase of potato tubers. Remediation for breaking the dormancy, such as subjecting dormant tubers to darkness (Claassens et al. 2005), was examined; however, it caused shorter sprouts; thus, that kind of physical treatment was rejected. With this problem, NMACLRC cannot cater to all the farmers' needs to supply planting materials on the spot. With that, limiting active tuber purchases are made to have enough tubers available to other farmers. This case is difficult since other large potato farms are limited with planting materials; thus, farmers are encouraged to take a portion of their harvest (almost 20-40%) to sustain the next cropping season and induce dormant tubers on their own.

While the effects of phytohormones/chemical treatments such as Gibberellic Acid (GA₃) (Hassani et al. 2014; Mustefa et al. 2017; Deligios et al. 2019), Benzyl-Adenine (BA) (Marjeed and Bano 2006), Ethanol (Wrobel et al. 2017), Rindite with GA₃ (Esztergalyos and Polgar 2020), Cytokinin and GA₃ (Hartmann et al. 2011), and Thiourea (Hosseini et al. 2011) have been studied in breaking dormancy, on the other hand, mechanical/physical treatments have limited attention, such as scarification (slicing/cutting and/or wounding) after harvest. It was also studied by Deligios et al. (2019), which found that cutting tubers also significantly resulted in releasing bud sprouts. However, there are few studies involving tubers' wounding (skin scarring) to break the dormancy (Jansky and Hameruiik 2014). Normally, excising of tuber buds and cutting of tubers are involved, but the prior technique (scarring) is out of topic.

Today, no existing protocols alleviate this concern in Northern Mindanao, Philippines. With that, this study used the Granola cultivar since it has a three to four months dormancy phase and is the most produced and purchased at NMACLRC. Furthermore, the study served as a guide in breaking the dormancy of potatoes that can be adapted in Northern Mindanao, specifically in NMACLRC under Potato Laboratory, where the seed multiplication program is prioritized.

MATERIALS AND METHODS

Experimental design

The experiment setup was 2 x 3 x 3 factorial in a Completely Randomized Design. Randomization was done via draw lots during the conduct of the study. Each treatment combination (Table 1) was triplicated with 15 tubers per replication. Experimental treatments were composed of mechanical treatment (wounded and unwounded), GA₃ (0, 50, 100 mg/L) (Esztergalyos and Polgar 2020), and BAP (0, 50, 100 mg/L) (Nuraini et al. 2016).

Treatment application

One-week-old minitubers (15-20 g) were washed using tap water and placed in a tray for 10 minutes to dry excess water before applying the treatments. After that, mechanical scarification was applied by putting the tubers (70 minitubers) in the improvised scarring machine for 80 revolutions/turns. Afterward, wounded and unwounded seed minitubers were placed in plastic meshed bags and immersed in different treatment solutions for one hour (Nasiruddin et al. 2016; Lizarazo-Peña et al. 2020). While minitubers under treatment combination having zero/untreated with GA₃ and BAP (Benzylaminopurine), and wounded and unwounded (T1 and T10) were not soaked in distilled water as it served as the control variable in the experiment (practiced in Northern Mindanao). Minitubers were stored in the seed storage screenhouse of NMACLRC for 100 days. Observations were done twice a week at 3 to 4-day intervals for the whole duration of the study.

Table 1. List of hormone and mechanical treatment combinations

Mechanical Scarification (Factor A)	Factors			Treatment combination	Treatment code
	GA ₃ (Factor B)	CK (Factor C)			
A1-Wounded	B1-0 mg/L	C1-0 mg/L	A1B1C1	T ₁	
		C2-50 mg/L	A1B1C2	T ₂	
		C3-100 mg/L	A1B1C3	T ₃	
	B2-50 mg/L	C1-0 mg/L	A1B2C1	T ₄	
		C2-50 mg/L	A1B2C2	T ₅	
		C3-100 mg/L	A1B2C3	T ₆	
	B3-100 mg/L	C1-0 mg/L	A1B3C1	T ₇	
		C2-50 mg/L	A1B3C2	T ₈	
		C3-100 mg/L	A1B3C3	T ₉	
A2-Unwounded	B1-0 mg/L	C1-0 mg/L	A2B1C1	T ₁₀	
		C2-50 mg/L	A2B1C2	T ₁₁	
		C3-100 mg/L	A2B1C3	T ₁₂	
	B2-50 mg/L	C1-0 mg/L	A2B2C1	T ₁₃	
		C2-50 mg/L	A2B2C2	T ₁₄	
		C3-100 mg/L	A2B2C3	T ₁₅	
	B3-100 mg/L	C1-0 mg/L	A2B3C1	T ₁₆	
		C2-50 mg/L	A2B3C2	T ₁₇	
		C3-100 mg/L	A2B3C3	T ₁₈	

Data analysis

Percent sprouting. After 100 days, the sprouted minitubers were recorded, considering that there were 45 minitubers per treatment combination. The data were expressed as a percentage.

Dormancy period. The dormancy period was counted as the number of days from treatment application to sprouting (initiation of bud outgrowth < 2 mm sprout length) (Hosseini et al. 2011). The average mean time (day) to complete germination/sprouting (\bar{t}) formula by Bewley and Black (1943) was used to calculate the mean time/day as of when the dormancy period ended in minitubers per treatment combination.

$$(\bar{t}) = \sum (t \times n) / \sum n$$

Where t is the time in days starting from day 0, the day of sowing (or minituber treatment application), and n is the number of minitubers completing germination/sprouting on day t .

Days to dormancy period break time. Days to dormancy period break time was counted and considered when minitubers in each replication attained at least one sprout with more than 2 mm sprout length (Hosseini et al. 2011). The day has taken to achieve 50% (E_{50}) minituber sprouting was calculated using the modified formula of Farooq et al. (2005).

$$E_{50} = t_j + \frac{\left(\frac{N}{2} - n_i\right)(t_j - t_i)}{n_j - n_i}$$

Where N is the final number of the sprouted minitubers, and n_i and n_j are the cumulative number of sprouted minitubers by adjacent counts at times t_i and t_j , respectively, when $n_i < N/2 < n_j$.

Tuber weight loss. Tuber weight loss was examined using a sensitive digital weighing scale. After treatment exposure, the average weight of one-week-old minitubers was labeled as initial weight, and after termination (approximately 100 days), sprouted tubers were weighed for final weight.

The average number of sprouts per minituber. The average number of sprouts per individual minituber was counted and recorded when 95% (Degebasa 2012) of the minitubers sprouted (100 days after treatment application).

Average sprout length (mm). The initial average sprout length was measured in each tuber after breaking dormancy or having 50% sprouted tubers in each replication (> 7 minitubers). The final average sprout length was recorded after 100 days.

Fresh mass of sprouts (mg/minituber). After 100 days, minitubers were de-sprouted and weighed using a sensitive digital weighing scale for accuracy.

Dry mass of sprouts (mg/minituber). After measuring the fresh weight, the dry weight was recorded after oven drying at 80°C for three consecutive days. The dry matter percentage was then determined.

RESULTS AND DISCUSSION

Percent sprouting

Table 2 summarizes the Analysis of the Variance of all the parameters in this study. Data showed a high percent sprouting of 97.67-100% regardless of the application of MS, levels of GA₃ and BAP, and interaction among factors. That was proven in the analysis of variance, where it showed that the percent sprouting was not influenced by the treatment combinations applied. However, it was observed that most of the minitubers applied with GA₃ showed a trend of faster sprout emergence. The earliest observation of sprouting was one week after treatment application. This observed trend could be supported by the study of Rehman et al. (2003), who stated that GA₃ “accelerates sprouting emergence and accessory shoots.

Dormancy period

Statistical analysis showed that applying GA₃ into the dipping solution significantly shortens the dormancy period of “Granola” minitubers. Although, when it comes to the concentration of GA₃ (50 and 100 mg/L), there is no significant difference in its level, both have the same effects in the dormancy period attaining an average day of 26.99-27.81 days, much shorter compared to the average dormancy period in control (69.27 days), which shows GA₃ has a sole effect on this parameter compared to the other two individually applied factors. The same results were found by Degebasa (2012), where tubers treated with 40 and 50 ppm shortened the dormancy period by 18 to 20 days on Ethiopian potato varieties.

Table 2. Summary of the ANOVA

SS	Percent sprouting	Dormancy period	Days to dormancy break time	Tuber weight loss (%)	Average number of sprouts	Average sprout length (mm)	Fresh mass of sprouts	Dry mass of sprouts
Factor A	0.78 ^{ns}	3.54 ^{ns}	1.10 ^{ns}	50.58 ^{**}	0.35 ^{ns}	90.57 ^{**}	36.47 ^{**}	40.09 ^{**}
Factor B	0.67 ^{ns}	82.42 ^{**}	111.61 ^{**}	3.88 [*]	5.47 ^{**}	139.31 ^{**}	69.18 ^{**}	71.74 ^{**}
Factor C	2.34 ^{ns}	1.34 ^{ns}	1.09 ^{ns}	0.56 ^{ns}	1.42 ^{ns}	2.38 ^{ns}	2.21 ^{ns}	1.09 ^{ns}
A x B	0.13 ^{ns}	1.68 ^{ns}	0.31 ^{ns}	0.11 ^{ns}	0.40 ^{ns}	19.28 ^{**}	6.36 ^{**}	6.76 ^{**}
A x C	0.77 ^{ns}	4.43 [*]	4.30 [*]	0.00 ^{ns}	0.73 ^{ns}	0.32 ^{ns}	0.29 ^{ns}	0.53 ^{ns}
B x C	1.10 ^{ns}	3.94 ^{**}	2.01 ^{ns}	1.68 ^{ns}	1.59 ^{ns}	1.41 ^{ns}	1.81 ^{ns}	1.40 ^{ns}
A x B x C	1.80 ^{ns}	3.75 [*]	5.29 ^{**}	0.08 ^{ns}	0.58 ^{ns}	0.40 ^{ns}	0.89 ^{ns}	0.84 ^{ns}
C.V.	2.17 %	18.15 %	15.67 %	26.31 %	28.65 %	21.18 %	31.27 %	30.46 %

When it comes to interaction effects, factors, A (MS) and C (BAP), B (GA₃) and C (BAP), and the combination of these three factors (A x B x C) had a significant influence on shortening the dormancy period. Figure 1 demonstrates the interaction effect of factors A and C. Both levels of MS (wounded and unwounded) and BAP (50 and 100 mg/L) attained a dormancy period ranging from 26.32-27.70 days and almost the same average got by factor B alone.

While on the interaction between two factors B and C (GA₃ and BAP), the mean values under treatments having 50 mg/L and 100 mg/L of both phytohormones attained a dormancy period ranging from 25.71-28.44 days much shorter compared to the treatment where levels of BAP were only applied (Figure 2) (42.15-59.19 days).

On the side of the combination of the three factors, lower mean average day values on dormancy period were achieved when both levels had hormone supplementation regardless if the minitubers were scarified or non-scarified. Figure 3 showed that without the hormone application, Factor A alone did not affect the shortening of the dormancy period in minitubers. Regardless of the levels of hormones (50 and 100 mg/L) in wounded minitubers, the dormancy period was significantly shortened by more than 45 days.

These observations proved that these treatment factors could be used as a remediation for attaining a shorter dormancy period in G₀ “Granola” minitubers. Furthermore, the result in this parameter was in line with the studies of Barani et al. (2009), where the effect of GA₃ was positively correlated to the release of tuber sprouting by hydrolyzing tuber starch into renewable sugars; Aksenova et al. (2012a,b) where CK facilitates tuber dormancy to bud outgrowth; and Ostroshy (2006) which accordingly, wounding minituber skin is effective in the penetration of externally applied to promote hormones.

Days to dormancy break time

Statistical analysis showed factor B (GA₃), the interaction between factors A (MS) and C (BAP), and the combination of these three factors (A x B x C) (Figure 5) attained a positive influence on faster dormancy break time. With GA₃ supplementation alone, it showed that 50 and 100 mg/L have the same effect on dormancy break time. Under this factor, 50% was achieved by 37.41 (50 mg/L) and 34.96 (100 mg/L) days. While in the interaction between factors A and C, dormancy break time was faster when minitubers were scarified regardless of the levels of BAP (34.35-34.54 days). However, the application of BAP alone on unwounded minitubers obtained 35.84-37.35 days, faster than wounded/unwounded minitubers without CK (66.12-68.00 days) (Figure 3).

On the other hand, Figure 4 revealed the significant interaction among the three factors (A x B x C). It was demonstrated that minitubers applied with these treatment combinations are closer to the baseline on average day-35.

This average day (35 days) was used to obtain a clear comparison as to when minitubers under these treatments attained 50% > 2 mm sprouts in just 35 days (50 days or shorter compared to the control in this study-84.69 days).

That shows a significant reduction in breaking the dormancy of “Granola” minitubers. In addition, GA₃ was also found to have a strong positive influence on this parameter.

Thus, this finding was about the studies of Hassani et al. (2014), where GA₃ supplementation resulted in “breaking minituber dormancy of potato for a short time”; Suttle (2007), where CK (BA) induced dormancy break and sprouting; and Hosseini et al. (2011) which stated that wounds on minituber skin (thin skin/wounds caused by harvesting) facilitates entry of chemicals, but in this case, hormones.

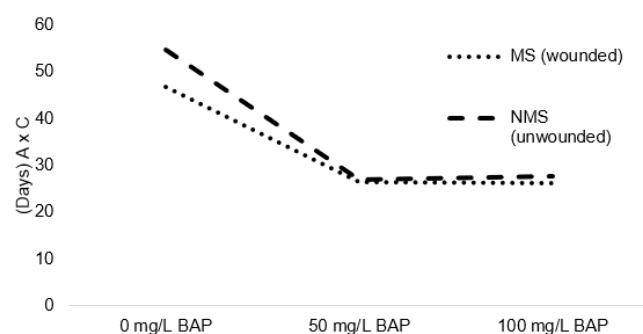


Figure 1. Interaction between Mechanical scarification and BAP levels in breaking the dormancy period (days) of “Granola” minitubers

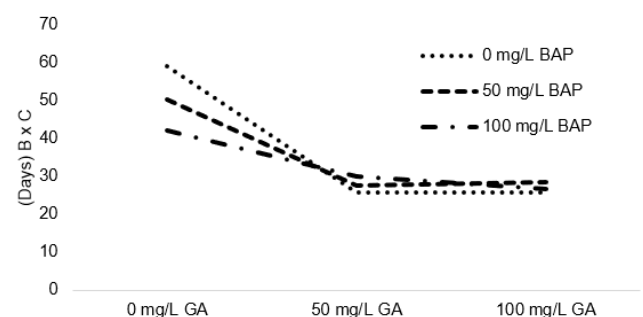


Figure 2. Interaction between GA₃ and BAP in breaking the dormancy period (days) of “Granola” minitubers

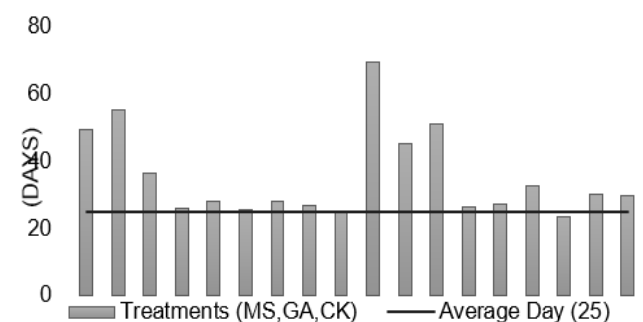


Figure 3. Interaction between three factors-(MS-With Mechanical Scarification; NMS-Without Mechanical Scarification (A); GA (0, 50, 100 mg/L) (B), CK (0, 50, 100 mg/L) (C), in breaking the dormancy period of “Granola” minitubers

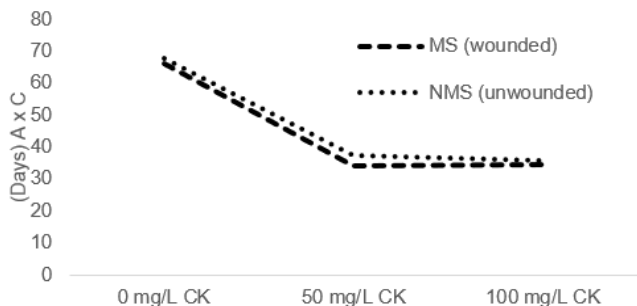


Figure 4. Interaction between Factor A (MS) and Factor C (BAP levels) in days to 50% dormancy break time (> 2 mm sprout length) of “Granola” minitubers

Tuber weight loss

Tuber weight loss was higher when minitubers were subjected to wounding and GA₃. Statistical analysis demonstrated that single factor A (MS) and regardless of factor B (GA₃) levels significantly affect the weight loss of minitubers, respectively. In contrast, levels of BAP (Factor C) and interaction among factors did not show a notable difference in this parameter.

Results in this study revealed that wounding applied on minitubers accelerated starch hydrolysis by efficiently delimiting the penetration of GA, which is the main stimulator of accumulating renewable sugars from hydrolyzing the starch content in minitubers (Alexopolous et al. 2008). Furthermore, this result also replicated the findings of Ostroshy (2006), which observed that hormone application is effective on the mechanically damaged skin of potatoes.

With limited attention to previous studies conducted, wounding of minituber skin (MS) has been proven in this result that it has the potential to enhance sprouting, contributing to a higher tuber weight loss. That indicates that when minitubers attain excessive weight loss, they are correlated to vigorous and lengthy sprouts (Ezekiel et al. 2007), which is vital for a positive performance in field propagation.

On the other hand, on Factor B, mean values were found in descending order. In contrast, as the level increased, the mean values were smaller, which is a speculative finding knowing that statistically, this factor is significant. However, compared to another factor (Factor C) and interactions among the factors, the tuber weight loss under the levels of GA₃ (50 and 100 mg/L) was still higher compared to having none (Control-4 %).

Average number of sprouts per minituber

The statistical analysis showed that the number of sprouts was only influenced when GA₃ was supplemented into the dipping solution regardless of the mechanical (Factor A), BAP (Factor C), and the interaction between factors applied. Furthermore, the HSD test revealed that 50 and 100 mg/L of GA₃ were both effective and did not have significant differences in their effect on an average number of sprouts.

This result implies that GA₃ plays an important role in tuber bud outgrowth. Furthermore, high levels of GA₃ (50

mg/L and 100 mg/L) were sufficient in enhancing the number of sprouting in minitubers. These findings showed accuracy to the study of Rehman et al. (2003), where GA₃ supports the acceleration of sprout emergence and is in line with its significant effect on the tuber weight loss parameter where the role of GA₃ in hydrolyzing starch and accumulation of renewable sugars in minitubers (Alexopolous et al. 2008) was mentioned.

In contrast to this result, the study failed to replicate the observation of Suttle (2007), where CK also facilitates sprouting and the effect of mechanical scarification that differed from the results of the earlier parameter (tuber weight loss). Thus, in this case, the addition of GA₃ has been strengthened to be vital in releasing a high sprouting number per minituber compared to the control (no hormone applied).

Average sprout length

Statistical analysis demonstrated that MS (Factor A), specifically wounding, attained a highly significant effect on the sprout length of minitubers. However, without GA₃ (Factor B) supplementation, it was observed that sprouts were shorter even if BAP (Factor C) was applied. With this, based on the analysis, the combination of Factor A (MS) and Factor B (GA₃ at 50 mg/L, 100 mg/L) highly influenced the average sprout length of minitubers (Figure 6). While For Factor C, the analysis showed a non-significant effect in the average sprout length.

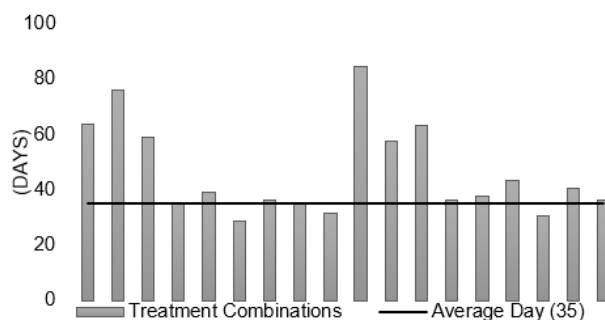


Figure 5. Interaction between three factors-(MS-With Mechanical Scarification; NMS-Without Mechanical Scarification; GA (0,50,100 mg/L), BAP (0,50,100 mg/L), on days to 50 % dormancy break time of “Granola” minituber

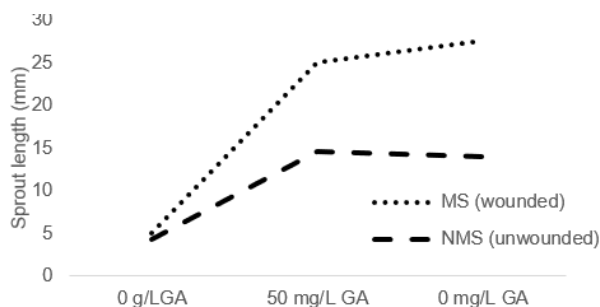


Figure 6. Interaction between Factor A (Mechanical Scarification) and Factor B (GA₃ levels) on average sprout length of “Granola” minitubers

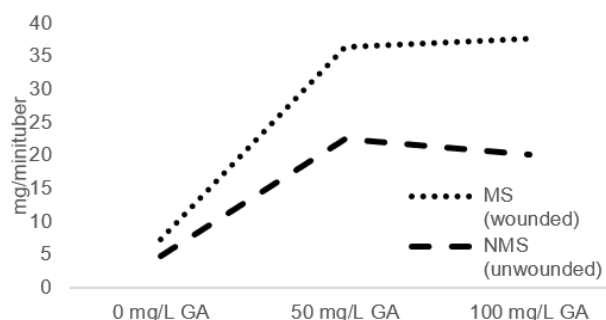


Figure 7. Interaction between Factor A (Mechanical Scarification) and Factor B (50 and 100 mg/L GA₃) on Dry mass of sprout of “Granola” minituber

In this parameter, the observation specifically on the interaction between GA₃ and BAP, where these two phytohormones are known to work together, especially in its application on in vitro seed germination, had created a speculative finding. However, studies involving the incompatibility effect of CK (BAP), specifically on the role of GA₃ in tuber dormancy breaking, are nonexistent today. With this, a thorough study approach on this matter can be done to excavate the factors why CK (BAP) did not complement GA₃ and/or scarred minitubers in this experiment. However, it is still important to note that there are other physiological reasons why CK did not prevail on its positive claims from studies conducted on breaking the tuber dormancy, specifically on tuber sprouting.

In terms of the interaction between Factor A and Factor B (Figure 5), analysis, once again, revealed a highly significant effect in this parameter. Minituber sprout length ranged from 25.14-27.67 mm when minitubers were wounded and dipped at 50 mg/L and 100 mg/L of GA₃. Conversely, minitubers wounded but not dipped in GA₃ got a mean value of 5.04 mm.

This result shows continuity to the findings observed from the tuber weight loss parameter, where it proved that the role of MS greatly maximized the effects of GA₃ by allowing the hormone applied externally to penetrate the internal tissues of minitubers which contributed to a faster and longer sprouting rate (Ostroshy 2006 and Barani et al. 2009).

Fresh mass of sprout

The fresh mass of the sprout (mg/minituber) result was in line with the previous parameter’s findings (average sprout length). Statistical analysis revealed that MS (wounding) and GA₃ (50 and 100 mg/L), and in a combination of those two (Factor A x Factor B), highly influenced the fresh mass of sprout of “Granola” minitubers. Treatment means were significantly higher when MS and 100 mg/L of GA₃ was applied regardless of the levels of BAP (Factor C).

This result shows the same trend in its compatibility with MS and GA₃ in allowing the externally applied hormone to maximize its effects on sprouting. With limited attention to studies conducted in this parameter, MS has been proven again to be a reliable treatment for minitubers

to have a higher fresh mass of sprouts, indicating higher sprout quality than the control.

Thus, the result in this study shows a relationship to the findings of Ostroshy (2006) and Jansky and Hameruk (2014) and the findings from the previous parameter in this study (average sprout length) where MS and GA₃ are best combined to achieve hormonal effects, tuber viability, and sprout quality.

Dry mass of sprouts

The minitubers attained the highest dry mass means applied with Factor A (wounded) and regardless of the levels of Factor B (GA₃ at 50 mg/L, 100 mg/L).

Statistical analysis demonstrated that MS and GA₃ and the interaction between the two factors (Figure 7) highly influenced the dry mass of sprouts. In comparison, supplementation of BAP on wounded minitubers and/or in a combination of GA₃ did not affect this parameter which is a consistent finding from the previous parameters discussed.

The same trend was shown in the two previous parameters; there is a high mean value on minitubers under the interaction of wounding and levels of GA₃ (either 50 or 100 mg/L) compared to other treatment combinations. That proves the importance of GA₃ as the hormone intervention for sprout quality parameters and the complementary effects of delimiting hormone penetration with MS on minitubers.

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