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Full Paper

Grape (*Vitis vinifera* L.) propagation using different types of cuttings and root-initiating substances

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Abstract

Grapes can be propagated through hardwood or semi-hardwood cuttings using various root-initiating substances. This study investigated different types of cuttings and root-initiating substances for grape propagation. The research evaluated the rooting response of different types of cuttings (semi-hardwood cuttings with one, two, three, or four buds and hardwood cuttings with one, two, three, or four buds) and various types of root-initiating substances (moringa leaf extract, young coconut water, honey, apple cider vinegar, aloe vera gel extract, and α-naphthalene acetic acid (NAA)). Cuttings were taken from a healthy, disease-free grapevine mother plant (White Malaga variety), and carried out in a Completely Randomized Design replicated three times. Hardwood cuttings resulted in significantly more roots, longer shoots, and more leaves compared to semi-hardwood cuttings. Moreover, cuttings with three or four buds had significantly higher number of roots, rooting percentage, root length, number of leaves, and percentage survival compared to cuttings with one bud. Furthermore, grapevine cuttings applied with NAA had the highest rooting percentage, root length, and survivability. Comparable results were found in cuttings applied with young coconut water or apple cider vinegar. Based on these results, the selection of hardwood grapevine cuttings with three or four buds and the application of NAA, coconut water or apple cider vinegar as root-initiating substances are recommended.

Keywords - grapes, hardwood cutting, semi-hardwood cutting, root-initiating

Introduction

Grapes of the genus Vitis originated from North America formed an important part of the diet of many Native Americans, and became an ingredient for wine production by European colonists (Hartmann et al., 1983). Today, the bulk of grape production is sourced from temperate countries, mostly from China, France, and Italy (Food and Agriculture Organization [FAO], 2022). It may seem unlikely that grapes can grow in a tropical country, but grapes are indeed cultivated in the Philippines (Wen et al., 2013), in the provinces of Cebu, Iloilo, Cotabato, Masbate, and La Union (Yap, 2018). The Philippines has a total grape area of 369 hectares, with a total production of 212 tons (FAO, 2020).

The propagation of grapevines is an important consideration in commercial viticulture and winemaking. Compared to sexual propagation, asexual propagation such as through cuttings is often preferred because the method is easier and faster, and can result in a large plant in a short span of time (North Carolina State Extension, 2018). Since grapes are prolific growers, cuttings are easily available. Softwood cuttings are available during the growing season, when plants are actively putting out new shoots, while hardwood and semi-hardwood cuttings are available offseason when plants are dormant. Grapes can be grown from softwood cuttings (Warmund et al., 1986), but the more common method of propagation is through hardwood cuttings (Waite et al., 2014).

Despite the importance of high-quality grapevine planting material, a large proportion of studies on grapevines have focused on disease or disease control (Martelli, 1999; Rego et al., 2009; Stamp, 2001), and more recently, on the effects of climate change on grapevine production or on climate change adaptations (Delrot et al., 2020; Duchêne et al., 2010; Tomasi et al., 2011; Trbic et al., 2021). Relatively few studies have investigated factors and

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strategies that can improve the quality of planting material, but most of these studies focused on hot- or cold-water treatments or on storage and hygienic practices in grapevine nurseries (Borsellino et al., 2012; Gramaje & Armengol, 2012; Waite & Morton, 2007). One study compared Vidal blanc (Vitis x sp.) hardwood and softwood grapevine cuttings, and found that hardwood cuttings resulted in larger roots and shoots (Warmund et al., 1986). This paper investigated growth parameters of hardwood and semi-hardwood grape cuttings (White Malaga variety) with varying number of buds. It is important to assess various types of cuttings to obtain lower mortality rates and facilitate grape propagation. Although there are no wide-scale scientific studies, grape growers observe that the first six months are most crucial for survival (Gumba, 2019). The use of appropriate type of cuttings is relevant to determine which type of cuttings will produce more vigorous grape seedlings.

This study likewise compared the effects of various root-initiating substances on survivability and on growth parameters of grapevines. Over the years, researchers have proposed theories about the role of plant growth and regulators and metabolites in promoting roots formation (Galavi et al., 2013; Kassahun & Mekonnen, 2012). Understanding the physiology of unrooted cuttings and the role of endogenous and exogenous hormones is crucial for successful plant propagation of grapes (Alem 2010; Pijut et al., 2011).

Grape growers commonly use synthetic hormones in propagating grapes, but there are other substances with promising effects on the rooting initiation of asexually propagated cuttings. Some growth substances investigated in the literature include moringa extract (Elzaawey et al., 2017), coconut water (Sandoval Prando et al., 2014), honey (Dunsin et al., 2018), and apple cider vinegar (Wang & Millner, 2009). However, these studies examined crops other than grapes. The second objective of this study is to investigate the rooting of grapevines using the aforementioned root-initiating substances.

Methodology

Study Site

Two experiments were conducted at the University of Southern Mindanao Research and Development Center, Kabacan, North Cotabato, Philippines (7°7′12″N, 124°49′12″E) from December 2018 to March 2019. The area has an elevation of 21 m.

Planting Material

Cuttings were obtained from 3.5-year-old healthy and fruit bearing vines of White Malaga cultivar. These vines were procured from a grape vineyard farm in Purok 2 Sto. Niño, Brgy. Glamang, Polomolok. The vines were maintained as stock plant for the experimental set-up, and were planted in Purok Saranay, Datu Piang Streets, Brgy. Poblacion, Kabacan, North Cotabato.

Experimental Set-up

Grapevine Cutting Experiment

The growth of grapevine cuttings using hardwood and semi-hardwood with different number of buds as planting material was investigated (Figure 1). Hardwood grapevines with one-bud cutting (7-11 cm long), two-bud cutting (12-16 cm long), three-bud cutting (17-21 cm long), and four-bud cutting (22-26 cm long) were prepared by cutting the vine with 22-23 mm diameter at the desired length from the shoot tip. The semi-hardwood grapevine was prepared with one, two, three and four buds (the same length with the hardwood grapevine) by cutting the vine with 20-21 mm diameter at the desired length from the shoot tip. The cuttings were covered with paper moist with distilled water for 24 hours.

The growing media composed of fine river sand and coco coir (1:1 ratio) was heat sterilized at 90°C for at least 30 minutes. Cuttings were planted by inserting the basal portion of the stem into the potting media at 5 cm depth. Watering was done a day before planting. The vine cuttings or sample plants were placed under a partially shaded area in an enclosed system propagation (*kulob* condition) in which the potted plants were placed inside clear plastic with string support on the top portion (Figure 2). Cuttings were not fertilized.

Plants were arranged in a Complete Randomized Design (CRD) with three replications per treatment. There was a total of 8 treatments with 10 sample grapevine cuttings used per replicate.

Rooting Experiment

The growth parameters of hardwood grapevine

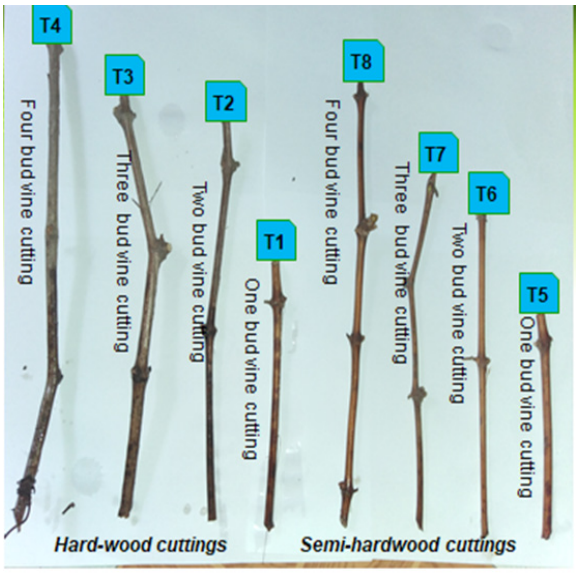

Figure 1. Different types of grapevine cuttings. T1, T2, T3, and T4 are hardwood cuttings with 1, 2, 3, and buds, respectively. T5, T6, T7, and T8 are semi-hardwood cuttings with 1, 2, 3, and 4 buds, respectively.

Figure 2. Grapevines propagated in transparent plastic bags (*kulob*) condition.

cuttings with root-initiating substances were investigated. Hardwood grapevine cuttings with four buds were covered with paper moist with distilled water for 24 hours. Thirty cuttings were transferred per container and soaked with 1000 mL of the following root-initiating substances: moringa leaf extract (100%), young coconut water (100%), pure honey (100%), apple cider vinegar (10 mL^{-1} of water), aloe vera gel extract (100%), distilled water (as untreated control), and α-Naphthalene Acetic Acid (NAA, 10 mL^{-1} of water, standard control). After 12 hours in these treatments, the grapevine cuttings were planted in sterilized media and maintained in an enclosed system propagation for 30 days as previously described. Plants were arranged in a Complete Randomized Design (CRD) with three replications per treatment. There were seven treatments with 10 sample grapevine cuttings used per replicate.

Plant Growth Assessment

Growth parameters were measured in both experiments at 30 days after planting. Plants were uprooted and washed with running water. The number of roots was counted per plant. The rooting percentage was computed using the formula

$$\frac{\text{Number of survived cuttings}}{\text{Total number of cuttings}} \times 100\%$$

Root length and shoot length were measured with a ruler. The number of leaves and number of shoots were determined by counting the number of newly developed leaves and shoots per plant sample, respectively.

The percentage survival (%) was computed using the formula

$$\frac{\text{Number of rooted cuttings}}{\text{Total number of cuttings}} \times 100\%$$

Data Analysis

For the grapevine cutting experiment, the data were analyzed using a 2x4 ANOVA (type of cutting: hardwoord or semi-hardwood × number of buds: 1,2,3,4). For the rooting experiment, one-way ANOVA was used to compare the effects of the different root-initiating substances on the growth and rooting parameters of the cuttings. The level of significance was set at 5%, and significant differences were analyzed using Scheffe's post-hoc test.

Results and Discussion

Grapevine Cutting Experiment

Tables 1 through 7 respectively present the number of roots, rooting percentage, root length, shoot length, number of leaves, number of shoots, and percentage survival of the grapevine cuttings (White Malaga variety) after 30 days. With respect to the type of cutting, significant differences were found only in the number of roots, shoot length, and number of leaves. Hardwood cuttings produced significantly more roots (on average, 15.22), longer

shoots (on average, 3.44 cm), and more leaves (on average, 5.26) compared to semi-hardwood cuttings (on average, 11.32 roots, 2.31 cm shoots, and 3.38 leaves). Sample cuttings after 30 days are shown in Figure 3.

The results agree with the findings of Rema and Pandey (1990) who found out that hardwood cuttings produced more roots compared to the softwood cuttings of grapes, resulting in higher availability of photosynthates. Similar results were obtained by Habib (1980) who observed that the rooting percentage of grapevines was significantly higher in hardwood cuttings compared to softwood cuttings, noting that a higher content of total carbohydrates and carbon/nitrogen ratio were important for high rooting capacity of grapevine cuttings. Moreover, Munoz et al. (1976) found hardwood grape cuttings c.v. Sultamina rooted better than softwood cuttings. Hardwood cuttings were also applicable for propagation of Concord grapes and other varieties according to Lima et al. (2006), Mayer and Pereira (2003), and Fischer et al. (2003). The better performance of the hardwood cuttings could be due to the presence of auxin, which can promote mobilization of nutritional reserves to the region of root formation (Galavi et al., 2013). Further, higher auxins in hardwood cuttings are involved in protein synthesis regulation and metabolism at the rooting zone, thereby promoting root regeneration (Gregory & Samantharai, 1950). Karakurt (2009) and Waite et al. (2015) also noted that grapevines are generally propagated through hardwood cuttings due to its high success rate even without the use of special a rooting hormone.

The number of buds in grapevine cuttings was also found to be a determining factor that improved root production. Significant differences were found in the number of roots, rooting percentage, root length, number of leaves, and percentage survival. In these

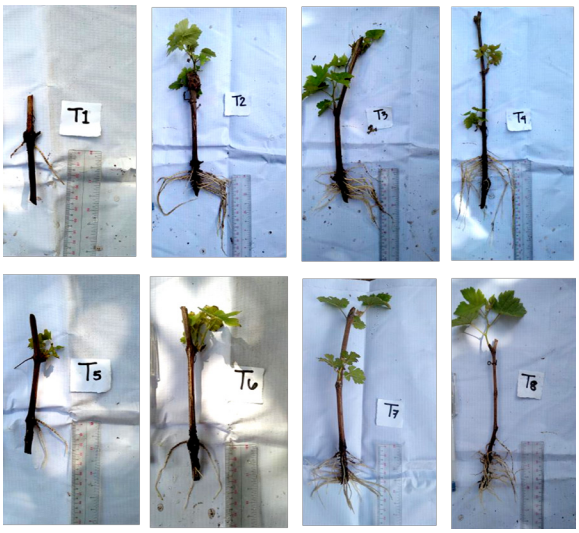

Figure 3. Roots, shoots and leaves of grape cuttings as influenced by the different types of cuttings 30 days after planting.

parameters, cuttings with three or four buds had significantly higher values compared to cuttings with one bud. These results indicate that a higher number of buds is a contributing factor that enhances the growth and rooting parameters of grapes. There were significantly fewer roots in grapevine cuttings with one or two buds (on average, 5.43 and 10.11, respectively) compared to cuttings with three or four buds (on average, 17.16, and 20.39, respectively). Similarly, the rooting percentage of grapevine cuttings with one bud (on average, 30.00%) was significantly lower than that of two, three or four buds (on average, 70.00%, 71.67%, and 80.00%, respectively). Grapevine cuttings with one bud or two buds had significantly shorter roots (on average, 4.67 and 7.91 cm, respectively) compared to cuttings with three or four buds (on average, 11.77 and 8.90 cm, respectively). There were significantly fewer leaves in grapevine cuttings with one bud (on average, 2.67) compared to cuttings with four buds (on average, 5.96). The percentage

Table 1. Average number of roots of grape vine cuttings as influenced by different types of cuttings progpagated for 30 days under *kulob* condition.

	1	2	3	4	
Hardwood	4.69	12.51	19.07	24.63	15.22[a]
Semi-hardwood	6.16	7.71	15.25	16.15	11.32[b]
	5.43[a]	10.11[a]	17.16[b]	20.39[b]	

*Column or row means having different superscripts are significantly different ($p < 0.05$)

Table 2. Average rooting percentage (%) of grape vine cuttings as influenced by different types of cuttings propagated for 30 days under *kulob* condition.

	1	2	3	4	
Hardwood	40.00	60.00	70.00	86.67	64.17
Semi-hardwood	20.00	80.00	73.33	73.33	61.67
	30.00[a]	70.00[b.]	71.67[b]	80.00[b]	

*Means in the same row having different superscripts are significantly different ($p < 0.05$)

Table 3. Average root length (cm) of grape vine cuttings as influenced by different types of cuttings propagated for 30 days under *kulob* condition.

	1	2	3	4	
Hardwood	5.26	7.64	9.87	10.05	8.21
Semi-hardwood	4.08	8.18	13.66	7.74	8.42
	4.67[a]	7.91[ab]	11.77[b]	8.90[b]	

*Means in the same row having different superscripts are significantly different ($p < 0.05$)

Table 4. Average shoot length (cm) of grape vine cuttings as influenced by different types of cuttings propagated for 30 days under *kulob* condition.

	1	2	3	4	
Hardwood	2.24	3.51	4.03	3.96	3.44[a]
Semi-hardwood	1.69	2.77	2.57	2.19	2,31[b]
	1.97	3.14	3.3	3.08	

*Means in the same column having different superscripts are significantly different ($p < 0.05$)

Table 5. Average number of leaves of grape vine cuttings as influenced by different types of cuttings propagated for 30 days under *kulob* condition.

	1	2	3	4	
Hardwood	2.67	5.16	4.43	8.87	5.26[a]
Semi-hardwood	2.67	3.71	4.11	3.05	3.38[b]
	2.67[a]	4.43[ab]	4.22[ab]	5.96[b]	

*Means in the same row and means in the same column having different superscripts are significantly different ($p < 0.05$)

Table 6. Average number of shoots of grape vine cuttings as influenced by different types of cuttings progpagated for 30 days under *kulob* condition.

	1	2	3	4	
Hardwood	1.00	1.25	1.26	1.56	1.27
Semi-hardwood	1.00	1.92	1.33	1.06	1.33
	1.00	1.59	1.30	1.31	

Table 7. Average percentage survival (%) of grape vine cuttings as influenced by different types of cuttings propagated for 30 days under *kulob* condition.

	1	2	3	4	
Hardwood	56.67	63.33	76.67	90.00	71.67
Semi-Hardwood	26.67	76.67	80.00	83.33	66.67
	41.67[a]	70.00[b]	78.34[b]	86.67[b]	

*Means in the same row having different superscripts are significantly different ($p < 0.05$)

survival of the grapevine cuttings with one bud (on average, 41.67%), especially for the semi-hardwood grapevine cuttings (on average, 26.67%), was significantly lower than that of the grapevine cuttings with two, three or four buds (on average, 70.00%, 78.34%, and 86.67%, respectively).

The larger number of leaves found in cuttings with multiple buds promoted photosynthetic activities that enhanced growth and root parameters, leading to increased nutritional reserves and survivability. Moreover, the leaves possibly supplied the required amount of endogenous hormones, particularly auxins, for promoting root formation and induction (Galavi et al., 2013). The higher auxin content in cuttings with multiple buds could explain the higher rooting percentage in these cuttings, resulting to fast mobilization of carbohydrates. The result of the study is consistent with findings of Patil et al. (2001) who observed the maximum number of leaves, shoot number, shoot length and leaf area in hardwood cuttings with 20-25 cm long cane.

The better performance of hardwood cuttings with multiple buds is consistent with results of Dulta (1982) who found that hardwood cuttings with multiple buds (basal portion cuttings) had better rooting percentage compared to softwood cuttings (apical portion cuttings). Hartman and Kester (1983) also noted that the best rooting was usually found in hardwood cuttings, due to the possibility of higher accumulation of carbohydrates and concentration of endogenous root-promoting substances in buds and leaves.

Rooting Experiment

Table 8 shows various growth parameters of hardwood vine cuttings applied with different root-initiating substances after 30 days of experimental period. The only significant differences were found in rooting percentage, root length, and percentage survival. In each of these three parameters, hardwood grapevine cuttings applied with NAA registered the highest percentage survival (83.33%), rooting percentage (83.33%) and root length (5.07 cm). Grapevine treatments applied with young coconut water and apple cider vinegar had comparable values with cuttings applied with NAA across these three parameters. Carusetta (2014) argued that coconut water, moringa leaf extract and aloe vera gel are natural materials that possess the ability to stimulate the rooting of cuttings, and are suitable substitutes to the synthetic hormones such as cytokinins, auxins and gibberellins. However, in this study, grapevine cuttings applied with moringa leaf extract and aloe vera gel had significantly lower survivability compared to cuttings applied with NAA.

The increased survivability and faster root elongation of grape cuttings applied with NAA, coconut water or apple cider vinegar can be associated with essential hormones and beneficial plant growth regulators in these substances. Plant regulators such as NAA contain auxin, which is essential for root induction and root formation (Galavi et al., 2013; Tofanelli et al., 2014). Coconut water naturally contains plant growth regulators such as cytokinin and indole acetic acid which promotes survival and rooting of cuttings (Agampodi, 2009). Yong et al. (2013) and Oluwagbenga et al. (2016) found that coconut water supports root growth of African locust bean (*Parkia biglobosa*) due to presence of cytokinin and auxins. The beneficial effects of apple cider vinegar may be explained by its composition of trace elements that are beneficial to plant growth (Gillen, 2021) and disease control (Chi & Anh, 2019).

Conclusion and Recommendations

This study investigated the effect of various types of grapevine cuttings and various root-initiating substances on survival, rooting, and growth performance of grapevine cuttings. The

Table 8. Growth parameters of grapevine cuttings applied with various root-initiating substrances propagated for 30 days.

Treatments	Percentage** survival (%)	Rooting** percentage (%)	Root** length (cm)	Number of^{ns} roots	Number of^{ns} leaves	Number of^{ns} shoots	Shoot^{ns} length (cm)
Control (untreated)	50.00^{abc}	50.00^{ab}	2.50	2.28	3.06	1.11	2.44
Standard control (NAA) (ml L^{-1})	83.33^{a}	83.33^{a}	5.07	3.20	2.85	1.00	2.38
Moringa leaf extract (100%)	53.00^{abc}	53.00^{ab}	3.09	2.51	2.62	1.06	2.21
Young woconut water (100%)	76.67^{ab}	73.00^{ab}	4.90	4.71	4.44	1.77	2.61
Pure honey (100%)	33.33^{c}	33.33^{b}	2.33	2.25	2.33	1.08	2.00
Apple cider vinegar (ml L^{-1} of water)	66.67^{abc}	66.67^{ab}	3.46	4.73	4.18	1.41	2.46
Aloe vera gel extract (100%)	46.67^{abc}	46.68^{ab}	2.67	3.25	3.23	1.23	2.38
Mean	58.57	58.00	3.43	3.27	3.24	1.23	2.37
CV	7.54	7.34	8.12	8.06	5.99	5.43	2.09

** signifcant
^{ns} not significant
Means in a column with different superscripts are significantly different ($p < 0.05$) (Scheffe's test)

best performance in terms of rooting, growth, and survival were found in hardwood grapevine cuttings with three or four buds. In particular, hardwood grapevine cuttings resulted to significantly more roots and longer shoots compared to semi-hardwood grapevine cuttings. Multiple buds also significantly increased the number of roots, rooting percentage, root length, number of leaves, and survivability.

Among the various root-initiating substances investigated, grapevine cuttings applied with NAA had the highest survivability, rooting percentage, and root length. The results were comparable to cuttings applied with young coconut water or apple cider vinegar. Based on these results, the selection of hardwood grapevine cuttings with three or four buds and the application of NAA, coconut water or apple cider vinegar as root-initiating substances are recommended.

Disclosure Statement

No potential conflict of interest was declared by the author.

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