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RESEARCH PAPER

Effect of Gibberellic Acid (GA3) and Stratification on Seed Germination and Seedling Growth of Grape (*Vitis vinifera* L.)

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A B S T R A C T:

The research was carried out at the lath-house of the college of Agricultural Engineering Sciences, Salahaddin University-Erbil for the period from August 2020 to February 2021 using grape (*Vitis vinifera* L.) cv. Rash-meri seeds to study the effect of (GA3 concentration and stratification period) on germination and seedling vigor to obtain good and healthy seedlings within a relatively short time. The seeds were soaked in 3 concentrations of GA3, which are (500, 100, 1500 mg. l^{-1}), in addition to the control (0 mg. l^{-1}) for 24 hours, then treated seeds were stratified for (1, 2 and 3 months) at 5±1oC. Complete Randomized Design (C.R.D) was used as a factorial experiment as three replications with 25 seeds for each treatment. The SAS statistical tool (SAS 2003) was used to perform an analysis of variance (ANOVA) on the obtained data, when significant differences were found, the means were correctly separated, and mean values were assessed at the $p<0.05$ level of significance using Duncan's Multiple Range Test. The results showed that there were significant differences in the germination percent and seedling growth, with the increase in GA3 concentration, the differences increased significantly compared with control, the concentration (1500mg.l^{-1}) gave the highest increase in all studied traits (Seed germination%, Stem length (cm), Stem diameter (mm), Root length (cm), Vigor index, shoot and root dry weight(g), Total dry weight (g) and quality index. With increasing stratification period differences increased significantly, the highest values were recorded in seeds stratified for (3 months). As for the interaction effect of 2 studied factors, the treatment that exceeded the other was (1500 mg.l^{-1}) GA3 and (2 and 3 months) stratification period compared with seeds not treated with GA3.

KEY WORDS: Keywords Rash-meri, Seed, Grape, GA3, Stratification
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1. INTRODUCTION :

The germination of seeds is a complex process that begins with the absorption of water and after a short time the activity of enzymes begins (Matilla and Matilla-Vazquez, 2008).

Seed dormancy is the preventing of seed germination under favorable conditions. The primary causes of seed dormancy can be categorized as physical factors within the seed structure, internal factors within the seeds, and external factors outside the seeds, to break dormancy, growth-regulating techniques such as washing, drying, temperature, light, mechanical, stratification, and scarification are used, as well as combinations of one or more of these techniques (Yalvaç, 2006).

Grapevine (*Vitis vinifera* L.) seeds germinate with some difficulty due to the dormancy period. These difficulties arise particularly in breeding studies. Therefore, determining the practices that induce germination rate is very important to production seedlings with a strong root system resistant to soil conditions, especially when the phylloxera insect spreads, which infects the roots of seedlings by budding, grafting and also for breeding programs (Akkurt et al., 2013). Cultivation from seed to seedling is one of the most important determinants of reproductive output (Yang et al., 2021). Germination rate of grape seeds is usually

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30-50% (Gan et al., 2008). Low germination rates may be due to parental selection, poorly developed seed embryos in female parents, or hard seed coats that do not divide easily (Gan et al., 2009). Furthermore, studies on grape seeds (*Vitis spp*. L.) have shown that dormancy release during stratification is controlled not only by stratification temperature but also by the duration of stratification suggesting that cold stratification affects seed growth, t it is responsible for the dormant. Rash-meri cultivar considered to be an excellent table grape is widely grown in several areas of Kurdistan Region/ Iraq. It is particularly well-known for its thickly-skinned grapes with good-fleshy juice that can be used for both fruit and pulp. The cultivar has good commercial properties if it is grown with flowers that are physiologically female or functionally pistillate, so it needs pollinizers to produce seed. The berries are oval-shaped, large in size, black at maturity, juicy and textured, covered with bloom, containing 1-4 seeds (Al-Atrushy, 2018). Previous studies on grape seed germination and seedlings have addressed differences in germination rates between different cultivars, differences in seedling rates between different cultivation and transplanting methods, or resolving the physiology of germination and dormancy characterization of the best way to release seed dormancy (Wang et al., 2009 and Zhang et al., 2008). Plant hormones, which are produced by both plants and soil microbes, and their interactions with plant genes are some of the processes regulating seed germination and dormancy, according to study (Miransari and Smith 2014). The fundamental cause of seed dormancy, according to research, is hormones, ABA is the endogenous inhibitor of seed dormancy, and GA and IAA are germination promoters and the equilibrium between endogenous growth inhibitors and growth, hormone agents is necessary for seed dormancy and germination (Carr, 2009 and Wang et al., 2009).

Gibberellic acid (GA3) is growth regulator that promotes germination by stimulating enzyme activation (Hartman and Kester, 2017). Using GA3 was investigated to enhance seed germination in orchards and to obtain uniform seedling size in nurseries (Dhupper, 2013). Cold treatments are often used to promote germination of dormant seeds, wet-chill treatment is thought to alter the balance between inhibitors and promoters and they are often used to promote germination of dormant seeds (Hassan and Fetouh 2014). During stratification, the seed shell softens and absorbs oxygen and water more easily. The temperature should be kept between 0 and 10°C during the stratification period. For grape seeds, the optimal stratification temperature is 5 °C (Ağaoğlu, 2002)

Grape seeds' dormancy traits have been known for a long time, according to the majority of studies, cold stratification is the most effective method for releasing grape seed's dormancy and the removed embryo can result in a healthy seedling (Gan et al., 2008). Consequently, grape seed dormancy may be of the physiological dormancy type (Baskin and Baskin, 2014). Although cold stratification is the usual technique for releasing the physiological dormancy of grape seeds, the temperature and duration of the stratification differ significantly amongst different investigations. Breaking the dormancy of Rash-meri and other local cultivars has not been studied up to now, so trials were connected to determine the effect of using four concentrations of GA3 and three periods of stratification to increase germination capability.

2. MATERIALS AND METHODS

To study seed germination of Rash-meri grape cultivar using Gibberellic acid (GA3) treatment as well as stratification periods fully ripened fruits were brought from the local market in the month of August-2020 and transported to the laboratory of Horticultural Department / Agricultural Engineering Sciences, University of Salahaddin, Erbil, Iraq. After seeds were separated from the flesh of berry, they washed well with water several times to get rid of sticky substances stuck to them, the survival of which leads to the growth of fungi and rotting, where the seeds were placed in a container of water to get rid of the empty seeds, and they were treated with hot water at a temperature of (50-52 ^{o}C) for 10 minutes in order to prevent or reduce the incidence of diseases Fungal infection (Al-Khafaji et al., 1990). The seeds were dried and then soaked in 4 concentrations of GA3 (control, 500, 1000 and 1500 mg.l^{-1}) in control the seeds were put into pure water. After that they were held there for 24 hours and moist chilling treatments consisted of (1, 2 and 3 months) the seeds were placed into refrigerator at 5±1oC (Hartmann and Kester ,2017), using plastic bags containing moistened

peat-moss, under lath house conditions every one month seeds were removed from the refrigerator and sowed. The seeds were sown from (19-9, 19-10 and 18-11-2020) after each stratification period inside the boxes filled with one part peat-moss and one part sand in lines at the rate of 25 seeds for each treatment and for each replicate. The seeds were covered with the same planting soil and slightly pressed to stabilize them. The treatments were randomly distributed and irrigated with water a day after planting with a low-pressure spray in order to preserve the seeds in their locations without being scattered by irrigation water and the seed was irrigated as needed.

2.1. Experimental design

Factorial experiment was carried out according to a Complete Randomized Design (C.R.D) with two factors (GA3 concentration and stratification period) with overlap between them as well as the control treatment, the number of treatments (12) with three replicates, so the number of experimental units became 36 (3* 4* 3). The SAS statistical tool (SAS 2003) was used to perform an analysis of variance (ANOVA) on the obtained data, when significant differences were found, the means were correctly separated, and mean values were assessed at the $p<0.05$ level of significance using Duncan's Multiple Range Test (Roger and Hasted, 2003).

2.2. Studied traits

After 2, 4 and 6 weeks of sowing, seed germination percentages were recorded and continued till the germination ceased, accordance to the guidelines for seed testing, the percentages of germination of the seeds were calculated, after three months of growth, 5 representative seedlings from each replication of a treatment were chosen in order to measure the parameters of growth: shoot and root length (cm), shoot diameter (mm), and shoot and rot dry weight (g). Shoots and roots were oven dried for 72 hours at 70 °C to measure dry weight. A vigor index was calculated according to Abdul-Baki and Anderson (1973). = germination percentage*seedling total length (shoot + root length).

3. RESULTS AND DISCUSSION

3.1. 3.1. Effect of GA3 on seed germination and seedling vigor of grape:

It is clear from the results of Table (1) that increasing the concentration of GA3 increased the percentage of grape seed germination cv. Rash-mer. The difference between treatments and control are significance during seed germination at (2nd, 4th and 6th weeks), the highest value was noted in (1500 $mg.l^{-1}$) GA3 (28.66, 42.33, and 52.33 %) comparing with control (6.52, 12.62 and 26.17%) respectively, in addition to the superiority of all treatments with GA3 over the control.

In same table results are shown the positive effects of GA3 treatment on shoot length and diameter, root length, vigor index, shoot and root dry weight, total dry weight and quality index after seed germinated, The treatment (1500 $mg.l^{-1}$) GA3 gave the best values starting from (8.21 cm ,1.67 mm, 10.30 cm, 969.11, 0.24 g, 0.12 g, 0.36 g and 0.05) comparing with the control (5.83 cm, 1.09 mm, 6.27 cm ,317.06, 0.16 g, 0.04 g 0.20 g and 0.02) respectively. Grape seeds treated with (500, 1000 and 1500 $mg.l^{-1}$) GA3 germinated and seedling grown quickly comparing with control, the current results are in agreement with the investigation of (Pan., et al. 2010) that GA3 significantly improved the germination rate, germination capacity and germination index of some wine and table grape seeds, and shortened the germination time.

By encouraging the release of calcium from the cell membrane and its movement into the cytoplasm, where conditions are favorable for water uptake and cell growth, GA3 increases the size of the cell. After growth, GA3 is deactivated, and calcium is reintroduced to the semipermeable membrane to stiffen it. The embryo produces GA3, which prompts aleurone cells to provide hydrolytic enzymes like α and β amylases that hydrolyze starch into glucose that the embryo may consume. This occurs after the seed has absorbed water and following an active absorption phase. By supplying enzymes and influencing the production of mRNA by proteins, GA3 promotes DNA replication and causes analysis of seed endosperm material (Lahuti et al., 2003). In a wide variety of plant species, GA is recognized to promote seed germination; the predominant active GA differs by species. In a wide variety of plant species, GA is recognized to promote seed germination; the predominant active GA differs by species. By weakening barrier tissues including the endosperm and seed coat, GA promotes seed

reservoir recruitment and releases hydrolytic enzymes that promote embryo growth (Thomas et al., 2005).

Table (1) Effect of GA3 on seed germination and seedling vigor of grape*

GA3 concentration mg.l^{-1}	Week 2	Week 4	Week 8	Stem length (cm)	Stem diameter (mm)	Root length (cm)	Vigor index	shoot Dry weight	root Dry weight (g)	Total dry weight (g)	Quality index
0 Control (water)	6.52 d	12.62 d	26.17 d	5.83 d	1.09 b	6.27 d	317.06 d	0.16 c	0.04 b	0.20 b	0.02 b
500	16.92 c	22.93 c	36 c	7.14 c	1.36 ab	7.71 c	534.11 c	0.19 b	0.06 ab	0.25 b	0.03 ab
1000	25.54 b	31.39 b	48.02 b	8.56 b	1.16 b	8.72 b	830.41 b	0.21 a	0.08 ab	0.30 ab	0.03 ab
1500	28.66 a	42.33 a	52.33 a	8.21 a	1.67 a	10.30 a	969.11 a	0.24 a	0.12 a	0.36 a	0.05 a

*Values within each column followed by the same letter are not significantly different from each other according to Duncan's Multiple Range Test (P≤0.05).

3.2. Effect of stratification period on seed germination and seedling growth of grape:

It is evident from the results of the Table (2) that seed germination percent was increased with increasing stratification periods, the heights significant increase was obtained within (3 months) of stratification period (16.00, 23.30 and 30.60 %), and it was also gave high significant values for all parameters of shoot diameter, root length and shoot dry weight, (1.36 mm, 6.57 cm, 0.19 g and 30.60 %) respectively, and (2months) of stratification period gave heights significant value of shoot length (6.70 cm) and vigor index (400.50), while were illustrated in samples after 2 months of stratification period most of parameters was increased compared with 1 month period. In contrast the results explained that no changed significantly in parameters of root dry weight, total dry weight and quality index after (1, 2 and 3 months) of stratification.

Table (2) Effect of stratification period on seed germination and seedling vigor of grape.*

Stratification period (Month)	Week 2	Week 4	Week 8	Stem length (cm)	Stem diameter (mm)	Root length (cm)	Vigor index	shoot Dry weight	root Dry weight (g)	Total dry weight (g)	Quality index
0 (Control)	6.52 c	12.62 c	26.17 c	5.83 b	1.09 b	6.27 c	317.06 b	0.16 b	0.04 a	0.20 a	0.02 a
1	11.67 b	17.68 b	30.33 b	6.70 a	1.24 ab	6.49 b	400.50 a	0.16 ab	0.03 a	0.19 a	0.02 a
2	16.00 a	23.30 a	30.60 a	6.34 a	1.36 a	6.57 a	394.88 a	0.19 a	0.04 a	0.23 a	0.02 a

*Values within each column followed by the same letter are not significantly different from each other according to Duncan's Multiple Range Test (P≤0.05).

3.3. Effect of interaction between GA3 and soaking period on seed germination and seedling vigor of grape:

GA3 concentrations and stratification periods interaction together gave the best results than each treatment alone on germination percent and seedling vigor as shown in Table (3). Highest values of germination percentage were obtained in (15000 mg.l^{-1}) GA3 with (3 months) stratification period (44.70, .50.67 and 59.67 %) within the (2nd, 4th and 6th weeks), after 6th weeks

percent were reached (59.67 %), it was still lower , these result is indicating that dormancy had not been fully broken by any of the treatments. Shoot length (10.59 cm), root length (11.34 cm) and vigor index (1311.18) registered for the same interaction, while the lowest value was founded in (1 month) stratification without GA3 (6.52, 12.62 and 26.17 %). Seedling showed more shoot diameter (1.90 mm) when seeds treated with (500 $mg.l^{-1}$) then stratified for (3 months) compared with another treatments, the lowest was recorded in (1 month) stratification period and control (1.09 mm). Interaction between (1500 $mg.l^{-1}$) GA3 and (2 months) stratification gave highest shoot dry weight (0.25 g), root dry weight (0.20 g), total dry weight (0.65 g) and quality index (0.09), it differed from the control and 1-month stratification. This increase is due to the combined effect of GA3 and stratification. The promoting effects of GA3 and stratification period on root parameters can be explained by the role of GA3 and stratification in promoting gibberellin synthesis, this also leads to increased growth and root branching, and increased overall root fresh weight (Penfield et al., 2005). According to this results it may be possible that higher concentration of GA3 (1500 $mg.l^{-1}$) could not shorten the stratification period completely.

Table (3) Effect of interaction between GA3 and stratification period on seed germination and seedling vigor of grape*

GA3 Concentration $mg.l^{-1}$	Stratification Period Month	Week 2	Week 3	Week 4	Stem length (cm)	Stem diameter (mm)	Root length (cm)	Vigor index	shoot Dry weight	root Dry weight (g)	Total dry weight (g)	Quality index
0 Control (water)	0 (Control)	6.52 h	12.62 g	26.17 f	5.83 g	1.10 d	6.27 f	317.06 g	0.16 d	0.04 c	0.20 c	0.02 b
	1	11.67 g	17.68 f	30.33 f	6.70 e	1.24 cd	6.49 f	400.50 g	0.16 d	0.03 c	0.19 c	0.02 b
	2	16.00 f	23.30 e	30.60 f	6.34 f	1.36 bcd	6.57 f	394.88 g	0.19 c	0.04 c	0.23 c	0.02 b
500	0 (Control)	16.92 f	22.93 e	36.00 e	7.14 e	1.36 bcd	7.71 e	534.11 f	0.19 c	0.06 bc	0.25 c	0.03 b
	1	18.76 ef	22.50 e	39.33 e	7.68 d	1.51 bc	7.84 e	609.85 f	0.19 c	0.06 bc	0.25 c	0.03 b
	2	21.66 e	29.67 d	46.67 d	7.85 c	1.90 a	8.48 d	762.75 e	0.23 ab	0.11 b	0.33 bc	0.06 ab
1000	0 (Control)	25.54 d	31.39 d	48.02 cd	8.56 bc	1.16 cd	8.72 cd	830.41 de	0.21 bc	0.08 bc	0.30 bc	0.03 b
	1	30.41 c	38.11 c	47.26 cd	9.13 b	1.29 cd	9.04 cd	858.60 cde	0.24 a	0.12 b	0.36 b	0.04 ab
	2	29.44 c	37.01 c	50.00 cd	9.50 b	1.21 cd	9.24 c	937.80 cd	0.22 ab	0.10 b	0.33 b	0.03 b
1500	0 (Control)	28.67 cd	42.33 b	52.33 bc	8.21 c	1.67 ab	10.30 b	969.11 c	0.24 a	0.12 b	0.36 b	0.05 ab
	1	36.00 b	47.59 a	55.33 ab	9.83 b	1.47 b	10.96 ab	1149.60 b	0.25 a	0.20 a	0.65 a	0.09 a
	2	44.70 a	50.67 a	59.67 a	10.59 a	1.72 ab	11.34 a	1311.18 a	0.23 ab	0.13 b	0.36 b	0.05 ab

*Values within each column followed by the same letter are not significantly different from each other according to Duncan's Multiple Range Test ($P \leq 0.05$).

4. CONCLUSIONS

Germination and seedling vigor of germinated grape cultivar were affected by different concentrations of GA3 and stratification periods. Application of GA3, especially (1500 $mg.l^{-1}$) to Rash-meri grape seeds just prior to stratification was more effective than controls in enhancing germination rate and seedling vigor. The best response was obtained with stratification for (3 months), however, a more significant effect interaction was obtained with (1500 $mg.l^{-1}$) GA3 for (2 and 3 months) stratification periods compared to stratified seeds only, with this finding of the current investigation, the combination of an appropriate stratification duration and an efficient level of GA3 will significantly improve seed germination. Our understanding of seed dormancy, germination and seedling vigor in local grape cultivars is still lacking and requires more extensive research.

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