Enhancing Seed Germination and Seedling Growth in Two Cultivars of Grape by Salicylic Acid and Scarification

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Abstract

Kurdistan/ Iraqi are famous for its different varieties of grapes, especially in the mountainous areas where they are grown on the mountain slopes. The main method of grapevine propagation is hard wood stem cuttings, for effective plant management and to obtain new grape varieties and resistant rootstocks in areas where phylloxera is prevalent a general grasp of the mechanisms releasing seed dormancy is crucial.

This study was undertaken during the period October 1st 2024 at a private plastic house located in Mirkan xdeer village, Qushtapa town, Erbil province, Kurdistan region, Iraq to check the effect of different pre-sowing treatments on seed germination of two local grape cultivars (Halawani and Zaitony). Box trials were conducted using completely randomized design (CRD). Seeds of two cultivars were subjected to three different concentrations of Salicylic acid (SA) (0, 1 and 2 mM) held for 24 hours, as well as Scarification (SC) (Control, thermal and mechanical), control (Without scarification), in combined treatment seeds scarified followed by soaking in Salicylic acid. Additionally for seeds storage moist chilling treatments consisted of (2 months) the seeds were placed into refrigerator at 5±1oC using plastic bags containing moistened peat-moss.

The results showed that grape cultivars had no significant effect on days taken for germination, germination (%), rate of germination, seedling survival (%) and vitality index1 11 (SVI 11) while for SVI 1 Halawani cultivar contributed the maximum value (4.75 cm) and the minimum value in the Zaitony cultivar (4.20 cm) and for SVI 111 highest value was recorded as in Zaitony cultivar (0.32 mg) and the lowest in Halawani (0.27 mg).

Effect of SA treatment on seed germination was more effective on increasing the seed germination percentage and decreasing the average day of germination by higher concentration, days to early emergence of the seedling were recorded in (2 mM) concentration (18.25 days) when compared to control (21.97 days), significance differences of germination percentage (88.51 %), rate of germination (5.05), seedling survival (75.28%), SVI1 (5.25cm), SVI 11 (53.95g) were obtained compared to control (77.10%), (3.74), (67.33 %), (4.17 cm) and (37.24g) respectively.

In scarification (Sc) more days to seedling emergence (21.19 days) were obtained in non scarified seeds when compared to scarified seeds (19.74 days) with sandpaper and (19.95 days) with hot water. Scarified

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seeds with sandpaper produced highest significance differences of germination percentage (83.92 %), rate of germination (4.58), seedling survival (72.08%), SVI 1 (4.75cm), SVI 11 (49.44g) and SV1 111(0.36 mg) compared to control (78.69%), (3.93), (68.44 %), (4.20 cm), (42.07g) and (0.23 mg) respectively.

Keywords: Seed; Dormancy, *Vitis vinifera* L.; Salicylic Acid; Scarification; Sandpaper; Thermal.

Introduction

Grapevine is one of the most important fruits in the world. The history of Viticulture started seven to eight thousand years ago when the grape (*Vitis sp*.) was domesticated in some areas in middle Asia [8] Commercial grapes belonging to the genus Vitis which is one of the 14 genuses belonging to the Vitaceae family [1]. Also, grape is linked to a lower risk of heart disease, cancer, high blood pressure, allergies, diabetes, constipation, and other conditions [23]. There are more than 700 species and more than 14,000 cultivated varieties in the world. In the world, grapes are grown for several purposes 71% of the grapes are used for making wine, 27% is used for eaten fresh, and the remaining 2% is being consumed as dried fruit [6].

In Iraq, about 100 cultivars were found, of which 70 in the Kurdistan region [14]. The most often grown grape fruit in Iraq are several types of the genus *Vitus vinifera*, also referred to as the European grape or the grape of the old world [28].

Seed germination is a complicated process that starts with water absorption, and after a brief period, enzyme activity commences [17]. Cultivation from seed to seedling is one of the most important determinants of reproductive output [28]. Seed dormancy is a survival mechanism that prevents germination under adverse environmental conditions. Breaking dormancy involves overcoming physiological and physical barriers [10].

The *Vitis* seeds generally have very low germination rates unless end dormancy has been overcome; Seed dormancy in grape is often ascribed to a thick and tough seed coat that can be a mechanical barrier to germination [19]. It has long been known that grape seeds have dormant characteristics [26]. Simply put, seed dormancy is a barrier that prevents seeds from fully germinating under favorable conditions. For germination of seeds in grape breeding programs, it is required to break seed dormancy. The main factors causes of dormancy in seeds can be summed up as follows: external (environmental) factors outside the seeds, internal (biochemical) factors inside the seeds, and physical (mechanic) factors in the seed structure [18].

Grape seed germination is impacted by various factors, including the specific grape cultivar and pre-treatment methods. Different cultivar exhibit

varying germination rates and responses to treatments like scarification, salicylic acid gibberellic acid (GA3) or cold stratification [13]. Optimal germination can be achieved by tailoring pre-treatment methods to the specific grape variety.

Salicylic acid (SA) is a phytohormone known for its role in plant growth, development, and defense responses. It also plays a significant role in breaking seed dormancy and promoting germination. Salicylic acid is known to reduce Abscisic acid (ABA) levels, enhance enzyme activities related to germination, and modulate reactive oxygen species (ROS) levels [3], [15]. Several studies have explored the effects of SA on grape seed germination. A study by [16] showed that pre-soaking grape seeds in salicylic acid solutions (ranging from 0.1 mM to 1 mM) significantly improved germination rates compared to untreated seeds. SA may also stimulate the release of certain enzymes like amylase, which break down starches stored in seeds, facilitating seedling growth.

Scarification is a method used to break seed dormancy, can potentially enhance germination rates and improve seedling vigor [7]. Seed dormancy in grapes is often attributed to the hard seed coat, which restricts absorption of the water and gas exchange, and the presence of germination inhibitors within the seed. Various scarification methods have been explored to overcome these barriers. Mechanical scarification includes physically shattering or weakening the seed coat using abrasives or cutting. Studies have shown that mechanical scarification can greatly improve water uptake and germination rates in hard-seeded species [6]. Thermal Scarification, involves subjecting seeds to high or alternating temperatures to break dormancy. Thermal treatments have been effective in some seeds by disrupting seed coat integrity and altering internal seed physiology [5]. Studies on grape seed scarification have indicated that mechanical scarification is particularly useful in promoting germination. According to [27], mechanical scarification of grape seeds significantly improved germination percentages compared to untreated seeds, which often exhibit delayed or poor germination due to the impermeability of their seed coat.

Many studies have been conducted on the release of dormant seeds from Vitis species [25]. Dormancy of seed in grape is a crucial physiological mechanism that prevents premature germination, ensuring that seeds only germinate under favorable environmental conditions. Grapevine seeds typically have a deep physiological dormancy that is influenced by environmental signals, particularly temperature and light, which they sense to time their germination appropriately. Understanding seed dormancy in grapevines is a key for effective

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cultivation, breeding, and seedling production [20].

The present study aimed to investigate the combined effects of salicylic acid and scarification on breaking dormancy in grape seeds, enhancing germination rates and seedling vigor, and the findings will have practical implications for grape seed propagation and breeding programs and to enhance germination rate and improve vineyard establishment.

Materials and Methods

The clusters of two local cultivars of table grape (*Vitis vinifera* L.) cvs 'Halawani and Zaitony' were taken from a private vineyard at the road of Kirkuk - Erbil in Hamzakor village, Qushtapa town, Erbil province, Kurdistan region, Iraq, located on (28km) South-east of Erbil Altitude of elevation 410 m above sea level Latitude N 35.970 and Longitude E 44.070.

Experimental materials

To study seed germination of (Halawani (C1) and Zaitony (C2)) grape cultivars using Salicylic acid (SA) treatment as well as Scarification (Sc), grape seeds were collected from mature, healthy grape berries from fully ripened clusters the vines are 4 years old cultivated in (2020) and trained as T-Trellis training method, with 2 x 4 meters apart, and 2 meters high above soil surface, system irrigated with drip system. Clusters were brought from a private vineyard transported to the laboratory of Horticultural and Landscape Design Department / Agricultural Engineering Sciences, University of Salahaddin, Erbil, Iraq.

Seeds were separated from the flesh of berry then washed well with water several times to get rid of sticky in order to prevent or lessen the incidence of diseases, the seeds were treated with hot water at a temperature of 50 to 52°C for 10 minutes after substances adhered to them, the survival of which causes fungus growth and rotting. The seeds were then placed in a container of water to remove the empty seeds [2].

Treatments: Salicylic acid (SA) with three concentrations (Control (0), 1mM (1) and 2mM (2)) seeds were held for 24 hours, in control the seeds placed in distilled water, as well as Scarification (Sc) (Control, thermal and mechanical), control (Without scarification), in mechanical scarification, seeds were rubbed with medium grade of sandpaper for 2 minutes [9]. In thermal scarification seeds were exposed to hot water (80°C) for intervals 15 minutes [11], in control the dry seeds were sown without any treatment, in combined treatment seeds scarified followed by soaking in Salicylic acid.

Seed storage

Moist chilling treatments consisted of (2 months) the seeds were placed into refrigerator at 5±1^{o}C

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using plastic bags containing moistened peat-moss [12].

After seed storage, the seeds were washed and placed in (12×40×15 cm) in boxes with germination media (12kg of soil that contained one part sand (6 kg) and one part peat-moss (6 kg).

The number of planting plastic boxes used in the research were (54) for two grape cultivars (27 box for Halawani and 27 box for Zaitony), at a rate of 20 seeds per box. This research was undertaken during the period October 1st 2024 at a private greenhouse located in Mirkan xdeer village, Qushtapa town in Iraq's Kurdistan region's Erbil province, located on (37 km) western north of Erbil Altitude of 399m elevation above mean sea level Latitude N 36°00'11.6"and Longitude E 44°12'31.5. Under growing conditions (28 ± 2°C and 65% humidity), germination was realized. The seeds germinated after 2 weeks of sowing and continue until the germination ceased in compliance with the seed testing guidelines. The indication of germinated seed was determined as the cotyledons emerging through the medium. The data collected included:

Germination %:

$$\text{Germination}(\%) = \frac{number\ of\ seed\ germinated}{number\ of\ seed\ sown} \times 100$$

Seedling Survival (%):

$$\text{Seedling survival}(\%) = \frac{\text{Number of seedling survived}}{\text{Number of seedling emerged}} \times 100$$

Days taken for Germination:

$$\text{Mean days} = \frac{N1\ \ T1 + N2T2 + \ldots\ldots + \ N21T21}{\text{Total number of germinating seeds}}$$

Mean days = the average number of days required for seed germination.

N = the number of seeds germinating within consecutive interval of time.

T = the time between the start of the test and the particular measurement interval.

In common practice, three modifications of seed vitality (SVI) can be used, depending on which parameter you use from the seed. At the end of seed cultivation, seedling lengths or grape biomass or dried biomass weights can be measured:

1- SVI I is calculated by multiplying seed germination (SG) (%) with the total length of seedling (cm).
2- SVI II is calculated by multiplying seed germination (SG) (%) with the total fresh weight of seedling (g).

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3- SVI I11 is calculated by multiplying seed germination (SG) (%) with the total dry weight of seedling (mg).

Experimental Design:

The experiment was performed in a Factorial experiment according to a Complete Randomized Design (C.R.D) with three factors (Cultivar, SA concentrations and Scarification methods) the number of treatments (18) each having 20 seeds with three replicates, the overlap between them as well as the control treatment, the number of experimental units became 54 (2* 3* 3*3).

The SAS statistical tool (SAS 2003) was used to perform an analysis of variance (ANOVA) on the data obtained, and when significant variations are found, the mean values assess at the $p<0.05$ level of significance using Duncan's Multiple Range Test [21].

Results and Discussion

Effect of Grape Cultivar on Grape Seed Germination and Seedling Vitality Index (SVI):

Days taken for seed germination, germination percentage (%) and rate of germination

The days needed for seed germination, germination percentage, and rate of germination in grape seeds were not significantly impacted by grape cultivar, according to table (1).

Data indicates that cultivar had a substantial impact on seedling survival; the Zaitony cultivar had the highest percentage (71.30 %), while the Halawani cultivar had the lowest (69.05%).

SVI 1, SVI 11 and SVI 111:

The effect of grape cultivar on SVI1 has significant effect as shown in table (1), the Halawani cultivar contributed the maximum value (4.75 cm) and the minimum value in the Zaitony cultivar (4.20 cm). For SVI 11(g) the data explained that the effect of grape cultivar has non-significant effect. The highest value of SVI 111 was recorded as in Zaitony cultivar (0.32 mg) and the lowest in Halawani (0.27 mg).

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Table 1: Effect of grape cultivar on grape seed germination and seedling vitality index (SVI) *

Cultivar	Parameters						
	Days taken for germination	**Germination %**	**Rate of germination**	**Seedling survival %**	**SVI 1 (cm)**	**SVI 11 (g)**	**SVI 111 (mg)**
C1	20.52 a	80.92 a	4.25 a	69.05 b	4.75 a	45.76 a	0.27 b
C2	20.07a	81.16 a	4.25 a	71.30 a	4.20 b	46.62 a	0.32 a

*Values within each column followed by the same letter are not significantly different from each other according to Duncan's Multiple Range Test at 5% level of probability.

Effect of Salicylic Acid on Grape Seed Germination and Seedling Vitality Index (SVI):

Days taken for seed germination, germination percentage (%) and rate of germination.

The impact of SA on days taken for grape seed germination is illustrated in table (2), soaking seeds in SA had a highly significant effect on number of days required for germination, soaking in SA (2 mM) resulted in fewer days for germination (18.25 days), while the control resulted in the highest number of days for germination (21.97 days) .

This experiment was mainly conducted to determine the optimum range of SA concentration for seed germination. Germination percentage was significantly affected by soaking seeds in SA (2 mM) (88.51%) resulted in the highest value, the control had the lowest germination percentage (77.10%). No significant differences were observed between soaking in SA (1 mM) and control.

Soaking seeds in SA had a highly significant effect on rate of germination in grape seeds; soaking seeds in SA (2 mM) recorded the highest value (5.05), while control recorded the lowest value (3.74).

The impact of SA on the seedling survival in grape seeds was shown maximum value recorded in SA (2 mM) (75.28**%**), whereas, the minimum value recorded in the control (67.33%).

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SVI 1, SVI 11 and SVI 111:

The table (2) show us that the effect of SA on SVI 1, SV1 11 and SV1 111 in the grape seedlings. SA (2 mM) treatment had the highest significant influence value (5.25 cm, 53.95 g and 0.40 mg), while the SA (1mM) had the lowest value (4.01cm, 37.24 g and 0.22 mg) respectively.

Table 2: Effect of Salicylic acid on grape seed germination and seedling vitality index (SVI) *

	Parameters						
SA(mM)	**Days taken for germination**	**Germination (%)**	**Rate of germination**	**Seedling survival (%)**	**SVI1 (cm)**	**SVI 11 (g)**	**SVI 111 (mg)**
0	21.97 a	77.10 b	3.74 c	67.33 b	4.17 b	37.24 c	0.27 b
1	20.67 b	77.51 b	3.97 b	67.93 b	4.01 b	47.37 b	0.22 c
2	18.25 c	88.51 a	5.05 a	75.28 a	5.25 a	53.95 a	0.40 a

*Values within each column followed by the same letter are not significantly different from each other according to Duncan's Multiple Range Test at 5% level of probability.

SA treatment may have resulted in improved or delayed seed germination depending on concentration. Some studies show that low concentrations of SA can accelerate seed germination by improving water uptake and enhancing metabolic activities [22]; [4].

The study may not have explored a wide range of SA concentrations. The effects of SA on plant growth can be dose-dependent, and certain concentrations might have stimulatory effects, while others could inhibit growth [29]. Including a more comprehensive gradient of SA concentrations could offer more insight. The effects of SA are often cultivar-dependent. For instance, a study on *Vitis vinifera* L. cultivars by [24]. Reported that SA enhanced seed germination and seedling growth in cultivars like Chardonnay and Cabernet Sauvignon. However, the optimal concentration and treatment duration varied for each cultivar.

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Effect of Scarification on Grape Seed Germination and Seedling Vitality Index (SVI):

Days taken for seed germination, germination percentage (%) and rate of germination.

Days taken for germination showed significant differences between submerging seeds in hot water and scarified seeds with sandpaper compared with control, table (3). Sandpaper resulted fewer days for germination (19.74 days), while non-scarified seeds recorded the higher number of days taken for germination (21.19 d days). Perusal of results presented indicates that the significantly highest germination percentage (83.92%) was observed in scarified seeds with sandpaper followed by (80.53%) soaking seeds in hot water which were significantly higher than non scarified seeds (78.69).

Scarified seeds given a significant result on rate of germination compared with non-scarified seeds, scarified seeds with **s**andpaper reached the highest value (4.58), followed by thermal scarification (4.25), while non scarified seeds recorded the lowest value (3.93). The highest significant value of seedling survival appeared with sandpaper scarification (72.08%), the second highest percentage was obtained with seeds scarified in hot water (70.01) compared with non scarified seeds (68.44%).

SVI 1, SVI 11 and SVI 111:

Scarification given a significant result on SVI 1, **s**andpaper reached the highest value (4.75cm). Where, the non scarified seeds recorded the lowest value (4.20cm).

Data showed significant differences between scarification treatments on SVI 11, Sandpaper recorded the highest value (49.44 g). Where, the control recorded the lowest value (42.07 g). Sandpaper recorded the highest value of SVI 111 (0.36 mg). Where, control recorded the lowest value (0.23 mg).

The thermal and sandpaper scarification treatments would likely lead to significantly higher germination rates compared to the control group. The study might show that seeds subjected to sandpaper treatments germinated more quickly, likely because of the softening the seed coat, which allows to improve the absorption of water absorption. Similarly, hot water scarification would physically disrupt the seed coat, facilitating earlier germination. [5]

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Table 3: Effect of Scarification on grape seed germination and seedling vitality index (SVI)*

Scarification	Parameters						
	Days taken for germination	Germination (%)	Rate of germination	Seedling survival (%)	SVI 1 (c m)	SVI 11 (g)	SVI 111 (mg)
Control	21.19 a	78.69 c	3.93 c	68.44 c	4.20 c	42.07 c	0.23c
Thermal	19.95 b	80.53 b	4.25 b	70.01b	4.47 b	47.05 b	0.29 b
Sand paper	19.74 b	83.92 a	4.58 a	72.08 a	4.75 a	49.44 a	0.36 a

*Values within each column followed by the same letter are not significantly different from each other according to Duncan's Multiple Range Test at 5% level of probability.

Effect of Interaction between Cultivars, Salicylic Acid and Scarification on Grape Seed Germination and Seedling Vitality Index (SVI):

Days taken for seed germination, germination percentage (%) and rate of germination.

The germination period significantly varies across interaction treatments between cultivar, salicylic acid and scarification on days taken for germination of grape seeds, the highest significant value appears in C2+ SA (0 mM) + non-scarified seeds (23.67 days) compared with other interactions, while lowest significant value appears in C2 + SA (2 mM) + scarified seeds with sand paper (17.17 days) as shown in table (4).

The present study indicates that mechanical scarification with sandpaper soaked in SA (2 mM) of grape seeds C2 produced the highest performance for all the parameters studied (germination percentage, rate of germination and seedling survival) compared with another interactions.

The highest percentage of germination from C2 + SA (2 mM) + scarified seeds with sandpaper (91.93 %) and the lowest value in C1 + SA (0 mM) + non scarified seeds (73.33%).

The highest rate of germination in C2+ SA (2 mM) + scarified seeds with sandpaper (5.49) which significantly difference with other interactions, the lowest percentage resulted from C1 + SA (1 mM) + non scarified seeds (3.46).

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The highest percentage of seedling survival appears in C2 + SA (2 mM) + scarified seeds with sandpaper (78.47%) compared with other interactions, while lowest value appears in C1 + SA (0 mM) + none scarified seeds (65.35%).

SVI 1, SVI 11 and SVI 111:

The results obtained showed that SVI 1, SVI 11 and SVI 111varied considerably depending on the different interaction treatments. The results of the analysis of variance showed the treatments differed significantly ($p < 0.05$).

The highest percentage of SVI 1 (cm) appears in C2+SA (2 mM) + scarified seeds with sand paper (5.86 cm) compare with other interactions, while the lower value recorded in C2+ SA (1 mM) + non scarified seeds (3.29 cm).

The table (4) indicated that the interaction between, cultivars salicylic acid and scarification on SVI 11. (mg) in the grape seeds had significantly affected, the highest value in C2+SA (2 mM) + scarified seeds with sandpaper recorded (57.02 g), and the lowest value recorded (27.99g) in C21+ SA (0 mM) + non scarified seeds.

For SVI 111 the highest value appears in C2+SA (2 mM) + scarified seeds with sand paper (0.51mg) with sand paper, and the lowest value recorded (0.15mg) in C1+ SA (1 mM) + non scarified seeds.

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Table 4: Effect of interaction between cultivars, Salicylic acid and scarification on grape seed germination and seedling vitality index (SVI)*

Cultivar	Treatments		Parameters						
	SA (mM)	Scarification (Sc)	Days taken for germination	Germination (%)	Rate of germination	Seedling survival (%)	SVI. 1 (cm)	SVI. 11 (g)	SVI.111 (mg)
C1	0	Control	23.67 a	73.33 g	3.56 ef	65.35 e	3.92 fg	27.99 i	0.19 hi
		Thermal	21.83 a-c	76.87 c-g	3.79 ef	66.97 e	4.79 cd	40.76 h	0.23 g-i
		Sand Paper	21.17 b-d	80.77 c	3.85 ef	67.83 de	4.96 cd	42.69 h	0.33 c-f
	1	Control	21.27 a-c	75.60 e-g	3.46 f	65.50 e	4.11 ef	44.40 gh	0.15 i
		Thermal	20.80 b-e	77.83 c-f	4.01 e	67.00 e	4.62 de	47.91fg	0.25 f-i
		Sand Paper	19.67 c-g	79.97 cd	4.57 cd	68.53 de	4.63 de	48.47 f	0.25 f-i
	2	Control	18.40 e-h	86.91 b	4.85 b-d	71.48 cd	5.62 ab	53.20 b-d	0.34 c-f
		Thermal	18.17 f-h	85.67 b	5.03 a-c	73.50 bc	4.79 cd	49.93 d-f	0.31 d-g
		Sand Paper	19.70 c-g	91.33 a	5.18 ab	75.30 ab	5.32 a-c	56.48 ab	0.42 a-c
C2	0	Control	23.07 ab	74.00 fg	3.57 ef	66.35 e	3.47 fg	28.26 i	0.21 g-i
		Thermal	21.10 b-d	76.87 c-g	3.81 ef	68.77 de	3.87 fg	40.91 h	0.27 e-h

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	Sand Paper	20.93 b-d	80.77 c	3.86 ef	68.70 de	3.98 f	42.84 h	0.38 b-d
1	**Control**	22.03 a-c	76.27 d-g	3.46 f	65.49 e	3.29 g	46.48 fg	0.16 i
	Thermal	20.40 c-f	76.67 c-g	3.82 ef	67.37 e	3.63 fg	47.81 fg	0.21 g-i
	Sand Paper	19.83 c-g	78.73 c-e	4.49 d	73.67 bc	3.76 fg	49.17 ef	0.30 d-g
2	**Control**	18.67 d-h	86.00 b	4.67 cd	76.43 ab	4.76 cd	52.08 c-e	0.36 b-e
	Thermal	17.40 gh	89.23 ab	5.06 a-c	76.47ab	5.14 b-d	54.99 a-c	0.45 ab
	Sand Paper	17.17 h	91.93 a	5.49 a	78.47a	5.86 a	57.02 a	0.51 a

*Values within each column followed by the same letter are not significantly different from each other according to Duncan's Multiple Range Test at 5% level of probability

Conclusion

The study's comparison of two grape cultivars, **'Halawani'** and **'Zaitony'**, provides valuable insight into how different grape cultivars may respond differently to SA treatment and scarification.

These experiment's overall results showed that grape seeds displayed hard seed coat dormancy. Softening the seed coat by soaking the seeds in SA significantly increased seed germination. The optimum mechanical treatment to break the dormancy imposed by the coat on those cultivars' seeds was found to be mechanical scarification, or sandpaper. Given that treatments to induce grape seed germination in this research were ones that could effectively break the seed coat, it can be concluded that the seed coat was the major barrier to grape seed germination. The increasing interest in using salicylic acid and scarification as practical tools for improving grape seedling establishment. For optimal results, research suggests tailoring these

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treatments based on the specific cultivar and environmental conditions.

More thorough research is required in order to fully comprehend grape seed dormancy, germination, and seedling production. No published work currently integrates all three variables in grape seeds, presenting a valuable research opportunity.

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